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The development and validation of a novel and quantitative 'Kremer' cleaning classification for reusable medical devices

By

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Doctor of Philosophy

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Based on the research carried out under the supervision of Professor Neil J Rowan and Dr Gerald McDonnell

Declaration

I, the undersigned, hereby declare that this thesis entitled “The development and validation of a novel and quantitative ‘Kremer’ cleaning classification for reusable medical devices” is entirely my own work. The thesis has not been submitted in whole or in part to any other University or Institution. All sources used have been acknowledged and referenced in text.

Terra A. Kremer 16 May, 2024

Confidentiality Statement

All of the information in this thesis is confidential and shall not be disclosed to any further parties without the permission of the first author due to intellectual property constraints. Details of the information presented shall be decided upon with the members of the project prior to public dissemination.

Abstract

For over half a century, Spaulding's microbial reduction categorization has been a guiding principle in the healthcare sector outlining necessary measures to safeguard patient safety for reusable medical devices. However, this classification system operates under an unreliable assumption that medical devices are initially clean before undergoing disinfection or sterilization procedures. This is supported by concerns regarding hospital-acquired infections (HAIs) originating from contaminated devices such as intricate endoscopes and robotic instruments. Despite medical device manufacturers validating their cleaning instructions, best-published literature highlights inconsistent adherence to effective device processing protocols within clinical settings leading to heightened risks to patient safety. Thus, the overarching aim of this novel study is to develop, test, and validate a new cleaning classification system (designated 'Kremer') for appropriate and effective standardized cleaning of reusable medical devices globally focusing on complex device features as a key challenge linked to patient risk.

Novel methods are developed and applied underpinning this simplified Kremer cleaning categorization system. An extensive suite of key device design features was evaluated (n = 23) for residual soils during cleaning validation to ensure their safety for human use. This risk-based approach evaluates the likelihood of residual soil remaining on or within different design features of a device after cleaning. For effective cleaning, the cleaning chemistry (comprising cleaning agent and water) must sufficiently access the soil, either through exposure (such as spraying or soaking) or force (like brushing, flushing, or sonication), to dissolve and remove it from the surface. The 'device feature' becomes a crucial variable influencing this relationship. Moreover, by focusing on the hardest to clean feature of the reusable medical device, the overall cleaning challenge can be established for the entire device. This more conservative approach for validation/verification of cleaning practices allows for the design of cleaning processes that are robust to quantify the risk of patient safety. By simplifying and streamlining classification criteria, users can swiftly assign items or concepts to specific categories, reducing complexity and the likelihood of errors. This simplicity expedites the categorization process, enhances clarity, and lessens cognitive load enabling users to make decisions based on their understanding of device's complexity. This cleaning classification proposes three risk categories: maximal, moderate, and minimal. Twenty-three of the most intricate device features were identified and rigorously tested in this study until they failed to clean effectively. Across the 150 experiments carried out (encompassing ca. 56,000 extractions/flushes for device feature validation and 2,695 individual analyte measurements for the 23 features experiment), each feature underwent evaluation concerning its impact on cleaning that considers geometry, material of construction, probability of soil drying, and fluid dynamics. Among these, the risk of soil drying emerged as the most crucial validation variable. Consequently, soil drying time and soil configuration were manipulated to adjust the cleaning challenge for the features. Manual cleaning, being the most variable method, served as the standardized cleaning approach. However, to explore the potential for excluding manual cleaning from the process, a semi-automated cleaning method was also tested to ascertain the feasibility of automation within the cleaning process. The results of cleaning validation for protein residuals were categorized into risk levels based on acceptance criteria outlined in ISO 15883-5.

The device feature categorization serves as the foundational element for risk assessment, but it is essential to also evaluate compound risks involving device geometry and material of construction. Compound risk occurs when multiple manageable risk factors converge or interact, creating a more complex level of risk. In the context of cleaning reusable medical devices, compound risk arises when various factors combine to make the cleaning process more challenging. This includes factors like complex device design, intricate components, and hard-to-reach areas. When these factors compound, they significantly increase the risk of incomplete cleaning, potentially endangering patient safety. As such, thirteen core topics were addressed using the risk assessment for medical devices outlined in ISO 14971 to quantify the compounding risks and sort reusable medical devices into the 'Kremer' cleaning classification for communicating device design risks across the entire device processing cycle. Medical device manufacturers can utilize this classification alongside Spaulding's antimicrobial criteria to evaluate risks associated with the entire processing cycle for reusable medical devices. For the first time, this integration can enhance validation methods for cleaning, disinfection, and sterilization, improve device design, and ensure effective risk communication and mitigation at healthcare facilities. The well-established Spaulding Classification, focusing on disinfection, sterilization, and patient risk, serves as a convenient means to link manufacturers and healthcare facilities regarding device validation and processing requirements.

The benefits of the conclusions from this novel research extend widely. In addition to completing ten peer-reviewed publications to disseminate the acquired knowledge, it is anticipated that future application of this cleaning classification will yield several advantages. These include enhancing the economics of processing reusable medical devices, fostering trust in sustainability practices related to device reuse, diminishing the occurrence of hospital-acquired infections (HAIs), and guiding the development of future device processing methods, including automation and machine learning. For example, a proposal for a new draft work item (NWI) for industry titled "ISO-NP TS 17664-3, Processing of healthcare products – Information to be provided by the medical device manufacturer for the processing of medical devices – Part 3: Guidance on the designation of a reusable medical device" was accepted by the ISO/TC 198 committee as part of an initiative with Kremer's cleaning classification. This endorsement by an international assembly of experts highlights the practicality and relevance of the classification to the healthcare industry. The introduction of this ISO document is expected to promote the adoption of the cleaning classification in various global guidance and standard documents, establishing it as a valuable tool for risk reduction in healthcare.

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In the relentless pursuit of knowledge, fueled by an insatiable curiosity and an unwavering desire to challenge assumptions, my dissertation emerges as a testament to challenge the status quo. It embodies a journey propelled by fervent determination to delve deeper and understand the complexity. Undoubtedly, the realization of this work owes its existence to the generous investment of time, expertise, and support from a myriad of individuals within my community. Their unwavering commitment and dedication have served as the cornerstone upon which this dissertation stands, highlighting the power of collaboration and the profound impact of collective effort. I am deeply grateful for their hard work, guidance, encouragement, and belief in the significance of this research.

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testing protocols. Christopher Ratanski, your steadfast support was indispensable in navigating through the complexities of this endeavor. Christopher Carfaro, your contributions at the project's inception and your dedication to training our budding scientists were invaluable. To Cindy Rodriguez, Saachi Shibad, Margaret McCauley, Stephen Kinyanjui, Gracie Ahrens, Holyfield Agyekum, Emily Garcia and Jaime Fogarty, I extend my warmest thanks for your tireless efforts in supporting this project. Your collective contributions have been instrumental in this journey, and I am profoundly thankful for each of you.

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List of Abbreviations

ATP: Adenosine triphosphate

CJD: Creutzfeldt-Jakob disease

GTA: Glutaraldehyde

IFU: Instructions for Use

OPA: Ortho-Phthalaldehyde

SKU: stock keeping units

SOP: Standard Operating Procedure

SPD: Sterile Processing Department

TOC: Total Organic Carbon

Glossary

Assurance of Sterility: Qualitative concept comprising all activities that provide confidence that product is sterile. Assurance of sterility implies an end-to-end concept that considers all processes that are required in the development, manufacture, and delivery of a microbiologically controlled or sterile labelled product for its intended use (McDonnell, G; Baseman, H; Cordi-Bancroft, L;, 2021).

Bioburden: Population of viable microorganisms on or in a product and/or sterile barrier system (International Organization for Standardization, 2018).

Biofilm: Communities of microorganisms. Biofilms can consist of single or multiple types of microorganisms, which can be multiplying, dormant, or generally associated with the biofilm structure. Biofilms can be "wet" (associated with water) or "dry" and typically develop on or are associated with surfaces or interfaces (e.g., water lines or storage systems). Biofilms are microbially derived communities characterized by cells that are irreversibly associated with a substratum interface, or each other; they are often embedded in a matrix of extracellular polymeric substances (EPSs) that they produce and exhibit mixed phenotypes with respect to growth rate, gene transcription, and resistance mechanisms (McDonnell, G; Baseman, H; Cordi-Bancroft, L;, 2021).

Clean: visually free of soil and quantified as being below specified levels of analytes. (International Organization for Standardization, 2018) Soil in this context can refer to any unwanted contaminate coming from the manufacturing process or product residuals.

Cleaning: Removal of contaminates to the extent necessary for further processing or for intended use (International Organization for Standardization, 2018).Removal of soil the extent necessary for further processing or for intended use. Modified. 'Soil; can include single or various forms of contaminants. Note that cleaning alone can provide a sufficient level of decontamination under many situations by physical removal and can o be a prerequisite to effective disinfection or sterilization. Contaminants can include unwanted materials between product batches, such as product residuals (McDonnell, G; Baseman, H; Cordi-Bancroft, L;, 2021).

Contaminant: Material (e.g., chemical, biochemical, or microorganism) not intended to be part of a product or process. Examples of contaminants can include soils, protein, dirt, detergent, product residuals, particulates, and microorganisms (McDonnell, G; Baseman, H; Cordi-Bancroft, L;, 2021).

Controlled Variable: A variable that is held constant or limited in a research study as it is not of interest in the research aim but may influence the outcome if not controlled.

Coupon: A coupon is a representative sample or piece of the material or component used to simulate a portion of a reusable medical device in a test system. Coupons allow for consistent and reproducible testing without using the full medical device. A coupon was used for the device feature validation.

Critical Water: Used in the final steps of processing (e.g., final rinse, thermal disinfection, and steam generation), and typically is extensively treated water. The treatment process may include carbon filtration, softening, deionization, reverse osmosis or distillation (Association for the Advancement of Medical Instrumentation, 2023).

Decontamination: Removes soil and pathogenic microorganisms from objects so they are safe to handle, subject to further processing, use or discard. (World Health Organization, 2016)

Disinfection: Process to inactivate viable microorganisms to a level previously specified as being appropriate for a defined purpose (International Organization for Standardization, 2018). Other terms are used internationally to describe disinfection processes including sanitization, germicidal, fumigation,

pasteurization, sterilant, and biodecontamination. These terms can have different regulatory requirements depending on the jurisdiction (McDonnell, G; Baseman, H; Cordi-Bancroft, L;, 2021).

Independent Variable: A variable whose variation does not depend on that of another.

Medical Devices: defined in part as instruments, machines or implants intended by the manufacturer to be used for human beings for a medical purpose. Medical devices are identified and labeled as a **single-use** medical device, defined as "medical device labelled or intended to be used on one individual during a single procedure". Alternatively, labeling may indicate a **reusable** medical device as per "medical device designated or intended by the manufacturer as suitable for processing and reuse" (International Organization for Standardization, 2018).

Microbiological Quality: *a qualitative concept comprising all activities that provide confidence that product is microbiologically safe according to its intended use.* (McDonnell & Hansen, 2020)

Processing: <Preparation of medical devices> activity to prepare a new or used health care product for its intended use (International Organization for Standardization, 2018).

Reprocessing: All steps that are necessary to make a contaminated reusable medical device ready for its intended use. These steps may include cleaning, functional testing, packaging, labelling, disinfection and sterilization. (World Health Organization, 2016)

Robustness: The robustness of an analytical procedure is a measure of its capacity to meet the expected performance criteria during normal use. Robustness is tested by deliberate variations of analytical procedure parameters. (U.S. Department of Health and Human Services Food and Drug Administration, 2024)

Soil: Natural or artificial contamination on a device or surface following its use or simulated use (McDonnell, G; Baseman, H; Cordi-Bancroft, L;, 2021).

Sterilization: Validated process used to render product free from viable microorganisms.

Note 1 to entry: In a sterilization process, the nature of microbial inactivation is exponential and thus the survival of a microorganism on an individual item can be expressed in terms of probability. While this probability can be reduced to a very low number, it can never be reduced to zero (International Organization for Standardization, 2018). A product can include components during a manufacturing process or a final product. Sterilization is a suitably designed, validated and controlled process that inactivates or physically removes viable microorganisms in a product until sterility is obtained. The process should meet pre-established specifications that will result in the inactivation or removal of microorganisms in a statistical reproducible manner/to predefined specification or to achieve sterility. Physical removal (filtration) process can be used but terminal sterilization (antimicrobial) processes are defined by a probability of survival in a final product (McDonnell, G; Baseman, H; Cordi-Bancroft, L;, 2021).

Test Article: Term used to describe the actual device or a specific feature of the device that is subjected to various testing protocols. Within this text, test article is used to describe the device being tested.

Utility Water: Used for precleaning, cleaning and rinsing, utility water is tap water with minimal treatment. As the quality of tap water can vary considerably, to reach the recommended acceptance criteria for utility water, tap water may require simple treatment processes such as the use of water softeners (to reduce hardness levels) and carbon filters (to reduce chlorine and conductivity levels) (Association for the Advancement of Medical Instrumentation, 2023).

Validation: Confirmation, through the provision of objective evidence, that the requirements for a specific intended use or application have been fulfilled (Association for the Advancement of Medical Instrumentation, 2022).

Worst Case: Condition or set of conditions that pose the highest risk(s) of process or product failure within the specified operating range. *Worst case does not mean working outside of normal or desired operating ranges or conditions nor should it necessarily mean those parameters or conditions that pose a high risk(s) of product or process failure. It is important when high risk parameters and conditions are identified, steps should be taken to migrate those risks rather than just including them in the validation studies. Therefore, worst case implies conditions that may have the highest potential to uncover any unaddressed process weaken or variability. As such it may mean working at the extremes of (but still within) the acceptable operating range or design space. It may include such parameters and conditions as exposure time, temperature, system configuration, container and closure design opening, operator presence and activity, etc.*

Chapter 1: Introduction

1.1. Background

Medical devices are of critical importance to patient health, where healthcare is constantly evolving to improve the quality of care provided to patients (McDonnell & Sheard, 2012). Medical devices are a common source of hospital-acquired infections (HAIs) and as of 2013 accounted for 60% to 80% of all bloodstreams, urinary tract, and pneumonia-related infections (Gold & Hitchins, 2013). Over the last 10 years, the occurrence of HAIs related to medical devices continue to be studied (Kola, et al., 2015) (Rahman, et al., 2019) (Rauwers, et al., 2018) (Ofstead, et al., 2020) (Okamoto, et al., 2022) (Southworth, 2014) (Ofstead, et al., 2015) (Lowman, et al., 2013). Medical devices intended to be processed at a healthcare facility must be cleaned, disinfected and/or sterilized prior to subsequent patient use (see Appendix 1). If a device is not cleaned effectively, not only can the disinfection or sterilization process step be ineffective (Alfa, 2019), but residual organic matter from clinical soil may be remaining in concentrations that elicit a biocompatibility risk, including potentially eliciting an undesirable immune response during/after the next patient use (McDonnell & Burke, 2011). Therefore, cleaning instructions are developed to ensure the removal of potential residual organic matter or soil (e.g., physical removal of blood, microorganisms, protein, detergents). This offers significant potential to align traditional established chemical and physical analysis along with microbiological factors so as to determine and inform cleaning efficacy. For example, determining microbiological load reductions including use of rapid *in vitro* approaches (e.g., ATP, biomarker reductions and so forth). Thus, science in this area should be considered as a holistic end-to-end approach as it extrapolates to many areas outside of device processing as cleaning is essential in various manufacturing environments.

1.2 Gaps in knowledge

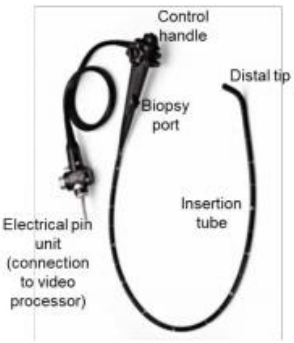
The validation of the instructions for use (IFUs) is designed to demonstrate the method of cleaning can consistently remove analytes to a pre-determined level. However, the validation global strategy employed today is at the discretion of the medical device manufacturer. Important industry regulations, and commensurate guidance were developed primarily using the validation experiences of industry members; however, these regulatory expectations surrounding device cleanliness may be different depending on the geographical location, local customs, regulator experience, cleaning chemistries and equipment. An example of this difference is demonstrated with the acceptance criteria for the cleaning analyte, protein residuals. In the United States the cleaning specification has been established as 6.4µg/cm^2 whereas in parts of Europe the value of 50-100µg/device is the required limit. Additionally, there will be significant interest in delivering effective device cleaning for processing of reusable devices (including sterilization) that will provide an opportunity to enhance 'reuse' options in healthcare where current preference is on meeting complex supply chain and logistics for single-use (disposable) items that have knock-on consequences for sustainable (environmental) waste management.

Cleaning validations supporting device processing instructions for use have historically been performed as prescribed in standards such as the Association for the Advancement of Medical Instruments (AAMI)

ANSI/AAMI ST98:2022 Cleaning Validation of Health Care Products – Requirements for Development and Validation of a Cleaning Process for Medical Devices (Association for the Advancement of Medical Instrumentation, 2022), ISO 15885 *Washer-disinfectors Part 1: General requirements, terms and definitions and tests* (International Organization for Standardization, 2009), and other regional guidance.

The objective of newer standard ANSI/AAMI ST98 is to define validation criteria for a device cleaning process for medical device manufacturers, and provide information on the development of cleaning protocols, test soil selection and determination of acceptance criteria (Association for the Advancement of Medical Instrumentation, 2022). Consensus standards such as ANIS/AAMI ST98 and the ISO 15883 series were developed based on published literature and the experience of those developing the standards. Supporting publications were often clinical surveillance experiments or opinion articles. However, there was a gap in published work supporting the foundational science, particularly controlled evidence-based experimentation.

The complexity of devices increases to meet emerging clinical needs. This coincides with an appropriate time to pursue novel research in order to ensure critical cleaning validation methods generate robust data in order to substantiate the efficacy of the medical device's IFU has consistent variables. Moreover, the criticality of the test variables investigated has a relationship to patient safety; thus, if the validation does not appropriately challenge the device, then patient safety is at risk (Rutala & Weber, 2019). Consider for example, two very different devices in complexity and the expectation that both have the same consideration for patient risk. The biopsy forceps (Image 1) has some complex features, such as hinges and mated surfaces, but is of one material (e.g., stainless steel) and can be terminally sterilized. The duodenoscope at the other end of the cleaning spectrum has extremely complex features such as long lumens, electrical parts, restrictive access areas (e.g., encased distal tip) and O-rings. It also is typically not routinely sterilized, but instead is treated with a liquid chemical disinfectant. In 2019 the US FDA prepared an executive summary titled, "Reducing the Risk of Infection from Reprocessed Duodenoscopes" where they document the patient infection and exposure risks caused by this complicated instrument.



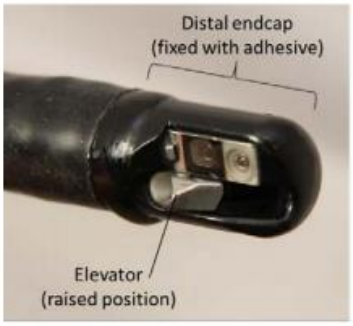


Figure 1.1: Biopsy Forceps **Figure 1.2: Duodenoscope**

Over 50 years ago, Dr. Earle H. Spaulding published a strategic approach still used today describing a risk-based classification of medical devices and recommending requirements for validating the appropriate cleaning, disinfection and/or sterilization of reusable medical devices (Spaulding, 1968); but limited research has been published so as to establish and to confirm such a strategy for the cleaning portion of the device processing instructions. Similar to the Spaulding Classification for disinfection and sterilization, a classification system can also be established for cleaning. However, unlike Spaulding system that used patient exposure to the device so as to establish the risk level, cleaning must consider a multiplicity of other factors such as device features and human variability when determining the potential patient risk. It is noteworthy that residual soil has in recent years been the independent variable tested during the cleaning validation that is known to far exceed the level challenged during a typical disinfection or sterilization validation process.

Consequently, there is a pressing need to establish a new (novel) classification system addressing the relationship between device feature and patient risk. Such a standardized cleaning validation approach should be established for each of the device categories. It is envisaged that this sought-after new standardization process should cater for the future development of device processing as an accurate evaluation of IFU cleaning performance and automated cleaning validations for the manufacture and performance verification for the user.

1.3 Research Aim and Objectives

1.3.1 Research Aim

The overarching aim of this novel study is to develop, test and validate a new 'Kremer' classification system for the effective standardized cleaning of reusable medical devices globally that focuses on complex device features as key challenge linked to patient risk. Thus, this research specifically elucidates, tests and validates the relationship between chemical and physical factors that influence the cleaning and validation of reusable medical devices. An extensive suite of key device design features is addressed that reflect introducing patient risk, such as for residual soils during subsequent use. This approach connects microbiological classification evaluation with this new cleaning process. The data generated will be utilized to establish a novel classification system for cleaning devices that will further facilitate future applications

of AI, machine learning, and automation. This addresses the query regarding the integration of cleaning validation with the overarching goal of processing devices to ensure their safety for human use.

1.3.2 Research Objectives

Operationally, this research has four inter-related novel objectives that are underpinned by commensurate cornerstones for informing alternative or complementary standardized cleaning process for devices.

(i) To establish if a novel cleaning classification for medical devices can be developed that incorporates patient safety risk compared to device design complexity.

(ii) To ascertain if device features can be used as an important novel independent variable within cleaning validations.

(iii) To determine if the aforementioned device feature variable relates to patient risk.

(iv) To determine if these novel device feature(s) can be applied as an approach for device processing validations to generate data that can be future proofed for artificial intelligence and machine learning to include verification testing in the healthcare setting.

1.3.3 Foundational Applied Cornerstones for Stakeholders that aligns with respective Research Objectives

These objectives are also captured and inter-connected via four foundational cornerstones that ensure clear messaging with stakeholders:

Cornerstone 1: Can a cleaning classification for medical devices be developed that incorporates patient safety risk related to device design complexity?

Within this cornerstone, a systematic research review was completed to understand the history of the device processing cleaning validation variables, associated test methods, and acceptance criteria. In addition, an investigation was conducted into how the Spaulding Classification was developed, along with exploring opportunities to apply this guiding framework philosophy to advance and potentially improve cleaning specifications. Fundamental information as to what defines cleaning, and current factors governing efficacy to inform acceptance criteria and standards is articulated. Evaluation of existing measurement test methods and associated requirements also in relation to patient safety demonstrates knowledge gaps in the literature and isolates the necessary research to further explore device cleaning classification. This includes addressing potential limitations, risks, and perceptions. The outcomes of cornerstone 1 provides insight into the knowledge gaps within this area and advance our understanding for how to improve methods of cleaning for medical devices, which considers addressing complexity of design and maintaining functionality post cleaning and decontamination.

Cornerstone 2: Can we isolate device features as the independent variable within cleaning validations?

Each testing variable for a cleaning validation is explored through original research so as to determine how the cleaning validation method may influence the outcome of a cleaning validation experiment. The relationship of the test method acceptance criteria and patient safety is established herein, so all variables in a cleaning validation can be controlled with the exception of the device feature in Cornerstone 3. Specifically, worst case device features are proposed based on complexity and test methods to support cleaning validation in conformance to developing standards. The hypothesis is the following: if control is established over the validation variables, then the device feature can be isolated as the independent variable predictive of patient safety. This novel research proposes new family groupings for device features and necessary equivalence evaluations; thus, new products are appropriately challenged. Data generated is used to inform modelling through indicative machine learning tools that will inform future automation and digital transformation. This has significant implications for future sustainability.

Cornerstone 3 [Objective 3] How is the device feature variable related to patient risk?

Using the device feature approach as the most challenging independent variable, worst case device features is established using the experimental design from Cornerstone 2 and categories are established through original research to demonstrate predictability of device cleanliness. Compounding features were also studied so accurate categories of patient risk can be determined. By categorizing devices using device features, patient exposure and intended procedure a new cleaning categorization system were generated to augment the Spaulding Classification for microbial reduction. The device category then defined recommended device cleaning validation parameters with associated confidence intervals to ensure patient risk is mitigated using the cleaning steps in the device IFU and provide structure to the healthcare facilities for process grouping and verification testing. This novel categorization approach closes the existing patient safety gap between device manufacturers and healthcare facilities for the processing of reusable medical devices.

Cornerstone 4 [Objective 4]: How can we apply the device feature approach for device processing validations of the future (AI/machine learning) and verification testing with digital training (augmented reality) in the healthcare setting?

The design of device categories for cleaning validations allows for more reliable application of the cleaning steps in clinical practice. As devices continue to become more complex, it is necessary to develop a body of evidence that will present rigorous data to give industry and regulatory organizations the scientific support for the consistency of device processing. The Spaulding Classification system is inadequate to address the complexity of all device processing steps (i.e., cleaning), so a subsequent classification must be established to ensure patient safety by driving manufacturers to design for cleaning and users to clean based on design. Operationally, the practical impact of meeting this cornerstone is that it is designed to determine device features of significance in terms of proposed risk to patients; thus, it may be that certain features may not be perceived as a significant risk that will inform a more reliable approach to device

cleaning that includes back-translation to initial design to engineer solutions to mitigate against this occurrence. For the industry, it essentially means that this timely project will inform design thinking by acceptable features for cleaning, and not just for confirming acceptability for clinical/surgical usage. Such information will inform critical data generation for automation, thus reducing further the potential risk to patients.

Within this cornerstone, a cleaning classification system to augment Spaulding's classification system for disinfection/sterilization will be established. This new classification system will be defined with recommendations for how we can ensure patient safety in future validations and build trust with the users for IFU performance using the device feature approach. Once the cleaning classification is established, application of the methodology will be investigated. Using the categories and associated method standardizations will allow for effective verification testing to be performed in a healthcare site using data intelligence with automation, and predictive modeling for device design.

1.4 Structure of Dissertation

Chapter 1 provides a succinct rationale and justification of undertaking this novel research on developing new cleaning classification for reusable medical devices based on device features along with extensive dissemination of key findings arising from same in journals, conferences presentations and new ISO standard. **Chapter 2** describes using a literature review the end-to-end device processing cycle and explores the development of the Spaulding Classification as a mechanism to communicate responsibilities for microbial reduction for reusable medical device between the medical device manufacturer and the healthcare user. In **Chapter 3** the foundational research for test variable selection is detailed with justifications and summaries of the preliminary research required for worst-case variable selection. This chapter describes the validation of the device feature approach and proves that cleaning efficacy data is normally distributed and device feature can be isolated as the independent variable in experimental designs supporting the cleaning classification. **Chapter 4** focuses on the materials and methods required for the experimental designs to challenge the device features. In **Chapter 5** the device feature validation results are described. In **Chapter 6**, the findings from Chapter 5 are analyzed, and a commensurate risk assessment is detailed incorporating acceptance criteria that shape the cleaning classification. This 'Kremer' classification system introduces three categories for devices based on their risk levels—minimal, moderate, and maximal using a quantitative approach to assign classifications. In **Chapter 7**, the application of the cleaning classification within the healthcare industry is elucidated, aiming to enhance risk communication among key stakeholders of reusable medical devices. The chapter emphasizes the integration of the cleaning classification into an ISO document with the intention of expediting its adoption within the industry. **Chapter 8** finalizes the work by arriving at related conclusions and insights addressing some pressing gaps identified from the literature review. The relevance of this novel work for informing ongoing and new industry standards, along with standard approaches to test and validate a device cleaning features framework are described. Finally, implications for future research are advocated along with key recommendations for informing design thinking (manufacturers) and universal uptake of

standardized cleaning approaches in healthcare for reusable medical devices including future provision for AI, and automation.

1.5 Novel contributions made to knowledge and innovation in this field of study

The findings of this novel research have been extensively published in appropriate channels by the author including in major journals, international conferences, industry workshops, and in a new ISO standard for industry. Novel disseminated findings represent all four objectives. It is noteworthy that the thesis in this verbose format is important for industry (especially Johnson & Johnson) as it will be used as a critical framework document to inform and guide future research for and with stakeholders.

1.5 1 Journal Publications arising from these novel studies

- **Kremer TA,** Felgar J, Rowen N, McDonnell G. Validation of the Device Feature Approach for Reusable Medical Device Cleaning Evaluations. Biomed Instrum Technol. 2023;57(4):143-152. doi: 10.2345/0899-8205-57.4.143. Epub 2024 Jan 3. PMID: 38170936; PMCID: PMC10764062.
- **Kremer T**, Rowan NJ, McDonnell G. A proposed cleaning classification system for reusable medical devices to complement the Spaulding classification. J Hosp Infect. 2024 Mar;145:88-98. doi: 10.1016/j.jhin.2023.11.018. Epub 2023 Dec 14. PMID: 38103694.
- **Kremer TA**, Bancroft R, Patel Z, Owen M, McDonnell G. A standardized method for evaluating test soils used to demonstrate cleaning efficacy. J Hosp Infect. 2022 Aug;126:52-55. doi: 10.1016/j.jhin.2022.04.012. Epub 2022 May 1. PMID: 35508206.
- **Kremer TA,** Ratanski CH. Test Soil and Material Affinity for Reusable Device Cleaning Validations. Biomed Instrum Technol. 2023;57(4):136-142. doi: 10.2345/0899-8205-57.4.136. Epub 2024 Jan 3. PMID: 38170937; PMCID: PMC10764060.
- **Kremer TA**, Carfaro C, Klacik S. Effects of Time, Temperature, and Humidity on Soil Drying on Medical Devices. Biomed Instrum Technol. 2023;57(2):58-66. doi: 10.2345/0899-8205-57.2.58. Epub 2023 Jun 21. PMID: 37343069; PMCID: PMC10512989.
- Kimble A, Ratanski C, **Kremer TA**. Chemical Changes Over Time Associated with Protein Drying. Biomed Instrum Technol. 2023;57(2):52-57. doi: 10.2345/0899-8205-57.2.52. Epub 2023 Jun 21. PMID: 37343070; PMCID: PMC10512996.
- Hoover J, Drosnock MA, Carfaro C, **Kremer TA.** Cleaning Challenges: Can Extended Soil Dry Times Be Reversed? Biomed Instrum Technol. 2023;57(2):44-51. doi: 10.2345/0899-8205-57.2.44. Epub 2023 Jun 21. PMID: 37343068; PMCID: PMC10508861.
- **Kremer TA,** Kimble A, Ratanski C. Improving Protein Assay Methods to More Accurately Assess Medical Device Cleanliness. Biomed Instrum Technol. 2023;57(4):122-128. doi: 10.2345/0899-8205-57.4.122. Epub 2024 Jan 3. PMID: 38170934; PMCID: PMC10764063.
- Rowan NJ, **Kremer T**, McDonnell G. A review of Spaulding's classification system for effective cleaning, disinfection and sterilization of reusable medical devices: Viewed through a modern-day

lens that will inform and enable future sustainability. Sci Total Environ. 2023 Jun 20;878:162976. doi: 10.1016/j.scitotenv.2023.162976. Epub 2023 Mar 22. PMID: 36963674.

- **Kremer T**, Murray N, Buckley J, Rowan NJ. Use of real-time immersive digital training and educational technologies to improve patient safety during the processing of reusable medical devices: Quo Vadis? Sci Total Environ. 2023 Nov 20;900:165673. doi: 10.1016/j.scitotenv.2023.165673. Epub 2023 Jul 20. PMID: 37481083.

1.5.2 Journal Manuscripts under review

- **Kremer, T.A**., Rowan, N., McDonnell, G. (2024) A quantitative method for determination of reusable medical device categorization into Kremer's classification. Journal of Hospital Infection. [noting, this is sequel paper to initial cleaning classification published in JHI).

1.5.3 Industry Presentations [medical devices and linked healthcare focus]

- **Kremer, T. A**. (2023) A Spaulding Classification System for Establishing Cleaning Limits. 2023 ASTM Cleaning Workshop
- **Kremer, T. A**. (2023). Utilizing Appropriate Endpoint Analysis into Device Cleaning Evaluations by Incorporating Device Biocompatibility Practices. 2023 AAMI Cleaning Verification Summit.

1.5.4 Oral Presentations

- **Kremer, T. A**. (2022). Thinking Differently to Unlock and Mitigate Risk in the End-to-End Device Processing Supply Chain. 2022 Kilmer Conference
- **Kremer, T. A**. (2022). Establishing a relationship between an RMM analyte and the CFU. 2022 Kilmer Conference
- **Kremer, T. A**. (2022) Time is Running Out: Importance of Environmental Conditions During Transport and Storage of Soiled Medical Devices. 2022 Healthcare Sterile Processing Association Annual Conference
- **Kremer, T. A**. (2022) Strengthening the Science of Device Processing. 2022 TUV-SUD
- **Kremer, T. A**. (2022) Importance of environmental conditions within the healthcare setting during the transport and storage of soiled medical devices. 2022 OR Manager Conference
- **Kremer, T. A**. (2022) The Impact of Time and Environmental Conditions on Contaminated Instrumentation. 2022 23rd World Sterilization Congress
- **Kremer, T. A**. (2023) Mitigating infection risk: What does the evidence really say about POU Instrument Treatment? 2023 AORN Annual Conference
- **Kremer, T. A**. (2023) Collaborating to Innovate Effective Disinfectant Rotation for Contamination Control. 2023 PDA Pharmaceutical Microbiology Conference
- **Kremer, T. A**. (2024) Practical Approaches for Validation of Cleaning Processes. 2024 Nexus

1.5.5 Poster Presentations

- **Kremer, T** (2023). New cleaning classification system to complement the Spaulding classification for disinfection/sterilization. 2023 24th WFHSS Sterilization Congress
- Rowan, N.J., **Kremer, T.A.** (2024). Use of graphic imagery to inform multi-actor understanding for improved understanding of medical device design and processing for patient safety. Graphic Medicine Conference 2024, TUS, Ireland, July 6-9.

1.5.6 ISO Standard Development and Industry Representation

- US ISO Delegate for TC198 WG12
- Author of new work item proposal for ISO 17664-3 Guidance on the designation of a reusable medical device to a quantitative cleaning classification
- Main contributor to working draft 1 of ISO 17664-3 Guidance on the designation of a reusable medical device to a quantitative cleaning classification

1.5.7 National and International Recognition and Awards

- 2023 recipient of the AAMI Standards Developer Award.

1.5.8 University Lectures

- Water quality: Read paper by **Kremer** and Mcdonnell (2020) and be prepared to discuss the microbiological and chemical risks (WATER method). Infection Control Africa Network: Postgraduate Diploma in Infection Control 2022
- **Kremer, T. A**. (2022) Robotic equipment/devices. Infection Control Africa Network: Postgraduate Diploma in Infection Control 2022
- **Kremer, T. A**. (2022) Case Studies on Typical Challenges in Decontamination in Outpatient Facilities. Infection Control Africa Network: Postgraduate Diploma in Infection Control 2022
- **Kremer, T. A**. (2022) Recycling of single use devices. Infection Control Africa Network: Postgraduate Diploma in Infection Control 2022
- **Kremer, T. A**. (2023) A Spaulding Classification System for Establishing Cleaning Limits. 2023 Northeastern University

Chapter 2: End-to-End Device Processing

Medical devices play a critical role in standardized healthcare, and their appropriate use is vital for patient safety. It is the medical device manufacturer's responsibility to properly label and provide instructions for use (IFU) to the user for appropriate patient use whether they be single use or reusable medical devices, so the healthcare facility has a clear understanding for how handle the device post use. Labeled single-use devices are disposed of as medical waste directly after use. The device is provided by the medical device manufacturer as ready-to-use and is only validated to be safe and effective after a single use. These devices are typically manufactured from materials that are relatively available (e.g., plastic). Complexity in the design of single-use devices vary; for example, both a medical examination glove and balloon catheter are manufactured as single-use and will undergo different manufacturing processes to ensure they are safe and effective for their intended use. Some examples of single use medical devices and the varying complexity are shown in Figure 2.1.

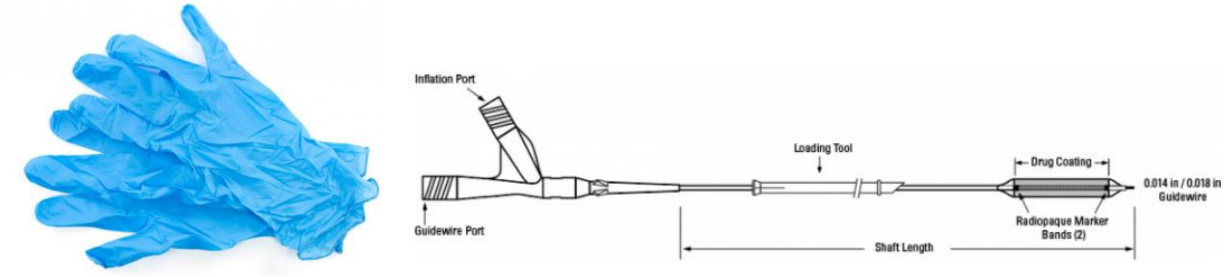


Figure 2.1: Image of Single Use Medical Device Examples

Despite their associated labelling, it still remains commonplace for single use devices to be reused within healthcare facilities. Unfortunately, the uncontrolled reuse of single-use devices has been reported to lead to patient complications (Guh, et al., 2012) (Olsson, 2009) (Wong, et al., 2010). Over the last 20 years such practices have been the subject of greater scrutiny. As an example, the US Food and Drug Administration began regulating the practice in the early 2000s (US Food and Drug Administration, 2006) (US Food and Drug Administration, 2003) and many countries have put regulations and guidance in place to eliminate the uncontrolled reprocessing of single use devices (EU MDR, 2017). The issued guidance required a regulatory burden for the healthcare facility to demonstrate that the process to ready the device for subsequent use was sufficient (Fireman, 2006). To satisfy this new regulatory requirement, medical device manufacturers began clarifying their labelling to indicate products as being single use or reusable and, when applicable, healthcare facilities moved to contracting with external companies to reprocess single use devices (U.S. Department of Health and Human Services Food and Drug Administration, 2015) (International Organization for Standardization, 2017) (EU MDR, 2017).

Medical devices labeled as reusable are intended by the manufacturer to be used for more than one patient use and are required to be provided with instructions for how to prepare the devices to make ready for the next patient use. The activity required to prepare a used device for subsequent patient use

can include cleaning, disinfection, and/or sterilization as appropriate to the device. An overview of the literature describing this process can be found in Table 2.1.

Table 2.1: Examples of publications that describe device processing activities

Title	Reference
A Practical Guide to Decontamination in Healthcare	(McDonnell & Sheard, 2012)
Decontamination and Reprocessing of Medical Devices for Health-care Facilities	(World Health Organization, 2016)
Surgical Instrument Decontamination: A Multistep Process	(Chobin, 2019)
Processing of Reusable Medical Devices	(Mitzel, 2021)
Disinfection and Sterilisation	(McAuley, 2023)
ANSI/AAMI ST79:2017 & 2020 Amendments A1, A2, A3, A4 (Consolidated Text) Comprehensive Guide to Steam Sterilization and Sterility Assurance in Health Care Facilities	(Association for the Advancement of Medical Instrumentation, 2020)
Sterile Processing Technical Manual	(Healthcare Sterile Processing Association (HSPA), 2023)

The terminology that describes these activities can include both *Reprocessing* and *Decontamination* as defined by the World Health Organization (World Health Organization, 2016). However, there are many devices that include instructions for the healthcare facility to complete prior to the initial use, so the term *Processing* has achieved consensus by the medical device industry to describe actions required at the healthcare setting to ready a medical device for patient use (International Organization for Standardization, 2018). As discussed within the publication, *COVID-19, Processing, and the Importance of Definitions: Focus on Face Masks* (Kremer & McDonnell, 2020), the definition of a process is important when the intent is to communicate the intended goal to broad stakeholders. "Processing" is the general term and under this are various steps that can include cleaning, disinfection, and/or sterilization. Although "processing" has gained industry consensus, the authors McDonnell, et. al discuss in their article that 'decontamination' is often used synonymously with a combined process of cleaning and disinfection, 'disinfection' and 'sanitization' depending on the geographical location (McDonnell, G; Baseman, H; Cordi-Bancroft, L;, 2021). Within this text the terms "reprocessing", "processing" and "decontamination" are used to describe actions performed to prepare a reusable device for patient use.

Like single-use medical devices, reusable devices can also range widely in complexity and material composition as illustrated in Figure 2.2. However, as described in ISO 17664:2017 *Processing of health care products – Information to be provided by the medical device manufacturer for the processing of medical devices*, these devices adhere to the following: "A medical device requiring processing is supplied with detailed processing instructions to ensure that, when followed correctly, the risks of transmission of infectious agents are minimized. In addition, effective processing minimizes the risk of other adverse effects on medical devices." (International Organization for Standardization, 2017)

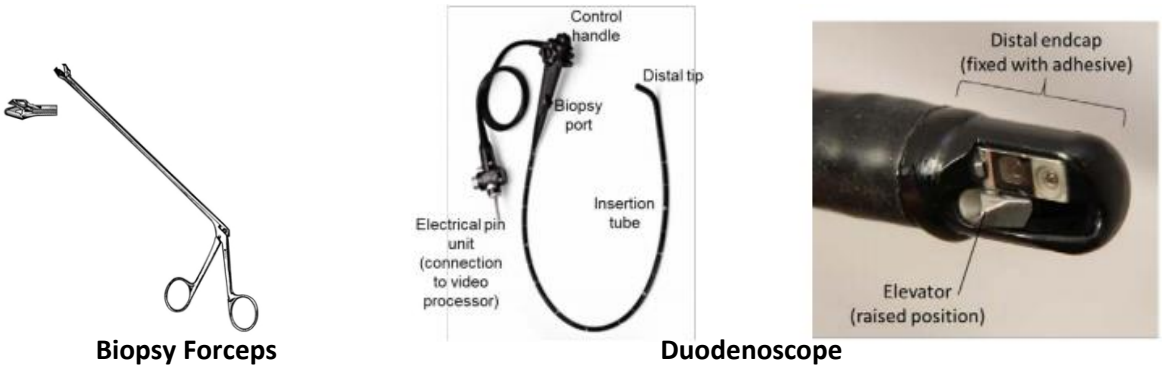


Figure 2.2: Image of Reusable Medical Device Examples

Advancements in technology and device design, specifically minimal invasive surgeries that have become the standard of care (John, et al., 2020), have resulted in a change to complex instrumentation that are not cost effective for single use (Malchesky, et al., 1995). As hospitals look for opportunities for cost reduction, waste disposal is a common area of evaluation. Sustainability programs and waste management regulations limit disposal choices, therefore the cost for single use medical device disposal can be a concern (Malchesky, et al., 1995) (Deprez, et al., 2000).

A previous study evaluated the cost difference between reusable forceps versus a single use disposable option (Hogan, et al., 2009). The high-volume endoscopy center, performed approximately 24,000 outpatient procedures per year, evaluated the device cost over a two-year period and found the reusable device to have a cost per procedure of $3.27 while the single use device was $10.00. By selecting the reusable forceps, the facility experienced a cost savings of $79,482 (Hogan, et al., 2009). In a European study by Deprez et. al the total cost of reusable device processing including purchase price, repair and all processing costs was $6.65 per forceps while the cost of disposable forceps ranged from $26.90 to $43.00 resulting in an annual savings of $78,377 (Deprez, et al., 2000). The differences in the cost analysis between the two studies is likely to the omission of all operating costs, such as utilities, and sourcing of single use forceps, but as indicated by these studies, as the complexity of devices increase, the practice of device processing will continue to be an increasing requirement for standardized healthcare. Therefore, the practice must be robust enough to ensure patient safety. A drive towards reuse is also informed by sustainability healthcare revolutionary practices that focuses on promoting enhanced 'reuse' options and reducing medical waste affecting our fragile environment, such as from disposal of single use devices.

2.1 Patient Risk

The true risk of infection is difficult to estimate due to a number of factors such as inadequate or no surveillance and low occurrence or absence of clinical symptoms (Kovaleva, et al., 2013). For example, an estimated risk of infection transmitted by endoscopy is 1 per 1.8 million procedures, and infectious agents such as *Helicobacter pylori, Salmonella spp., Pseudomonas aeruginosa, Strongyloides sterocoralis,* and hepatitis B and C viruses have been attributed to GI endoscopy (Fireman, 2006), but other publications have reported between a 6 to 23% infection rate (Kovaleva, et al., 2013) (Kenters, et al., 2015). These

examples demonstrate the complexity of the issue. Cost alone cannot influence this decision. Hogan et. al go on to explain that the cost of single use device may include influencers such as convenience, consistent performance, a lower risk of cross contamination. Ultimately, customers must consider cost along with device functionality and patient safety (Hogan, et al., 2009).

Hospital Acquired Infections (HAIs) are defined as infections developing after 48 hours of a stay at a healthcare facility that was not present or incubating at the time of admission when receiving care for another condition. HAIs are estimated to affect 1.7 million patients in the US annually leading to 99,000 deaths (Duarte, et al., 2009) (Hensley & Monson, 2015) (Gold & Hitchins, 2013). Medical devices are a common source of HAIs and have accounted for 60% to 80% of all bloodstreams, urinary tract, and pneumonia-related HAIs (Gold & Hitchins, 2013). Otter et. al describe in their literature review that transmission routes of pathogens are complicated and have been difficult to assign an assignable cause through investigation (Otter, et al., 2013). The use of data collected via modeling transmission, microbiological studies *in vitro* and *in situ*, observational epidemiological studies, intervention studies with improved decontamination and outbreak reports have provided insights into how to improve infection prevention (Otter, et al., 2013), so although biopsy forceps theoretically have a potential risk of prion transmission, there is currently no evidence in the literature that it has been transmitted this way to patients (Fireman, 2006). An overview of the literature investigating HAIs can be found in Table 2.2.

Table 2.2: HAI examples published the literature including device-related infections

Title	Reference	Industry Impact
An Outbreak of *Pseudomonas aeruginosa* Infections Associated with Flexible Bronchoscopes	(Srinivasan, et al., 2003)	Outbreak of *P. aeruginosa* caused by contaminated loose biopsy-port cap in bronchoscopes.
Reassessment of the Risk of Healthcare-Acquired Infection During Rigid Laryngoscopy	(Muscarella, 2008)	Literature review of HAIs as a result of laryngoscopy and call to action for increased standardization for manufacturer's processing instructions.
Control of a multi-hospital outbreak of KPC-producing *Klebsiella pneumonia* type 2 in France, September to October 2009	(Cabronne, et al., 2010)	Identified the duodenoscope as a source of HAIs when not processed effectively.
Contamination of Laryngoscope Handles	(Williams, et al., 2010)	Evidence demonstrating HAI risk from lack of decontamination of laryngoscope handles.
Prevalence of Healthcare-Associated Infections in Acute Care Hospitals in Jacksonville, Florida	(Magill, et al., 2012)	Study designed to detect HAIs in a healthcare system. 6% of the 851 patients in the study were identified with a HAI.
Surgical Site Infections Linked to Contaminated Surgical Instruments	(Dancer, et al., 2012)	Post-sterilization contamination of sets containing surgical instruments was linked with an increased rate of surgical site infections.

Transmission of Infection by Flexible Gastrointestinal Endoscopy and Bronchoscopy	(Kovaleva, et al., 2013)	Literature review of the challenges with flexible endoscopy. 6% of patients will experience a HAI due to improper infection control procedures.
Bacterial Contamination of Re-usable Laryngoscope Blades During the Course of Daily Anesthetic Practice	(Lowman, et al., 2013)	Ineffective decontamination of laryngoscope blades is a source of microbial contamination and patient risk.
Laryngoscope Blades and Handles as Sources of Cross-infection: An Integrative Review	(Negri de Sousa, et al., 2013)	Literature review of cross-infection risk from laryngoscope blades and handles.
Multistate Point-Prevalence Survey of Health Care-Associated Infections	(Magill, et al., 2014)	Additional study by authors to look at multiple facilities (183 hospitals) for the detection of HAIs. Of the 11,8282 patients, 4% were identified with a HAI.
An Outbreak of Carbapenem-resistant OXA-48-producing *Klebsiella pneumonia* Associated to Duodenoscopy	(Kola, et al., 2015)	Investigation of a German outbreak with the root cause being a contaminated duodenoscope.
Genomic Epidemiology of an Endoscope-Associated Outbreak of *Klebsiella pneumoniae* Carbapenemase (KPC)-Producing *K. pneumonia*	(Marsh, et al., 2015)	HAI outbreak investigation with root cause being endoscopes.
Persistent Contamination on Colonoscopes and Gastroscopes Detected by Biologic Cultures and Rapid Indicators Despite Reprocessing Performed in Accordance with Guidelines	(Ofstead, et al., 2015)	Monitoring of 60 colonoscopes and gastroscopes in a clinical setting with demonstration of microbial contamination despite compendial processing protocols.
Retained Bioburden on Surgical Instruments After Reprocessing: Are We Just Scraping the Surface?	(Davis, 2017)	Compilation of data reporting the rate of bioburden per patient claim records. There is an increasing trend of bioburden prevalence.
A Perfect Storm: Understanding Hemostasis, Coagulation and Inflammation with Artificial Material	(Padera, 2017)	Investigates thrombosis caused by foreign particulates within the vascular system.
High Prevalence Rate of Digestive Tract Bacteria in Duodenoscopes: A Nationwide Study	(Rauwers, et al., 2018)	Dutch study where duodenoscopes within the clinical setting were cultured and demonstrated high levels of bioburden post processing
Duodenoscope-Associated Infections: Update on an Emerging Problem	(Rahman, et al., 2019)	Literature review of duodenoscope associated HAIs due to difficult to remove biofilm.
Transmission of Mobile Colistin Resistance (*mcr*-1) by Duodenoscope	(Shenoy, et al., 2019)	HAI investigation with transmission associated with a duodenoscope.
Duodenoscope-Associated Infection Prevention: A Call for Evidence-Based Decision Making	(Ofstead, et al., 2020)	Literature review of duodenoscope related HAIs and discussion around risk mitigation.

A Prospective, Multicenter, Clinical Study of Duodenoscope Contamination after Reprocessing	(Okamoto , et al., 2022)	US study investigating the contamination rate of duodenoscopes after processing.

In 2012, *A Practical Guide to Decontamination in Healthcare* defined *disease* as any effect that impairs/harms the body's normal function and therefore has an impact on our health (mild, moderate, or even severe). The authors go on to explain that diseases or complications following a clinical procedure can be infectious or non-infectious (McDonnell & Sheard, 2012). Immune responses can occur both from microbiological contamination or other toxic compounds from the surface or eluting from the device (Truscott, 2004). For example, an immune response may occur from toxins produced from bacteria or the presence of other foreign organic components like protein. Milo found that bacteria, yeast, and mammalian cells have an estimated range of 2-4 million proteins per cubic micron (Milo, 2013). So, even if bacteria are inactive, the presence of protein can elicit an immune response. In a study performed by Kremer et. al (2019) protein concentrations were measured for patient safety using the cytotoxicity test. This study found the when the concentration of known toxic proteins was increased to greater than 8µg/cm^2, cell death occurred resulting in cytotoxicity test failure (Kremer, et al., 2019). Although this is an exaggerated response, L29 mouse cells within the study have no immune system, the evidence demonstrates that residual protein is cytotoxic. In addition, Tamashiro et. al and Kremer et. al demonstrate that chemical residue, such residual cleaning agent, can also be cytotoxic (Tamashiro, et al., 2013) (Kremer, et al., 2021).

Table 2.3: Examples describing protein immune response in the published literature

Title	Reference	Industry Impact
Neutrophil – Particle Interactions in Blood Circulation Drive Particle Clearance and Alter Neutrophil Responses in Acute Inflammation	(Fromen, et al., 2017)	Research demonstrating how neutrophil-particle complexes can suppress neutrophil interactions within an inflamed mesentery vascular wall.
Early Precursor T Cells Establish and Propagate T Cell Exhaustion in Chronic Infection	(Utzschneider, et al., 2020)	Study exploring the effects of T-cell exhaustion resulting from a high antigen load.

Adding to patient risk are residual proteins that are difficult to detect and can be severely harmful (Table 2.3). One such protein is the abnormally folded and protease-resistant form of a cellular protein known as the prion protein (PrP). Small amounts of these prion proteins can begin to accumulate throughout the central nervous system, disrupting structure and function (Green, 2019). Prions are associated with a family of diseases known as transmissible spongiform encephalopathies and specifically Creutzfeldt-Jakob disease (CJD). These diseases are degenerative neurological disorders and are invariably fatal. The prion protein is not normally broken down in the body and accumulates in various cells of the body, particularly CNS cells. Their presence promotes the further misfolding of natural forms of the PrP protein in these

cells, causing further precipitation that will progressively lead to cell death and local dissemination to other cells, leading to severe tissue damage. Known cases are approximately 3.2 cases in a million population per year (Klug, et al., June 2013). Approximately 85 percent of cases are sporadic (i.e., person with no known risk factors) (Department of Veteran Affairs Office of Inspector General, 2014). Prions have been demonstrated to have unique resistance profiles to cleaning, disinfection, and thermal or some gaseous sterilization modalities and detection of contamination instruments is currently not possible within the healthcare facility. It is therefore critical that enhanced cleaning and inactivation processes be established to mitigate the risk of prions (Table 2.4).

Table 2.4: Examples describing prions including links to contaminated medical devices

Title	Reference	Industry Impact
Novel Methods for Disinfection of Prion-Contaminated Medical Devices	(Fichet, et al., 2004)	Proposed methods of decontamination to reduce the risk of prion contamination.
Prion Inactivation Using a New Gaseous Hydrogen Peroxide Sterilisation Process	(Fichet, et al., 2007)	Demonstrated that gaseous peroxide may be a useful method for prion inactivation.
Quantitative Measurement of the Efficacy of Protein Removal by Cleaning Formulations: Comparative Evaluation of Prion-Directed Cleaning Chemistries	(Ungurs, et al., 2010)	Found that cleaning improved with a pre-soaking treatment followed by intermediate performing enzymes.
Effect of Fixation on Brain and Lymphoreticular vCJD Prions and Bioassay of Key Positive Specimens from a Retrospective vCJD Prevalence Study	(Wasworth, et al., 2011)	Investigation of the accumulation of abnormal prion protein.
Transmission of Infection by Flexible Gastrointestinal Endoscopy and Bronchoscopy	(Kovaleva, et al., 2013)	Documents that in a new variant of CJD, large amounts for protein are accumulated in the lymphoid tissue including the GI tract, so CJD transmission via a GI endoscope procedure is possible.
Healthcare Inspection: Potential Exposure to Creutzfeldt-Jakob Disease VA Connecticut Healthcare System West Haven, Connecticut	(Department of Veteran Affairs Office of Inspector General, 2014)	Case study describing the actions of a healthcare facility after identifying patient exposure to CJD.
RT-QulC: A New Test for Sporadic CJD	(Green, 2019)	Describes a novel method of prion detection. This assay recognized the misfolded protein PrP^{Sc}.

AAMI ST79 Comprehensive Guide to Steam Sterilization and Sterility Assurance in Healthcare Facilities – Annex C: Processing CJD-contaminated patient care equipment and environmental surfaces	(Association for the Advancement of Medical Instrumentation, 2020)	Provides a description of CJD and associated risk along with processing recommendations for devices potentially exposed.
A Cold Water, Ultrasonically Activated Stream Efficiently Removes Proteins and Prion-associated Amyloid from Surgical Stainless Steel	(Secker, et al., 2020)	Demonstrated the efficient removal of prion strains by an ultrasonically activated stream

The occurrence of HAIs has direct medical cost impact on hospital finances and many researchers have performed analysis to understand the economic benefits of the investment of an infection prevention and control program. For example, in a 2009 summary report based on 2002 HAI data, the consumer price index (CPI) for inpatient hospital service ranged from $35.7 billion to $45 billion, while the benefits of prevention ranged from $25.0 billion to $31.5 billion (Scott, 2009). While the cost impact from prions is unknown, the risk of a HAI can be mitigated by effective device processing.

2.2 The Device Processing Cycle

Devices that are designed for the health care facility to be processed before initial or subsequent patient use undergo processing in what the World Health Organization (WHO) describes as the Decontamination Life Cycle or the Device Processing Cycle (World Health Organization, 2016). This cycle is established and managed under a quality system to verify devices are safe and effective each time they are used. Devices that need to be cleaned, disinfected and/or sterilized between patient exposure can be those used directly on a patient during surgery (e.g., forceps, endoscopes, etc.), those items that have minimal contact (e.g., blood pressure cuff) or those that just share a room (e.g., monitor, piece of equipment).

The device processing cycle for critical devices (Figure 3) begins with patient use, whereby the device is in a ready state where it is safe and effective for patient use. Immediately following patient use, the preparation for cleaning should begin at the point of use (Association for the Advancement of Medical Instrumentation, 2020) (McDonnell & Sheard, 2012) (Association of periOperative Registered Nurses, 2022) (World Health Organization, 2016). This treatment includes the removal of the visible soil on the device, disassembly if required, flushing lumens, and brushing hard to reach areas on the device. To aid in the successful removal of residual soil the instruments should be covered with a wet cloth will keep them moist and prevent soil from drying as much as possible. Treatment at point of use is a critical first step to prevent a more challenging cleaning process, device damage, or the growth of microorganisms during the wait time prior to cleaning (Association for the Advancement of Medical Instrumentation, 2020).

The devices are then typically transferred to a centralized location within the healthcare facility to undergo the remainder of the processing cycle. The manufacturer's cleaning and disinfection instructions for use are followed to ensure the devices are appropriately cleaned and safe to handle. They will sometimes require a manual process or preclean that may involve removing them from a tray, rinsing, soaking, flushing, brushing, or sonication before loading them into a washer/disinfector for an automated cleaning process. A washer-disinfector is loaded carefully to ensure the best possible outcome for device cleanliness as well as to reduce the microbial load by the associated disinfection process. In an automated process, like a washer-disinfector or an automated endoscope reprocessor (AER), the final stage of the cycle may include a disinfection cycle. As described above by the WHO the cleaning and disinfection steps of the Processing Cycle is described as 'decontamination' (World Health Organization, 2016). In some cases, the device after the decontamination phase is ready for patient use, but others that are used more invasive, are recommended to undergo additional processing steps.

In the case of sets of devices provided for use in certain types of surgery, device trays are reassembled after close inspection of the components to ensure all residual soil has been removed even from devices with lumens or small openings. Devices with moving parts are actuated to mimic simulated use. This detailed inspection is intended to ensure continued functionality before being placed in a surgical tray or other packaging for terminal sterilization (Association for the Advancement of Medical Instrumentation, 2020). Packaging of devices prior to use requires the device to be placed in a sterile barrier system, defined as the *minimum package that minimizes the risk of ingress of microorganisms and allows aseptic presentation of the sterile contents at the point of use* (International Organization for Standardization, 2018). Aseptic presentation is defined as the *transfer of sterile contents from its sterile barrier system using conditions and procedures that minimize the risk of microbial contamination* (International Organization for Standardization, 2018). Once the devices are packaged in the appropriate packaging (e.g., rigid containers, wraps, pouches), they are loaded into the sterilizer for terminal sterilization. Sterilization can be achieved using a variety of modalities (e.g., moist heat/steam, vaporized hydrogen peroxide, ethylene oxide) that can be controlled in defined, enclosed processes. Once sterilized the devices are stored or transferred to be used on the next patient with the assurance that the device is safe and effective for the next patient use. A full description of the device processing steps can be found in Appendix 1.

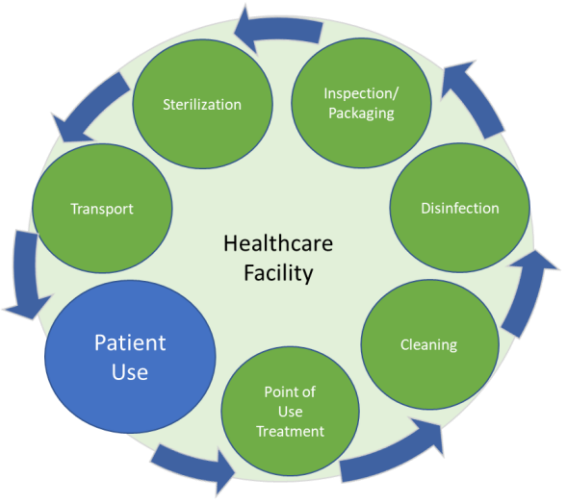


Figure 2.3: Typical Processing Cycle for Critical, Reusable Medical Devices

The processing cycle is typically viewed as a standalone circle or loop encompassing the processing steps depicted in Figure 2.3. However, the full end-to-end supply chain for reusable medical devices is far more complicated. If one were to follow a reusable medical device throughout its life, it is truly a complex system with the transfer of responsibility to ensure patient safety happening routinely as depicted in Figure 2.4. The process begins with the manufacturer. The medical device manufacturer has the responsibility to ensure that each device is manufactured with the intended microbiological quality and delivered to the Healthcare Facility with the instructions that allow for safe and effective throughout the device lifetime. The healthcare facility continues the cycle by taking ownership of the device or instrument set, and is responsible that after each patient use, the product is processed so that it may be used again on another patient. This processing includes the cleaning, disinfection and or sterilization of the device or instrument set.

Depending on the surgical procedures performed within a healthcare facility, it may not be feasible for them to own every type of surgical kit required. The cost of ownership for these kits may prohibit owning them outright. To address this barrier to use, medical device manufactures or other external companies, may offer a loaner set program. Where after patient use, the loaner kit is cleaned, disinfected, and packaged so it may be returned to an external distribution center or transported to another user. At some distribution centers, the set may be processed so it is ready for subsequent patient use. However, using best practices, the loaner kit would be processed again at the receiving healthcare facility to ensure it is in a ready state with fully processing traceability.

In some geographical locations, this effort of processing at an off-site location takes place at an external reprocessor or 3rd party reprocessor. These companies will transport devices after point of use treatment to an off-site location where they will be processed and returned back to the healthcare facility ready to

use. The companies that perform this service may or may not be affiliated with the healthcare facility or device manufacturer.

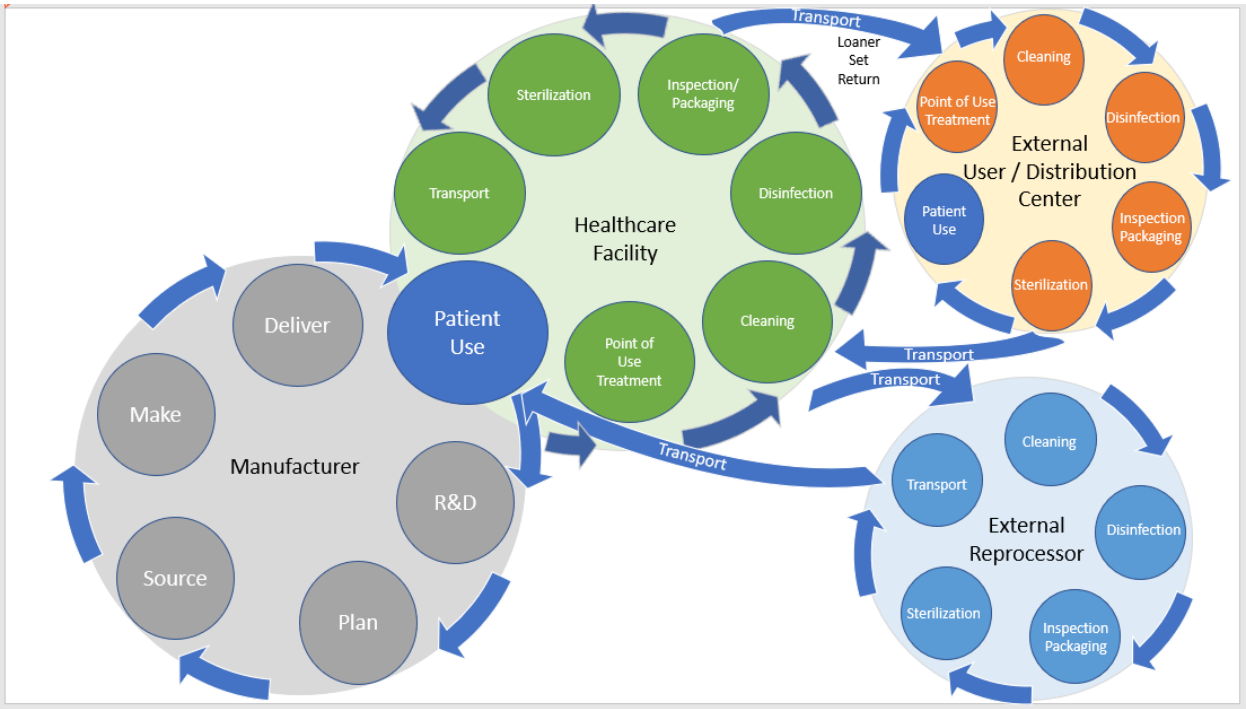


Figure 2.4: End to End Device Processing Cycle

By not acknowledging these additional loops for end-to-end device processing, the medical community work within their silos. Decisions, without taking the entire process into consideration, may lead to increased risk for patient safety. Therefore, each portion of the End-to-End Device Processing Cycle will be further explored to identify where the risk to patient safety is increased.

2.3 End-to-End Microbiological Quality & Sterility Assurance (MQSA)

The reusable medical device manufacturer has the initial responsibility for the microbiological quality is described in the technical information report (TIR) from the Association of the Advancement of Medical Instrumentation, *TIR100 End-to-End Microbiological Quality and Assurance of Sterility.* Wherein, the end-to-end process is described as beginning and ending with the customer. (Association for the Advancement of Medical Instrumentation, 2021)

The customer is defined as “a person or organization that receives a product”. Their safety when using the product influences the microbiological quality requirements for a healthcare product. The medical device manufacturer then moves the device through the end-to-end supply chain process consisting of research and development (R&D), plan, source, make, and deliver (Figure 2.5).

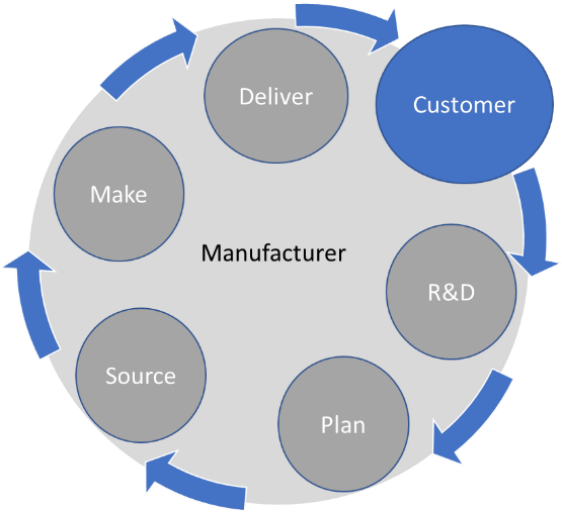


Figure 2.5: End to End Medical Device Supply Chain

In each phase of the end-to-end supply chain, steps are taken to ensure the device is of the appropriate cleanliness and microbiological quality to be safely used within a healthcare facility. Below are examples of actions taken by a medical device manufacture at each phase of the end-to-end medical device supply chain:

R&D – The medical device is designed with features that can be decontaminated. For instance, the device may be designed to be disassembled to facilitate easy cleaning and exposure to hard to sterilize locations. This would also encompass design innovations to improve efficacy of cleaning and subsequent sterilization steps.

The device feature is the major variable affecting the reuse of a device. Materials must be selected to allow for effective device processing. For example, insulating materials that crack or metals that can be subject to pitting can create an environment for residual soil buildup or microbial biofilm formation. Device features (e.g., mated surfaces, lumens) should allow for all processing steps (Malchesky, et al., 1995).

Plan – During the plan phase, the manufacturer will ensure the appropriate microbiological quality inputs are included in planning of '*Make*' step. The infrastructure, for example, in which the device will be manufactured may need to include the appropriate air and water quality that remains in a state of control.

Source – Materials and components that are included in the device manufacturing process must be of the appropriate chemical and microbiological quality. The source of raw materials intended to be included in the product must be consistently under a state of control. For example, if the following '*Make*' process is designed to clean a device to a specific level, the amount of soil in the raw material should not exceed what the process is able to reduce.

Make – The phase of '*Make*' is defined as the phase of the supply chain where the produce is produced to a finished state. (Association for the Advancement of Medical Instrumentation, 2021). The process in which a device is manufactured must be well defined, validated and remain in a state of control as defined by Good Manufacturing Practices and ISO 13485, Quality Management Systems. The MAKE process can occur all at one manufacturing location or with various steps at different locations. This ensures that the customer's need is fulfilled as expected. For example, if the device is labeled as sterile, the make process should deliver product free of microorganisms and ready for use.

Deliver – The phase of transporting the medical device to the customer should include the maintenance of conditions to ensure the cleanliness and microbiological quality are not impacted. The packaging of the product becomes a primary way to ensure effective delivery to the customer.

During the deliver phase of the supply chain, the reusable device manufacturer must provide product label by providing comprehensive written instructions for processing to the customer. The suitability of these instructions must be validated. When a medical device is sold to a healthcare facility it is the responsibility of the customer, referred to as the user for reusable devices, to ensure the device manufacturer's instruction for use (IFU) can be followed within the health care facility (Association for the Advancement of Medical Instrumentation, 2020). As required by ISO 13485 and country specific regulations, there is the added responsibility to report whenever they believe there is probability that a medical device has contributed to any negative impacts on patient health (e.g., death) and in some cases may be required to track certain devices from receipt, through patient use to disposal (Congress, United States of America 101st, 1990; EU MDR, 2017).

The focus on end-to-end microbiological quality and sterility assurance requires a trust between the customer and medical device manufacturer. The safe use of a medical device always requires collaboration between the manufacturer and the user. For single use, the product is provided sterile and ready for us, but safety can only be assured when the device is handled correctly during storage and use at the healthcare facility. Trust is even greater with reusable medical devices as a greater responsibility is placed on the healthcare facility as they essentially become the manufacturers.

Over the last 25 years, country-specific and global standardization committees have worked extensively to standardize how to validate the device processing instructions provided by the manufacturer (Table 2.5). The standardization of how instructions for use are validated allow for the successful transfer of the device microbiological quality from the device manufacturer to the healthcare facility. The standardization of validation requirements in this area requires a collaborative effort and has resulted in performance requirements documented in country-specific, regional, and international consensus standards and guidance. These documents are vitally important to device manufacturers when demonstrating cleaning efficacy and writing instructions for use and drive consistent requirements.

Table 2.5: Examples of validation standards and guidelines.

Document Number	Title	Reference
ISO 17665-1	Sterilization of heath care products – Moist heat – Part 1: Requirements for the development, validation and routine control of a sterilization process for medical devices	(International Organization for Standardization, 2006)
FDA Guidance for Industry and FDA Staff	Reprocessing Medical Devices in Health Care Settings: Validation Methods and Labeling	(U.S. Department of Health and Human Services Food and Drug Administration, 2015)
ISO 17664 -1	Processing of health care products – Information to be provided by the medical device manufacturer for the processing of medical devices Part 1: Critical and semi-critical medical devices	(International Standard Organization, 2021)
ISO 17664-2	Processing of health care products – Information to be provided by the medical device manufacturer for the processing of medical devices Part 2: Non-critical medical devices	(International Organization for Standardization, 2021)
ASTM F3293-18	Standard Guide for Application of Test Soils for the Validation of Cleaning Methods for Reusable Medical Devices	(ASTM International, 2018)
ASTM F3321-19	Standard Guide for Methods of Extraction of Test Soils for the Validation of Cleaning Methods for Reusable Medical Devices	(ASTM International, 2019)
ASTM F3208-20	Standard Guide for Selecting Test Soils for Validation of Cleaning Methods for Reusable Medical Devices	(ASTM, 2020)
AAMI ST98	Cleaning validation of healthcare products – Requirements for development and validation of a cleaning process for medical devices	(Association for the Advancement of Medical Instrumentation, 2022)
ASTM F3438-21	Standard Guide for Detection and Quantification of Cleaning Markers for Reusable Medical Devices	(ASTM International, 2021)
ISO 17664-2	Processing of health care products – Information to be provided by the medical device manufacturer for the processing of medical devices – Part 2: Non-critical medical device	(International Organization for Standardization, 2021)
ISO 15883-5	Washer-disinfectors – Part 5: Performance requirements and test method criteria for demonstrating cleaning efficacy	(International Organization for Standardization, 2021)

AAMI TIR55	Human factors engineering for processing medical devices	(Association for the Advancement of Medical Instrumentation, 2014)

To validate the device processing IFU the manufacturer must demonstrate the device can be cleaned, rendered microbiologically safe, biocompatible, and retain functionality throughout the device lifetime.

2.3.1 Cleaning Validation

To effectively demonstrate cleaning efficacy of the manufacturer's IFU, it is critical to understand and consider the experimental variables as much as possible. The cleaning instructions must be addressed as the independent variable and all other variables that may affect effective cleaning outcome must be isolated and controlled. The validation standards in Table 2.5 aim to provide guidance to medical device manufacturers for how to control these variables: for example, Jain et. al isolated the critical validation variables that may impact the test results if not fully controlled (Jain, et al., 2021). However, it is evident from review of the literature that additional standardization is needed to fully control key influencing variables.

The process steps for critical device cleaning instructions must be defined but are typically as follows: initial treatment at point of use (pre-treatment), preparation before cleaning (e.g., disassembly), manual cleaning, automated cleaning, rinsing, drying, and visual inspection. During the cleaning validation, test conditions are selected to mitigate the human factors that may impact the cleaning process within a healthcare setting. AAMI ST98, *Cleaning validation of health care products – Requirements for development and validation of a cleaning process for medical devices*, lists the following conditions for consideration (Association for the Advancement of Medical Instrumentation, 2022):

- Simulated Use - Repetition of soiling and cleaning and further processing, e.g., high-level disinfection and/or sterilization (if applicable) of the device that may cause soil accumulation over time.
- Soil Volume - Determination of the volume of test soil to adequately soil the device.
- Soiling Location - Identifying the most challenging areas of the device to clean, which can include areas of test soil and fluid ingress and accumulation.
- Soil Application - Methods of applying the test soil.
- Device Articulation - Physical manipulations (actuations, flexures, etc.) or the device during soiling to simulate clinical use.
- Soil Conditioning - Conditions such as heating and other conditions during use that can make the test soil more difficult to remove.
- Soil Drying - The length of time and environment conditions for drying of test soil on the device.

In addition to the preparation of the test samples, the cleaning parameters should also be selected to simulate a worst-case cleaning experience. If the cleaning instructions provide a range of processing parameters, the validation should be completed at worst case parameters (Association for the Advancement of Medical Instrumentation, 2022).

- Cleaning agent (i.e., detergent) preparation – If the instructions state to dilute the detergent to obtain a concentration range, then the weakest detergent concentration is used.
- Flushing – If the device is to be flushed for a specific time or until visibly clean, then the more subject indicator of visual cleanliness should be specified in the validation with a timing element employed.
- Soaking – If a device is intended to be submersed for a specified time range (e.g., sonicate for 5 to 10 minutes) then the time selected should be the most rigorous (e.g., 5-minute soak is selected for validation as the lowest contact time).
- Volumes – If volume of fluid is specified (e.g., flush lumen with 60mL of prepared detergent), then a volume slightly below the volume specified should be used in the validation (e.g., 59mL of detergent would be used in the validation procedure).
- Temperatures – If a temperature is specified, the condition representing the most rigorous challenge would be selected (e.g., devices specified to be cleaned in a cleaning chemistry at a specific temperature, 45°C ± 5°C), then the most extreme condition over the enzyme activity range should be selected (e.g., 40°C is selected as it is below the optimum temperature for enzyme performance).

By stacking worst case validation parameters, the risk of a failed cleaning procedure at the health care facility is mitigated. However, care should be taken to only select parameters that are critical to quality to prevent overchallenging the device during the validation. Cleaning validations include the following steps (Figure 2.6):

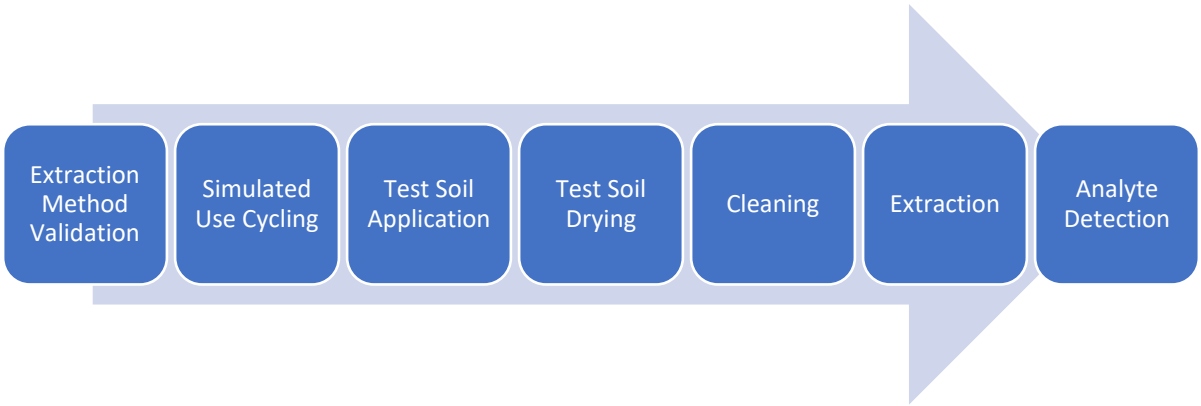


Figure 2.6: Cleaning Validation Steps

Each phase of the cleaning efficacy study is designed to appropriately challenge the cleaning instructions stated in the IFU.

- Extraction Method Validation – As required by AAMI ST98, the extraction method used for test soil recovery must be validated to demonstrate that it is capable of accurately quantifying the defined test soil analyte across a specified range of values that include the acceptance criteria. The correct use of extraction volume is assessed as part of the validation to avoid diluting the analyte and reporting inaccurate results.
- Simulated Use Cycling – Prior to validation, the samples and controls are conditioned, so they are in a used state to assess the potential of soil accumulation.
- Test Soil Application – The selection of a test soil and the application method can impact the outcome of a cleaning efficacy study. The test soil must be representative of the types of clinical procedures and the application method must reflect the intended use; worst case conditions prior to processing shall also be represented.
- Test Soil Drying – Drying of test soil has a significant impact on soil solubility. Therefore, drying conditions are an important consideration within cleaning validations.
- Cleaning – Cleaning is the physical removal of the test soil using the steps indicated as part of the medical device's IFU. The methods and cleaning agents used for the cleaning should be designed to remove such soil and contamination effectively. Cleaning can be achieved with one or a combination of the following:
    - Manual Cleaning: removal of contaminants from an item to the extent necessary for further processing or for intended use without the use of an automated process.
    - Mechanical Cleaning (i.e., automated cleaning): removal of contaminants from an item to the extent necessary for further processing for the intended use via a mechanical process such as a washer-disinfector or sonicator.
- Extraction - The validated method for test soil extraction is performed to remove residual analytes. The extraction parameters are validated to achieve the highest possible recovery rate of the test analyte.
- Analyte Detection – An analyte is defined as a chemical substance that is the subject of a chemical analysis. Analyte detection methods should be validated in accordance with ASTM F3438-21, *Standard Guide for Detection and Quantification of Cleaning Markers (Analytes) for the Validation of Cleaning Methods for Reusable Medical Devices*. The following analytes were evaluated within this study design.

Isolating test variables and controlling as many factors as possible are fundamental principles in experimental design and scientific research. When conducting experiments, researchers aim to understand the relationship between a specific variable and the observed outcomes. Isolation of test variables involves keeping all factors constant except the one under investigation. By doing so, researchers can attribute any changes in the results solely to the manipulated variable, enhancing the

validity and reliability of the findings. Controlling other variables means minimizing their potential influence on the experiment, reducing confounding factors that could obscure the true effects of the independent variable.

2.3.1.1 Analyte Clinical Relevance

When establishing the efficacy of cleaning instructions, the question of "how clean is safe" must first be addressed. Clinical soil is primarily comprised of high levels of protein, carbohydrate and lipids associated with tissue and bodily fluids. In a study investigating analyte concentrations by Cloutman-Green et. al, *Biochemical and microbial contamination of surgical devices: A quantitative study,* the contamination levels present on various types of devices following clinical use. The analytes measured in this study were Bacteria, Total Organic Carbon, Protein and Hemoglobin (Cloutman-Green, et al., 2015). This paper is foundational to demonstrating clinical relevancy of test analytes and provided clinical evidence supporting the level of analytes present on a reusable medical device after clinical use.

The measure of cleanliness evaluated as part of a cleaning efficacy study as governed by the international standards requires the measurement of two quantitative analytes. Because of its high presence in clinical soils (Cloutman-Green, et al., 2015), protein is always required as the primary analyte. The work by Cloutman-Green, et al. was a foundational paper detailing analyte concentration remaining on reusable medical devices after patient use and showed that depending on the surgery type, the amount of analyte can be highly variable. This research also demonstrated the effectiveness of the protein assay to measure effective cleaning as the most predominate analyte in clinical soil. Other analytes, such as TOC, Hemoglobin, Bacterial Endotoxin, Carbohydrates, Adenosine Triphosphate (ATP) or Bacteria are used as the second analyte as they might apply to the clinical use of the device (Association for the Advancement of Medical Instrumentation, 2022). Table 6 highlights literature that has investigated analyte levels after clinical use and set the groundwork for cleanliness levels within the international consensus standards.

Table 2.6: Examples investigating clinical analytes evident in the published literature

Title	Reference	Industry Impact
Natural bioburden levels detected on rigid lumened medical devices before and after cleaning	(Chan-Myers, et al., 1997)	One of the first articles to document levels of bacterial contamination present on a device before and after cleaning.
Comparison Study of Surgical Instruments from Sterile-Service Departments for Presence of Residual Gram-Negative Endotoxin and Proteinaceous Deposits	(Lipscomb, et al., 2006)	Documented levels of protein and bacterial endotoxin levels post cleaning.
Residual Total Protein and Total Organic Carbon Levels on Reprocessed Gastrointestinal (GI) Biopsy Forceps	(Lappalainen, et al., 2009)	Obtained measurements of protein and TOC on GI Biopsy Forceps before and after cleaning.
Analysis of the microbial load in instruments used in orthopedic surgeries	(Pinto, et al., 2010)	Documented levels of bioburden present on devices post-surgery.
Microbial Contamination of Surgical Instruments Used for Laparotomy	(Saito, et al., 2014)	Characterization (numbers and types) of the microorganisms present on surgical instruments after use in a laparotomy procedure.
Estimation of average bioburden values on flexible gastrointestinal endoscopes after clinical use and cleaning: Assessment of the efficiency of cleaning processes	(Hamed, et al., 2015)	Characterized the bioburden remaining on endoscopes after clinical use and demonstrated efficacy of the cleaning process to reduce the bioburden levels by ~4 $\log_{10}$.
Biochemical and microbial contamination of surgical devices: A quantitative analysis	(Cloutman-Green, et al., 2015)	Defined levels of analyte contamination on clinically used devices.
Analysis of Microbial Load on Surgical Instruments after Clinical Use and Following Manual and Automated Cleaning	(Evangelista, et al., 2015)	Results found the average microbial load to be 2 logs and to be reduced after cleaning.

For reusable medical devices, the acceptable levels of device cleanliness has not been directly evaluated for patient safety, with the exception of protein and detergent residuals. Kremer et. al evaluated protein residuals in the publication, *Protein Residuals on Reusable Medical Devices and Patient Safety Impact* (Kremer, et al., 2019) using the very sensitive biocompatibility assay described in ISO 10993-5 (International Organization for Standardization, 2009). In *Assessing Detergent Residuals for Reusable*

Device Cleaning Validations (Kremer, et al., 2021) researchers used cytotoxicity to assess patient safety if devices are not appropriately rinsed.

Within the research aim of this project, patient safety risk will be evaluated using the appropriate clinically relevant analytes. Therefore, selection of the analyte detection method will be critical component to project success.

2.3.1.2 Analyte Detection

The clinically relevant analyte detection methods can be grouped into four categories as described in Table 2.7.

Table 2.7: Analyte detection method categories (ASTM International, 2021)

Category	Test Name	Description
Biological Reduction	Microbial Reduction	Bacterial spores are inoculated on a device evaluated for a log reduction post cleaning using a heterotrophic plate count.
Biochemical Assay using UV- Visible Spectrophotometry	Protein Residuals	The three protein residual tests, Bicinchoninic (BCA) Assay, Bradford Assay and Ortho-Phthalaldehyde (OPA) Method, all rely on a chemical reaction with a method specific reagent with the primary amines in amino acids, peptides and proteins. The reaction is measured using a UV-vis Spectrophotometer and linear regression against a standard protein (e.g., bovine serum albumin (BSA).
	Hemoglobin	The four hemoglobin tests, Tetramethylbenzidine (TMB) Assay, Drabkin's Assay, Copper (II)-Phthalocyanine Complex Assay, and Triton/NaOH Assay use a chemical reaction to measure the activity of hemoglobin. This reaction is measured using a UV-vis Spectrophotometer and a linear regression against the reacting reagent curve (e.g., cyanmethemoglobin).
	Carbohydrates	A phenol-sulfuric acid method is performed using a reaction of concentrated sulfuric acid to produce furfural derivatives that further react with phenol. The reaction is measured using a UV-vis Spectrophotometer and a linear regressing against a glucose standard.
	Bacterial Endotoxin	Gram-negative microorganisms, which contain a lipid polysaccharide (LPS) layer as part of their cell walls, is inoculated onto a device. A reaction between the LPS and a clottable protein (e.g., limulus amebocyte lysate) followed by measurement with a UV-vis Spectrophotometer.
Oxidation	Total Organic Carbon	TOC utilizes a catalytic oxidation combustion technique at high temperatures (~720°C) to convert organic carbon into CO_2. The CO_2 generated is measured with a non-dispersive infra-red (NDIR) sensor. The CO_2 concentration is measured using linear regression against a sucrose standard.

Bioluminescence	ATP	ATP detection methods uses a lysis buffer in a luciferase assay to release ATP from cells within a sample. Bioluminescence is measured using a luminometer.

Prior to 2015 the microbiological (e.g., spore) reduction method was the primary analyte in cleaning efficacy studies in the USA as this marker was well supported by the literature (see table 2.6 for examples). However, in 2015 the US FDA published their guidance on reprocessing and included the following statement:

"FDA does not recommend the use of spore (or any other microbial marker) log reduction testing as a method to determine the effectiveness of the cleaning method. Currently, there is a lack of adequate scientific evidence regarding whether or not the removal of spores directly correlates to the remove of clinical organic soil from the devices. Such testing only indicates how well a process reduces spore count and provide no information on any other component of organic soil."

This statement accelerated a shift in the medical device industry away from traditional microbiology in the USA to harmonize globally using analytical chemistry for testing analytes (International Organization for Standardization, 2021).

The biochemical reactions within assays utilizing UV-vis spectrophotometry are sensitive and interference from instrument material or processing chemicals can impact the measurement. For example, in the study by Wehrl et. al (Wehrl, et al., 2014), two protein detection methods were used to challenge the cleaning efficacy of a robotic instrument. Although both methods provided comparable results interfering compounds that eluted from the tungsten cables resulted in false-positive protein results (Wehrl, et al., 2014). Table 2.8 describes additional literature investigating biochemical assays utilizing a UV-vis spectrophotometer measurement for cleaning efficacy studies.

The other two methods used during the cleaning efficacy validation as listed in AAMI ST98, namely TOC and ATP, use different instrumentation for detection; however, both approaches have their limitations. TOC testing is only compatible with a water extraction eluent that may leave water-insoluble residue remaining on the device undetected. The ATP method can demonstrate cellular activity of a test sample; but ATP detection typically uses swabs that may be limited in the extraction efficiency from the entirety of the device. Although there is no direct correlation between ATP and residual protein, ATP may provide value in monitoring of instrumentation in the healthcare setting. However, use of biological reduction studies, like ATP, may not be the best indicator of device cleanliness levels when considered on their own (Ohishi & Fushimi, 2019) (Winfried & Martiny, 2019).

Table 2.8: Literature examples investigating analyte detection methods

Title	Reference	Industry Impact
Determination of Protein Concentration by Total Organic Carbon Analysis	(Rouwenhorst, et al., 1991)	Demonstrated linear relationship between Protein and TOC between 25 and 1000 mg/L (µg/mL).
EN 1484 Water analysis – Guidelines for the determination of total organic carbon (TOC) and dissolved organic carbon (DOC)	(European Committee for Standardization, 1997)	Standard describing requirements for TOC analysis of water, including calibration curve, controls and preparation of the sample.
A Designed Experiment for Evaluation of the OPA Method for Cleaning Studies of Medical Devices	(McCormick, et al., 2007)	Development of the OPA method for residual protein determination using a test soil.
Analytical Procedures and Methods Validations for Drugs and Biologics	(U.S. Department of Health and Human Services Food and Drug Administration, 2015)	US FDA Guidance for industry for the validation of analytical procedures.
Assessment of Possible Disruptive Effects of Residues from Cleaning Agents on Medical Devices After Cleaning on Protein Assays	(Hubert & Schnieder, 2020)	Identified that the OPA protein assay is more susceptible to interference from detergents than the BCA protein assay.

Within the list of clinically relevant test analytes, protein is currently the only analyte with published literature correlating to patient safety for the biochemical analytes in cleaning studies (Kremer, et al., 2019), which is critical to the success of a cleaning validation performed by the medical device manufacturer. Other analytes are often used in addition to protein for investigative purposes for residual analytes that may be reflective of clinical use or processing steps (e.g., TOC used for detergent concentration analysis or when device material interferes with the biochemical assay; (Sagourin, et al., 2021)). When conducting this method, it is crucial to acknowledge the limitation of TOC in solely assessing water-soluble extraction. Haugen et al. (Haugen, et al., 2012) illustrated in their study that relying solely on water extraction may not be adequate for quantifying residual protein concentrations when dealing with test soils containing water-insoluble components. The protein test method also exhibits sensitivity issues, especially when measuring concentrations at the lower end of the curve (Kremer, et al., 2023). Therefore, enhancing the detection method is essential within this project to improve the accuracy and precision of data collection. Minimizing data variability is imperative for maximizing the effectiveness of data intelligence applications. For example, it was demonstrated when using test soils with water insoluble components that a water extraction alone is not sufficient to quantify residual protein concentrations (Haugen, et al., 2012).

2.3.1.3 Soil Formulation Standardization

The selection of the test soil used to mimic patient use is a critical variable during a device cleaning validation. The test soil should include representative concentrations of analytes (e.g., protein) and demonstrate performance characteristics (e.g., viscosity, surface adhesion, solubility) (Kremer, et al., 2021). Many research efforts have been made over the last 40 years to identify a soil recipe with performance criteria that can be used across the medical device industry. ISO TC 198 released a technical specification that provided examples of test soils used in various countries in 2006 (International Organization for Standardization, 2005); but there was little research performed at that time to compare soil performance. In 2008, Crutwell (Crutwell, 2008) published a comparison of the performance of the soils in ISO TS 15883-5 but did not use a washer-disinfector due to variability in equipment performance. Instead, a controlled flow of water was applied to a soiled surface and the percent soil remaining was calculated. The study results demonstrated that soils containing egg yolk, a mixture of proteins, carbohydrates, and lipids, were more difficult to remove than the blood-based test soils. However, Crutwell also indicated that soil drying was an uncontrolled variable in this experiment, so more research for soil performance should be performed (Crutwell, 2008). Table 2.9 describes literature related to the test soil formulation that highlights the complexity of this important determination.

Table 2.9: Literature investigating test soil formulation

Title	Reference	Industry Impact
A Comparison of Surgical Instrument Test Soils Published in ISO/TS 15883-5	(Crutwell, 2008)	Comparison of test soil recipes published in ISO guidance. German egg yolk soil was identified as the most difficult to remove.
Investigation of Atomic Level Patterns in Protein – Small Ligand Interactions	(Chen & Kurgan, 2009)	Investigated the role of covalent and non-covalent bonds in protein-small ligand interactions and found predictable patterns.
Comparison of Methods (and Test Soils) for Testing Washer-Disinfectors Used for Surgical Instruments under Everyday Conditions	(Buchrieser, et al., 2009)	Investigated test soil formulation performance for test soils published in ISO/TS 15883-5 within a clinical setting. Results were contradictory to those found within a laboratory setting as the test set up did not have the full complexity seen within everyday practice.
Ophthalmic Viscoelastic Devices as a Cleaning Challenge	(McCormick, et al., 2013)	Study to challenge the cleaning of lumens of an ophthalmic viscoelastic device using a test soil comprised of blood, hog mucin and egg yolk. Successful cleaning was achieved when the cleaning IFU was followed.

Mechanism of Fibrin Polymerization and Clinical Implications	(Weisel & Litvinov, 2013)	Investigated the chemical composition of fibrin.
Fibrin Formation, Structure and Properties	(Weisel & Litvinov, 2017)	
Physical and Composition Characteristics of Clinical Secretions Compared with Test Soils Used for Validation of Flexible Endoscope Cleaning	(Alfa & Olson, 2016)	Investigated the use of test soils for flexible endoscopes and found ATS2015 and Edinburgh-M (i.e., DBLSO) to be the most appropriate.
What is the Biological and Clinical Relevance of Fibrin?	(Litvinov & Weisel, 2016)	Paper describing fibrin, its function in the vascular system, and demonstration of thrombosis.
A Standardized Method for Evaluating Test Soils Used to Demonstrate Cleaning Efficacy	(Kremer, et al., 2022)	Validation of commonly used test soils found in ISO 1533-5 Annex A.

The ISO TC 198 committee has continued to work on the development of a soil performance standard and ISO 15883-5 *Washer-disinfectors – Part 5: Performance requirements and test method criteria for demonstrating cleaning efficacy* published in 2022. Contained within this standard is the normative Annex B, *Protein-based test soil performance assessment* which requires how test soil formulations are evaluated. This method was developed using the blood soil recipe, Coagulated Blood, from Annex A, *Examples of test soil,* of the same document, however, not all test soils from Annex A were evaluated, so it is unclear how they compare in performance (International Organization for Standardization, 2021). This evaluation was performed as part of this present study to establish the appropriate test soil to determine cleaning efficacy.

2.3.1.4 Soil Application & Drying

The application method of the test soil during a cleaning efficacy study can have major influence on the test results and must therefore be a controlled variable in the experimental design. When a surface is wetted with a layer of test soil, the oxygen layer between the material surface and soil can attract the protein to the material surface. For adsorbing material, such as stainless steel, material polarization and test soil adhesion demonstrated a linear relationship (Gettens & Gilbert, 2006) (Gettens & Gilbert, 2007) where Gonzalez et. al. provided evidence that surface texture (i.e., roughness) may substantially increase soil and bacterial adhesion (Gonzalez, et al., 2017). Although ASTM F3293-18 provides guidance for how to perform test soil application (ASTM International, 2018), very little was known about soil amount or thickness on different materials. Thus, these important variables were investigated within this present study to establish appropriate materials and methods in the experimental design.

Most medical device IFUs warn the end user against allowing soil to dry on a device prior to cleaning. This is due to the logical assertion that by allowing the soil to dry on the device the cleaning challenge increases. During a cleaning efficacy study, the device manufacturer must allow the test soil to dry as a

worst-case challenge to account for mistakes within the healthcare facility (Kremer, et al., 2023). Little evidence exists for explaining the chemical reactions that occur within a soil during drying and the possible effects on device cleanability, so this was explored within this project. However, the literature does describe how drying may lead to absorption of proteins into the material of the device and the accumulation of soil buildup over time (Table 2.10).

Table 2.10: Literature investigating the drying of test soil

Title	Reference	Industry Impact
Effect of Temperature on Dynamic Viscoelasticity During the Clotting Reaction of Fibrin	(Kaibara & Fukada, 1977)	Demonstration that the denaturation of fibrin occurs at high temperatures (45°C) and irreversible denaturation occurs with high ionic strength.
Effect of Drying Time, Ambient Temperature and Pre-Soaks on Prion-Infected Tissue Contamination Levels on Surgical Stainless Steel: Concerns Over Prolonged Transportation of Instruments from Theater to Central Sterile Service Departments	(Lipscomb, et al., 2006)	Protein is likely to adsorb into device material if soil is allowed to dry and could not be removed by immersion in deionized water. Pre-soaking the instrumentation significantly reduced (96%) prion-infected tissue contamination.
Adsorption of Prion and Tissue Proteins to Surgical Stainless Steel Surfaces and the Efficacy of Decontamination Following Dry and Wet Storage Conditions	(Secker, et al., 2011)	Storage of contaminated devices in a moist environment at room temperature can reduce protein and prion amyloid adsorption without the need for pre-soak chemistries.
Efficacy of Humidity Retention Bags for the Reduced Adsorption and Improved Cleaning of Tissue Proteins Including Prion-Associated Amyloid to Surgical Stainless Steel Surfaces	(Secker, et al., 2015)	Increased dry time increases the attachment of tissue proteins and prion-associated amyloid to surgical stainless steel and reduces the efficacy of cleaning chemistries. Use of humidity bags to keep devices in a moist state reduce protein attachment.
The Influence of the Dwell Times Including Transportation on the Cleaning of Surgical Instruments in the WD	(Michels, et al., 2022)	Clinical investigation on the impact to cleaning effectiveness with dried soil up to 14 hours. Study demonstrated increased cleaning challenge is correlated with time.

2.3.1.5 Soil Extraction

It is important that the extraction method be validated to demonstrate the effectiveness of residual analyte removal, which will inform the accurate measurement of the amount of test soil remaining on a device after cleaning. This is investigated by soiling the device with a known amount of analyte and by extracting the same in order to demonstrate the percent removal (i.e., recovery efficiency). This value is then used to estimate a correction factor. For example, if the extraction method yields a recovery efficiency of 70%, a correction factor of 1.3 would be applied to unknown results to adjust for the

method's inability to remove 100% of the residual analyte. Indeed, until the development of AAMI ST98, the correction factor was calculated using only the positive control, resulting in a large measure of uncertainty within the test system. Present state-of-the-art requires a minimum test sample number of three for extraction method validation (Association for the Advancement of Medical Instrumentation, 2022).

With the change in validation requirements, concerns have been raised that by increasing the sample size, the validation results may prove to be variable that adds to the uncertainty of the analyte residual result. Kremer et. al demonstrated that with tight control over the validation variables, consistent extraction efficiency results can be achieved; but, when variation is allowed within the method (e.g., shaking force, soil amount, inconsistent soiling technique), the extraction efficiency can be highly variable (Kremer, et al., 2021). Two methods of extraction method validation are described in AAMI ST98, namely Spike Recovery and Exhaustive Extraction Efficiency. The spike recovery method is performed by applying a known amount of test analyte to the device and calculating the ratio of the recovered analyte to the total amount of analyte recovered. For the exhaustive extraction, the test soil is applied and then extracted repeatedly. The recovery efficiency is calculated by dividing the first extraction amount by the total amount extracted.

Currently there is a gap in the literature that demonstrates equivalency between these methods. Therefore, this deficiency was also investigated within this present study. The eluent used to extract the analyte from the device can also contribute a level of uncertainty to the test system. The primary extraction eluent used in cleaning validations is water. However, using water alone as a polar diluent for analyte extraction may leave non-water-soluble proteins (e.g., fibrin) remaining on the surface that are unaccounted for as stated in the literature (Table 2.11). The extraction eluent was explored within this present study so an accurate protein measurement can be achieved. The eluent used to extract the analyte from the device can also contribute a level of uncertainty to the test system. The primary extraction eluent used in cleaning validations was water.

Table 2.11: Literature describing test soil extraction for reusable medical devices

Title	Reference	Industry Impact
Blood viscosity during coagulation at different shear rates	(Ranucci, et al., 2014)	Provides evidence that as blood coagulates the shear rate changes as platelets adhere to the material surface.
Test Method for Comparative Evaluation of Instrument Detergents for Manual Reprocessing of Surgical Instruments on the Basis of Fibrin	(Wehrl, et al., 2018)	Investigates the extraction techniques of the water insoluble component of clinical soil, fibrin. Study provide a novel method to remove all but the water insoluble component of blood soil on a coupon.

A test method to assess the contribution of fluid shear stress to the cleaning of reusable device surfaces	(Hariharan, et al., 2019)	Study evaluated fluid flow-induced shear stress for the detachment of soil remaining on a reusable medical device.
Connecting Across Competencies: Leveraging Best Practices for Processing	(Kremer, et al., 2021)	Demonstrated reproducible extraction efficiency results under controlled validation conditions.

2.3.1.6 Cleaning Agent (i.e., Detergent) Selection

Currently, there are no standards that define the performance of cleaning agents used to clean reusable medical devices. Cleaning agent manufacturers provide safety information while using the chemistries, but little information is often provided on the residual toxicity levels or efficacy of soil removal (Kremer, et al., 2021). This is problematic as it is not practical for medical device manufacturers to validate every possible cleaning agent that may be used on the device as chosen by the healthcare facility. Device manufacturers do not list specific cleaning agents in their IFU to allow for flexibility in the supply chain. As discussed by Kremer et. al. (Kremer, et al., 2021) it should be the responsibility of the cleaning agent manufacturer to demonstrate performance against standardized criteria.

For cleaning efficacy validations, a good quality neutral pH or mildly alkaline detergent (pH 7 to 9.5, with or without enzymes) from a well-known provider, recognized as a global provider (e.g., STERIS, Ecolab or Dr. Weigert), is utilized as this type of detergent used during cleaning validations will validate the minimum cleaning chemistry requirements for soil removal. Detergent products should be labelled for use on medical devices and be provided with safety and efficacy data. An alkaline detergent should be used for end-of-life studies to establish end-of-use criteria for device lifetime as these types of chemistry can often present a greater challenge to device material compatibility and influence the end-of-life performance of the device. Enzymatic detergents may also need to be considered for end-of-life studies evaluating the potential for residual (e.g., protein) buildup impacting patient safety. Although this validation strategy is common for device manufactures, little has been published comparing performance of the different cleaning chemistry formulations (Table 2.12).

Table 2.12: Literature investigating the performance of cleaning agents

Title	Reference	Industry Impact
Utilization of Enzymatic Detergents to Clean Inorganic Membranes Fouled by Whey Proteins	(Arguello, et al., 2005)	Optimization of the cleaning process for inorganic membranes. Use of enzymatic detergents increase the cleaning efficacy.
Quantitative Measurement of the Efficacy of Protein Removal by Cleaning Formulations; Comparative Evaluation of Prion-Directed Cleaning Chemistries	(Ungurs, et al., 2010)	Demonstrated alkaline cleaning chemistries are more effective at removal of prions than enzymatic detergents when pre-soaked and rinsed.

Ability of Manufacturers' Information on Efficacy and Compatibility of Detergents Used for Cleaning Dental Instruments	(Calvert, et al., 2012)	Literature and physico-chemical data review for commercially available detergent information. Investigation found that data is not supplied to users by cleaning agent manufacturers.
In Vitro Evaluation of Cleaning Efficacy of Detergents Recommended for Use on Dental Instruments	(Smith, et al., 2012)	Cleaning solutions range in performance in removing blood from stainless steel. Alkaline solutions showed the reversal of interactions with stainless steel surface with albumin by reversing the charge of the oxide layer.
Evaluation of Detergents and Contact Time on Biofilm Removal from Flexible Endoscopes	(Ren, et al., 2013)	Comparison of cleaning solutions against biofilm development. Data suggests that short time exposure to cleaning chemistries can improve efficiency of cleaning.
Enzymes Enhance Biofilm Removal Efficiency of Cleaners	(Stiefel, et al., 2016)	Validation of an enzymatic cleaning chemistry by the cleaning agent manufacturer. Study showed near complete removal of biofilms and reduced extracellular polymeric substances.
Assessing Detergent Residuals for Reusable Device Cleaning Validations	(Kremer, et al., 2021)	Documented differences in residual toxicity levels for detergent types using ISO 10993-5 performance criteria.

2.3.1.7 Sample Size & Conditioning

With the intent of reusable medical devices to be processed repeatedly, consideration must be given to the conditioning of the device samples used for testing. To deliver validation samples into a used state, the devices are repeatedly cycled through all processing steps. This repeat cycling process performs two functions, first to condition the device to a used-state, and second to assess the device features for the potential for soil accumulation (Association for the Advancement of Medical Instrumentation, 2022). However, there is currently no published literature that justifies an appropriate number of cycles for this conditioning process, and this is likely to vary depending on the device labelling. However, AAMI ST98 has specified a minimum number of 6 cycles through industry consensus. If cleaning instructions are validated and performed correctly, there should be little risk of soil accumulation, so only the conditioning of the samples should be necessary.

As described in AAMI ST98, the sample size used within a cleaning validation must be of a size to ensure reproducibility and confidence within the results (Association for the Advancement of Medical Instrumentation, 2022). Cleaning validations are time consuming and expensive, so medical device manufacturers default to the smallest sample size to demonstrate reproducibility, n=3. However, with the increase in complexity with device features, recent evidence in the literature (e.g., endoscopes and robotic devices) indicate a larger sample size may be necessary with some device designs to demonstrate the robustness of the cleaning validation (Association for the Advancement of Medical Instrumentation, 2022).

2.3.2 Device Processing Risk Mitigation

When designing the cleaning instructions for use, medical device manufacturers must account for human factors during the device processing steps. As described in AAMI TIR55, humans are fallible and prone to error. Therefore, the manufacturer must account for the risk of humans not fully following the cleaning instructions for use during processing (Association for the Advancement of Medical Instrumentation, 2014). To appropriately evaluate the independent variable within a cleaning validation, written cleaning instructions from the device manufacture must identify the worst-case conditions within the cleaning process. For example, most cleaning instructions include the requirement to rinse or apply a point of use treatment to prevent soil from drying on the device. Because this action takes place at the patient bedside immediately after use, human factors, like time, training or supplies to perform the required action may prevent the point of use treatment from occurring. The device manufacturer may therefore omit this processing step within the cleaning validation as a worst-case conditioning for the device (Association for the Advancement of Medical Instrumentation, 2022). By accounting for human error during the cleaning validation the device manufacturer mitigates the risks of the processing instructions not being followed exactly.

The selection of the 'worst-case' parameters is highly variable and at the discretion of the device manufacturer. Although guidance has been provided by industry standards, there is no consensus on what the worst-case variable configuration is for a specific device. Therefore, with an appropriate justification the manufacturer can select any variable combination they see fit. The device manufacturer must balance the intent to create a robust challenge for the device, without overchallenging. The compounding effects of selecting worst case for every step in the cleaning process will inevitably lead to circumstances where devices are unable to effectively cleaned. For instance, if the most challenging controlled variables are chosen, such as soil removal, (Kremer, et al., 2021), soil application method (Kremer, et al., 2021) (Kremer & Ratanski, 2023), soil drying conditions (Kremer, et al., 2023), and lowest detergent concentration with the least stringent cleaning instructions as independent variables, then the risk of failure becomes inadvertently high. In the event of failure, adjustments to the processing instructions are necessary to showcase cleaning effectiveness. The change in processing steps often results in transferring greater risk to the user by over processing the device. The cost of cleaning validations is high (~$35K per validation), so it is the best interest for the device manufacturer to select the combination of variables that can be justified as challenging to the device but does not compound the worst-case risk past the point of failure.

Although industry guidance recommends soliciting the voice of customers prior to designing the processing instructions, there is no requirement for what information should be collected or how the feedback should be interpreted based on risk. Lack of standardization within this area creates a disconnect between the end-user and device manufacturer leading to production of similar devices with different processing instructions. The expectation of the device manufacturer is that the processing instructions written within the IFU will be followed exactly; however, this expectation is not realized in healthcare facilities.

2.4 Device Processing at the Healthcare Facility

The manufacturer of single-use, sterile medical devices has the responsibility to maintain the Assurance of Sterility throughout the end-to-end supply chain. However, there is a greater shared responsibility between the device manufacturer and the health care facility when medical devices are intended to be processed by the customer. Like the MQSA supply-chain, each step in the device processing cycle is designed by the device manufacturer to ensure that after each device processing cycle the medical device is safe for patient use (Figure 2.3). A full description for all phases of the critical device processing cycle can be found in Appendix 1, but the following is a summary of the main steps.

Decontamination – Following patient use, the first three steps of the device processing cycle at the healthcare facility are generally point of use treatment, cleaning, and disinfection. Point of use treatment describes the actions taken to prevent soil from drying on the device directly after patient use. This process step is performed at the point of use and may include pre-cleaning the device to physically remove the soil by rinsing or wiping, applying a treatment product to prevent soil from drying, or packaging / covering the devices to generate a humid environment where conditions prevent drying. The next two decontamination steps are typically performed in a centralized decontamination area. Cleaning describes the physical removal of clinical soil and microorganisms using physical force. Disinfection is the process of inactivating microorganisms through chemical applications designed to destroy bacteria, viruses, and fungi.

Inspection/Packaging – This step verifies the efficacy of the decontamination step and readies the device for further processing or patient use. There are at least four components to inspection: cleanliness, no damage, dry (when applicable) and any maintenance requirements. Following inspection, the device or set of devices are packaged as per the specified sterilization modality.

Sterilization – Sterilization is the inactivation of microorganisms to reach a very low probability of viable microorganisms remaining on the product. The probability is so low that the definition is the device is free from problematical microorganisms.

Transport – The device is moved to/from storage to the point at which it will be used.

Global standards and guidance have been established to standardize how the processing steps and environments in which they are performed within the healthcare facility. Examples of such documents are noted in Table 2.13.

Table 2.13: Healthcare processing standards and guidance

Country	Title	Reference
International	World Health Organization: Decontamination and Reprocessing of Medical Devices for Health-care Facilities	(World Health Organization, 2016)
Australia	AS/NZS 4187:2014 Reprocessing of Reusable Devices in Healthcare Service Organization	(Australian Commission on Safety and Quality in Health Care, 2019)
Germany	Hygiene Requirements for Reprocessing Medical Devices	(German Society for Hospital Hygiene (DGKH), German Society for Sterile Supply (DGSV), Working Group Instrument Preparation (AKI), 2012)
United States of America	ANSI/AAMI ST79:2017 Comprehensive Guide to Steam Sterilization and Sterility Assurance in Health Care Facilities	(Association for the Advancement of Medical Instrumentation, 2020)
	AORN Guidelines for Perioperative Practice	(Association of periOperative Registered Nurses, 2022)
	ANSI/AAMI ST58: 2013 (R2018) Chemical Sterilization and High-Level Disinfection in Health Care Facilities	(Association for the Advancement of Medical Instrumentation, 2018)
United Kingdom	Health Technical Memorandum 01-01: Management and decontamination of surgical instruments (medical devices) used in acute care	(United Kingdom, 2013)
Global	ISO 17664-1_2021 Processing of health care products - Information to be provided by the medical device manufacturer for the processing of medical devices	(International Organization for Standardization, 2021)
Global	ISO 17664-2 Processing of Health Care Products - Information to be provided - Non-Critical Medical Devices	(International Organization for Standardization, 2021)

Within the literature, many articles qualitatively describe the realities of device processing within a Healthcare's Sterile Processing Department (SPD). However, the first quantitative studies were recently published by Alfred et. al documenting the editorial positions with data in 2021 (Alfred, et al., 2020) (Alfred, et al., 2021). In the publication, *Work systems analysis of sterile processing: decontamination* (Alfred, et al., 2020), device processing failures resulting from decontamination errors (3%) from two processing facilities processing approximately 23,000 units each month were characterized by the root cause as described below.

2.4.1 Staff Knowledge

Within the two large hospitals observed, the SPD technicians were expected to conduct complex cleaning tasks largely from memory for 250,000 unique instruments. These often low-paid individuals received limited formal training such as 20-minute in-service performed by the instrument sales representative leading to a high turnover rate. Cleaning instructions for each individual instrument were not referenced during decontamination, but instead the observed controls, standard operating procedure (SOP) displays and access to IFUs were established to help technicians clean the instruments effectively. It was noted in the study that is it unlikely that the most experienced and highly trained SPD technician would remember the decontamination instructions for every instrument (Alfred, et al., 2020).

The expectation of the device manufacturer is for the IFU to be followed exactly as instructed. However, evidence showed that point-of-use processing was only effective in approximately 54% of the instrument trays and the probability of SPD remembering the cleaning instructions for each instrument is low. With the increase in device feature complexity, it was noted that similar devices can have extremes in cleaning steps depending on the outcome of the decontamination IFU validation (Alfred, et al., 2020).

2.4.2 Production Pressures

OR time is expensive, and the instruments used in these surgeries are expected to be reprocessed with a speed that allows for optimization of the resources to keep the OR in service as much as possible. The decontamination steps include a combination of manual and automated methods that can be time consuming. Throughput constraints can affect the ability for SPD to meet surgical demands, resulting in priority shifts and deviations to SOPs to alleviate production pressures. For example, deviation observed by Alfred et. al describes a required sonication step being omitted from the workflow due to the equipment being down or at capacity (Alfred, et al., 2020).

Shifting priorities can also affect how fast the instruments are moved through the time sensitive decontamination phase. Soiled instruments sets arriving from the OR are prioritized based on need. Therefore, there may be a long delay before beginning the decontamination process for some instruments allowing for an opportunity for soil to dry or a biofilm to develop on used devices (Alfred, et al., 2020).

2.4.3 Instrument Design

It was observed by Alfred et al. that disassembly instructions were not intuitive or error tolerant. It is becoming increasingly common for instruments to include complex device features that may hide residual soil. These complex devices require additional processing time and increased instruction for disassembly, inspection, and assembly practices (Alfred, et al., 2020).

2.4.4 Tray Composition

OR case cancellations have a cost of $1,500 or more per hour, so delivering a defective instrument kit for a scheduled surgery can have significant cost implications for the health care facility. Instrument kits are often built based on the specification of the surgeon, so tray composition can be highly variable and require the SPD technician to have a high level of subject-matter knowledge to inspect and select the right

instruments for a case cart. Alfred et al. also observes that only 13% of the instruments in a tray are used in a surgical case. Thus, it would be possible for SPD to optimize tray configuration with general instruments in one case and specialty instruments in a smaller case (Alfred, et al., 2021). Stockert et. al reported that the total cost to reprocess one instrument is $0.51 and if a hospital with 13 operating rooms were to reduce the excess volume of instrumentation sent through sterile processing, then it is possible to achieve as cost saving of $156,461 per year (Stockert & Langerman, 2014).

2.4.5 Workstation Design

SPD is typically faced with physical compacity constraints leading to congestion of case carts containing used devices awaiting processing, However, there is frequently inadequate holding space, insufficient quantity of sinks or decontamination equipment allowing for poor line clearance and poor lighting resulting in insufficient inspections to meet appropriate processing in healthcare SPD (Alfred, et al., 2020). As defined by the requirements by the WHO, the Sterile Processing Department ideally is separated by function with physical barriers designating the physical areas where specific actions occur. The four main areas are decontamination, inspection/packaging, sterilization, and storage. In high throughput healthcare facilities, a wall of washer-disinfectors with a 2-door pass through may act as the barrier between decontamination and inspection allowing for a one-way process flow-through moving from dirty to clean.

Similarly, a bank of sterilizers may act as the barrier between inspection and storage, ensuring which cases have been terminally sterilized and are ready for use. (World Health Organization, 2016). However, some facilities do not have the space for this type of physical segregation and must therefore adapt line clearance measures appropriate to the space. These situations highlight the need for greater simplification of cleaning and processing in SPD internationally.

2.5 Workflow Standardization

It is appreciated that device manufacturers have a similar barrier to validating each device within their product portfolio, which may be comprised of thousands of SKUs. To lower this barrier for entry into market, device manufacturers are allowed to establish product families and validate the worst-case design with demonstrated commonality in device materials, design features, intended use and clinical soil exposure. Processing instructions must be the same for each device in the product family (Association for the Advancement of Medical Instrumentation, 2020). US FDA Guidance (Section VII, second paragraph) – “It is possible that similarities in design, materials and other factors may allow for establishing product families (e.g., devices with a range of available sized) for the purpose of minimizing processing validation efforts.” (U.S. Department of Health and Human Services Food and Drug Administration, 2015) (U.S. Department of Health and Human Services Food and Drug Administration, 2015)ISO 17664:2017 – “If a manufacturer supplies a number of different medical devices that share common attributes, then validation studies may be performed as a product family” (International Organization for Standardization, 2017) (International Organization for Standardization, 2017). There is currently no global industry guidance for how to adopt devices into a process within a Sterile Processing Department using a family

grouping strategy. ISO 17664-1 outlines what instructions must be included in the device IFU based upon risk to provide sufficient instructions for device processing (International Organization for Standardization, 2017). As such, it is left to the discretion of the device manufacturer to identify the level of detail provided. For example, robotic instruments may have pages of cleaning instructions, where simple devices have a single paragraph. It remains the device manufacture's expectation that the IFU will be followed exactly (Association for the Advancement of Medical Instrumentation, 2020), but this is not practical considering the number of devices processed each day through Sterile Processing Departments. Adopting an appropriate product family approach for device cleaning would greatly help to address many processing challenges, yet this information is currently lacking.

Standardization efforts to develop process flows using device risk for Sterile Processing have been an initiative by many standard committees over the last 10 years, but different strategies have been deployed based on the geographical region. For example, in the US guidance is provided to the device manufacturer in Annex D and E of AAMI TIR 12 for what processing instructions should be included depending on a device category (Association for the Advancement of Medical Instrumentation, 2020). While in Germany the responsibility shifts to the Healthcare Facility with the requirement of a process qualification for the process. The qualification is an assessment of cleaning performance for the processing steps and will typically use a worst-case device or surrogate device as the process challenge device. As many European countries strongly recommend a fully automated process for cleaning-disinfection, the qualification requirements for a cleaning process are described in ISO 15883-1 (International Organization for Standardization, 2021). However, it is still at the discretion of the healthcare facility to group devices (families) and adopt them into the appropriate processing process.

As discussed above, the pressures on healthcare's Sterile Processing Departments are immense to ensure that the processing of devices are quickly addressed meeting the need of OR that constantly depends on their safe reuse. To increase throughput, Sterile Processing will establish groups of devices that can be processed together using the same steps.

2.6 Spaulding Classification

In 1968 Earle H. Spaulding developed a classification system to address the microbiological quality of medical devices processed within a healthcare facility. This system has provided device manufacturers and healthcare facilities with the ability to easily identify device types and validate the appropriate disinfection / sterilization techniques to achieve an appropriate level of microbiological quality for patient safety. This system is used by infection control professionals and others when adopting devices into a disinfection / sterilization process (Rutala & Weber, 2013). The Spaulding Classification system for medical devices is based on the risk of the transmission of infections (International Standard Organization, 2021). This risk is based on the level of contact the device has with the patient and the device is classified accordingly. The three (3) levels of classification are "Critical", "Semi-critical", and "Non-critical". The Spaulding Classification system for medical devices is based on the risk of the transmission of infections (International Standard Organization, 2021). This risk is based on the level of contact the device has with

the patient and the device is classified accordingly. The three (3) levels of classification are “Critical”, “Semi-critical”, and “Non-critical”.

Critical Devices: Contacts ‘sterile’ tissues (including blood and internal body spaces) during their use. Examples include surgical devices. It is recommended that these devices are adequately cleaned and sterilized prior to patient use (Spaulding, 1968) (International Standard Organization, 2021) (U.S. Department of Health and Human Services Food and Drug Administration, 2015).

Semi-critical Devices: May only contact mucous membranes or non-intact skin. Examples include flexible colonoscopes, gastroscopes, and respiratory equipment. It is also recommended that these devices are adequately cleaned and sterilized prior to use, but they may also be subjected to a terminal high-level disinfection method (Spaulding, 1968) (International Standard Organization, 2021) (U.S. Department of Health and Human Services Food and Drug Administration, 2015).

Non-critical Devices: Devices or instruments that may only contact intact skin, but do not penetrate it. Examples include blood pressure cuffs, stethoscopes, and skin electrodes (non-critical patient care devices). They will also include a variety of equipment and environmental surfaces that may not directly contact the patient but can become contaminated during use or over time in clinical practice (non-critical environmental surfaces). Recommended processing steps can include cleaning and disinfection, where the level of disinfection can vary depending on the risk to patient or staff safety, as well as local requirements (Spaulding, 1968) (International Standard Organization, 2021) (U.S. Department of Health and Human Services Food and Drug Administration, 2015).

Most devices processed within a Sterile Processing Department are classified as either critical or semi-critical and should default to being adequately cleaned and sterilized prior to use. However, over the decades since the Spaulding Classification was first introduced, semi-critical devices have become increasingly complex and manufactures have defaulted to the unrecommended, high-level disinfection over terminal sterilization as the standard of care (Rutala & Weber, 2013). While the terminal sterilization process is designed to render a device free from microorganisms (International Organization for Standardization, 2018), disinfection is demonstrated by the reduction of specific microorganism types (U.S. Department of Health and Human Services Food and Drug Administration, 2015). An example in the USA is:

- **High-level disinfection:** 6-log reduction of a *Mycobacterium* species.
- **Intermediate-level disinfection:** 6-log reduction of vegetative bacteria and a 3-log reduction of a *Mycobacterium* species.
- **Low-level disinfection:** 6-log reduction of vegetative bacteria.

The rationale behind these microbial reduction requirements stems from the resistance profile of microorganisms (Figure 2.7) (Kremer, et al., 2023).

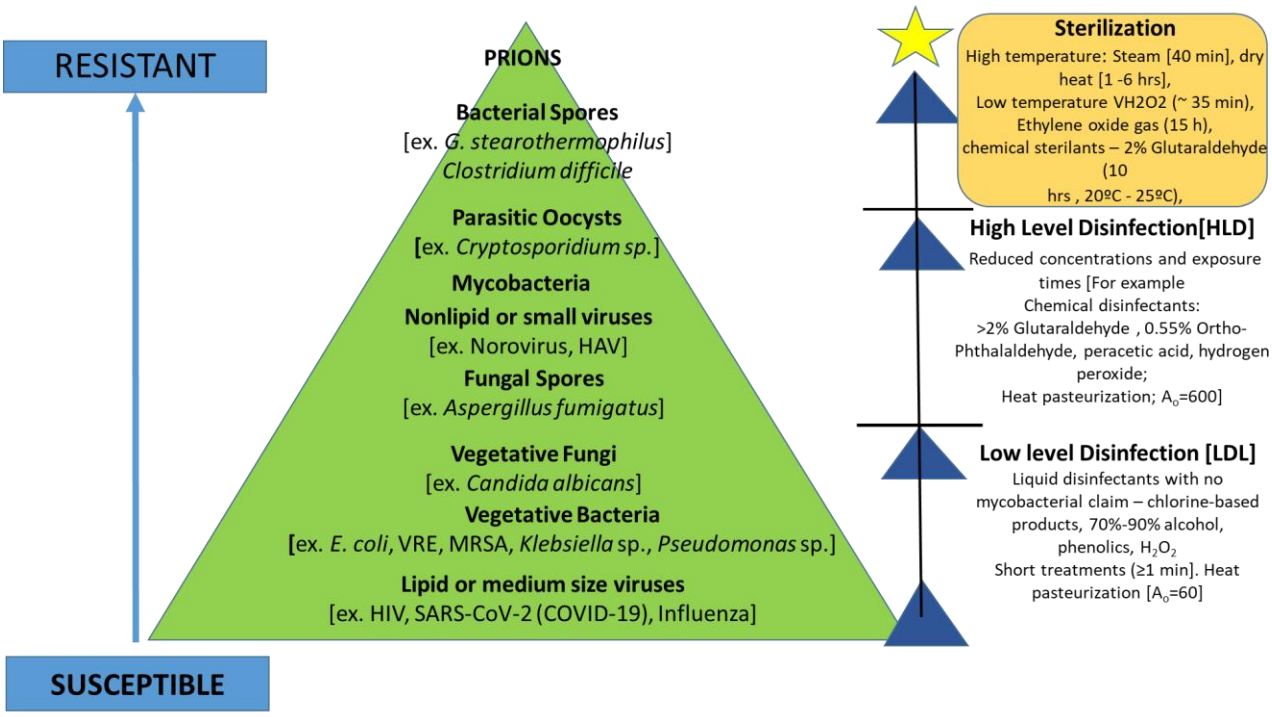


Figure 2.7: Microorganism Resistance Scale

The Spaulding Classification provided an easy mechanism to connect manufacturers and healthcare facilities to how devices must be validated and then processed to ensure the appropriate microbiological quality of the patient. Over the last 50 years, the Spaulding Classification is used globally in standards governing the activities of these two parts of the Device Processing supply chain (McDonnell & Burke, 2011). With the increasing complexity of medical devices combined with advanced knowledge of microorganisms' resistance to inactivation, the industry has identified some challenges with the current application of Spaulding's classification system. Within the literature the following three primary concerns are supported with evidence:

1. Improved understanding of microbial resistance profiles
2. Failures in high-level disinfection practices for semi-critical devices
3. Assumption that all devices are clean prior to sterilization / disinfection.

2.6.1 Microbial Resistance

McDonnell & Bruke challenged the Spaulding Classification in their paper, *Disinfection: Is It Time to Reconsider Spaulding?* The authors summarized how advances in our understanding of modern-day microbiology revealed increased patient risks (McDonnell & Burke, 2011). "Various types of viruses, bacterial strains and protozoa have been shown to survive existing high-level disinfection/sterilant processes, outside of what would be expected from the Spaulding classification system. Protozoa are rarely considered, and marker strains of viruses and bacteria may not always reflect disinfection activity against these groups of micro-organisms. The potential risks with atypical transmissible agents such as

prions and other protein-precipitation-associated diseases are already considered completely outside of such classification systems. The authors go on to explain how the microbial resistance profile that is foundational to the Spaulding Classification was developed and can be dependent how the microbes are presented for inactivation during the test set up. As discussed in the publication, current real-world conditions have demonstrated unexpected resistance profiles to inactivation. Examples are lipid enveloped viruses and vegetative bacteria that have a fairly low resistance when considered alone. However, if these pathogens are clumped together, and/or protected in residual soil (i.e. biofilm) then the resistance can exceed that of spores tested in isolation (McDonnell & Burke, 2011) (Rutala & Weber, 2019).

As per the wording of the Spaulding Classification, semi-critical items should be terminally sterilized if possible (Spaulding, 1968). Sterilization should be the default method of microbial reduction for these devices since how the devices are used can often impact patient safety. An example of a device that is difficult to categorize is a biopsy device. It may be considered a semi-critical device and processed accordingly, but if exposed to an unexpected internal bleed during patient use, it would actually be a critical device (McDonnell & Burke, 2011).

2.6.2 Inappropriate Disinfection Use

Authors, Rutala and Weber, have published extensively on this topic and the associated infection risks when semi-critical devices are not processed effectively (Rutala & Weber, 2013) (Rutala & Weber, 2013) (Rutala & Weber, 2019) (Rutala & Weber, 2019). In their 2019 article, they summarize investigations in the literature calculating the margin of safety for a disinfected endoscope. Examples of decontamination failures during the use of high-level disinfection include lack of cleaning, inappropriate use/prep of the disinfectant, inappropriate rinsing, detection of biofilm, detection of microorganisms not inactivated by the disinfectant. The starting concentration of microbial contamination on flexible endoscopes is on average between 4 $\log_{10}$ and 5 $\log_{10}$ (Cloutman-Green, et al., 2015). The cleaning process can be demonstrated to remove 2-4 $\log_{10}$ of microorganisms during the physical removal of the soil with another 4-6 $\log_{10}$ reduction of the challenge microorganisms *M. terrae* resulting in an overall reduction of 6-12 $\log_{10}$ of microorganisms within the test environment. Compare this to the estimated 17 $\log_{10}$ margin of safety on instruments cleaned and sterilized (Rutala & Weber, 2019). This reduced margin of safety is likely a contributing factor to the patient risk described above.

2.6.3 Assumption of Clean

At the time Spaulding's classification was widely adopted (1950s and 1960s), the measurement techniques for determining cleanliness had yet to be established. Visual cleanliness was the requirement at the time and the classification system was established under with the foundational assumption that all devices would be visibly clean prior to the microbial reduction step of disinfection or sterilization.

In the US, it was not until 1976 that when the Medical Device Amendment of 1976 to the Federal Food, Drug and Cosmetic Act was enacted that the FDA was allowed to impose production, distribution, and

sales rules on device manufacturers. Every device already on the market prior to 1976 was “grandfathered in”, requiring none of the safety testing that device would have to undergo moving forward. The Safe Medical Devices Act of 1990 further expanded the FDA’s authority to regulate medical devices and require hospitals to report serious injury, death, or other “adverse experiences” related to medical devices to both the device manufacturer and the FDA (US Food and Drug Adminitration, 2018).

Without oversight from regulators or medical device manufacturers, healthcare facilities self-regulated and established their own guidelines for safety. The Spaulding Classification provided the needed guidance to establish protocols for maintaining the appropriate microbiological quality levels necessary for patient safety. However, over the last 50 years medical devices have become increasingly complex with hidden areas that make the requirement of visual cleanliness difficult to verify.

The literature reviewed in Table 2.14 describes adverse events related to medical devices that were not clean. The terminology described in these articles refer to “bioburden” remaining on the device after processing. As defined in the standards, bioburden as the population of microorganisms on the device (International Organization for Standardization, 2018), but bioburden is also common vernacular used in the healthcare facility to describe the visible soil remaining on the device after processing. As discussed above, the risk of residual microorganisms and proteins are interrelated.

Microbial resistance used as the cornerstone of the Spaulding Classification was established under conditions with little (e.g., microorganism titer prepared in a 5% bovine serum) or no soil remaining on the device. As discussed by McDonnell & Burke, the resistance profile may change in the presence of soil and therefore should be investigated (McDonnell & Burke, 2011). In an experiment by Kaiser et. al, spore survival studies were performed using moist heat sterilization to sterilize spores dried in a calcium carbonate and iron oxide/hydroxide. The microorganism preparation was designed to simulate hard water deposits left on the device after processing, and the experiment demonstrated that as the “soil” dried on the device the calcium carbonate became insoluble. The steam, therefore, had to rehydrate the soil before it could be penetrated and inactivate the spores trapped within. The authors conclude that it is possible for spores to survive steam or gas sterilization if trapped within hard water deposits (Kaiser, et al., 2000).

Regardless of which definition is used for the word ‘bioburden’ (or the presence of biological material on a surface), it being present on a device post processing increases patient risk (see table 2.2). A high-risk example of this is with biofilms, defined as communities of microorganisms. Roberts discusses in the article, *The Role of Biofilms in Reprocessing Medical Devices,* the required conditions for biofilms to develop are the following: colonizing microorganisms present, surface to be colonized, sufficient nutrients and water, temperature conditions for growth and time required for development (Roberts, 2013) Figure 2.8). Within some reusable medical devices, it is possible for these conditions to occur, especially when devices are not processed right away after use, not properly cleaned and not completely dry before storage. If biofilms are allowed to develop within a device, the cleaning challenge is increased due to the presence of a dry biofilm (Alfa, 2019) (Roberts, 2013).

The assumption of cleanliness is a particular concern for medical devices classified as semi-critical. As discussed above, the margin of safety is lower with these devices as they can be chemically disinfected in lieu of terminal sterilization. In the healthcare facility today, most scopes and probes are considered semi-critical devices where the standard of care due to their complex and sensitive device features (e.g., camera, long lumens) are not compatible with terminal sterilization methods. The processing of these devices are so complicated that in the US ANSI/AAMI ST:91 *Flexible and Semi-Rigid Endoscope Processing in Health Care Facilities* and AAMI TIR99 *Processing of Dilators, Transesophageal and Ultrasound Probes in Health Care Facilities* are available as guidance for these complicated devices (Association for the Advancement of Medical Instrumentation, 2021) (Association for the Advancement of Medical Instrumentation, 2022).

Alfa et al has published extensively on the challenges with cleaning and monitoring of semi-critical devices like flexible endoscopes. The complex features of these devices make visual inspection and monitoring for cleanliness difficult, leading to an increased risk of soil accumulation and biofilm development. The behavior of traditional biofilms, forms under continuously hydrated conditions, differs from a dry surface biofilm, heterogenous accumulation of organisms and other material in a dry matrix, as it less difficult to clean/disinfect/sterilize (Alfa, 2019). Modeling of dry surface biofilms demonstrate that some disinfectants are not as effective if a dry surface biofilm is present (Alfa & Howie, 2009) and can be environmental reservoirs promoting microbial growth (Alfa, 2019). Literature describing increased risk for patient safety for devices not processed effectively can be found in Table 2.14.

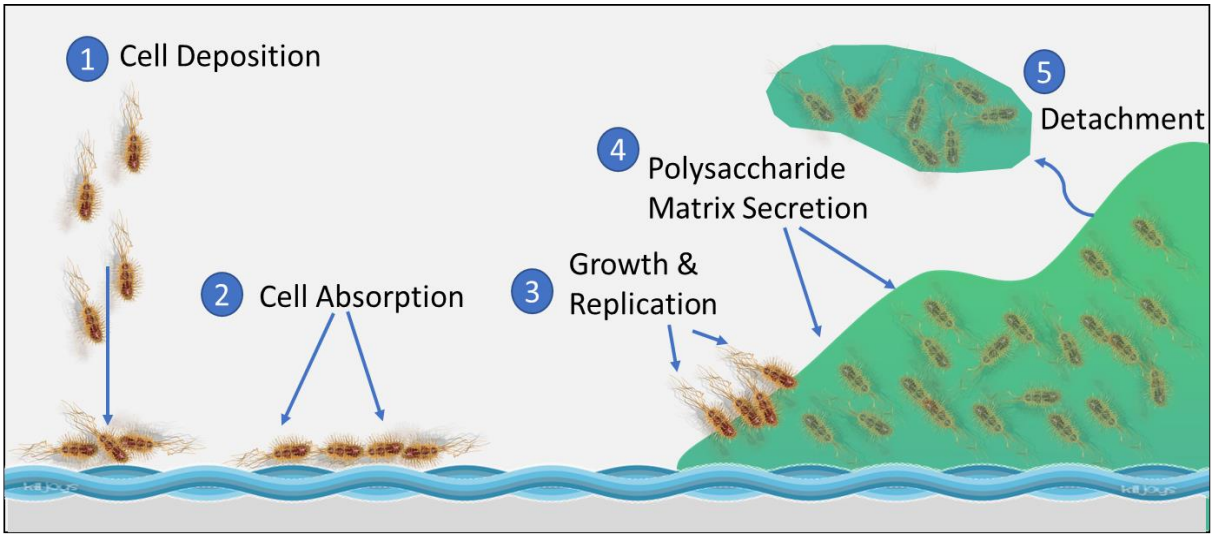


Figure 2.8: Depiction of Biofilm Development

Table 2.14: Literature describing ineffective processing

Title	Reference	Industry Impact
Contamination of Equipment in Emergency Settings: An Exploratory Study with a Targeted Automated Intervention	(Obasi, et al., 2009)	Demonstration that automated cleaning is more reproducible at removing microorganisms, 0% colonization rate, compared to manual cleaning, 25% colonization rate.
Comparative Cost-Efficiency of the EVOTECH Endoscope Cleaner and Reprocessor Versus Manual Cleaning Plus Automated Endoscope Reprocessing in a Real-World Canadian Hospital Endoscopy Setting	(Forte & Shum, 2011)	Automated reprocessing is more efficient with an increased time saving. Cost benefit analysis of supply cost versus labor cost demonstrated a cost savings.
Assessing the Risk of Disease Transmission to Patients when there is a Failure to Follow Recommended Disinfection and Sterilization Guidelines	(Weber & Rutala, 2013)	Process for how to identify risk of a device that has not been processed effectively.
Infections and Exposures: Reported Incidents Associated with Unsuccessful Decontamination of Reusable Surgical Instruments	(Southworth, 2014)	Literature review performed in 2014 of 21 articles reporting failures in decontamination. 43% of the failures were associated with surgical instruments and the procedure with most reported failures (21%) were eye surgeries.

2.7 External User / Distribution Center Processing

A large inventory system is a high-cost burden for the healthcare facility to manage. This can be especially true for specialized procedures. An affordable solution to the expense is for healthcare facilities to participate in a loaned device program with manufacturers. The definition of the term “loan” in this scenario is a single or group of devices that are used by a healthcare facility but are not part of that facility’s inventory (McDonnell & Sheard, 2012). Loaned devices (or loaner sets) can include implants, instruments, and equipment.

Loaner programs are specific to the inventory owner and healthcare facilities must work to proceduralize how loaned devices will be processed before and after patient use. The inventory owner may be the manufacturer of the device or a third party distribution company. The device processing requirements for the loaner program are established by the inventory owner and the applicable processes are proceduralized by the healthcare facility. Best practice for infection prevention dictates that point of use treatment should always occur directly after patient use so this processing step remains the responsibility of the healthcare faciltiy. By following this practice gross soil is removed from the device in a timely manner. This is a critical step in the decontamination process to ensure remaining of the device processing cycle is effective.

The remainining processing steps may occur within the healthcare facility or may occur after the transport to a centralized processing center. Currently, there is no standard or regulation for device loaner programs, so the responsibility for processing steps must be agreed upon by all parties involved in the program. For example, as depicted in Figure 2.9 a common loaner set program requirement is for the loaned instruments to be processed by the healthcare facility through the decontamination phase so they are in a clean state with a microbial reduction making them safe to handle for the inspection and packaing process prior to transport and reciept at the external reprocessing facility (i.e. Distribution Center). The devices may be transported in a clean, cleaned/disinfected or ready to use state. This means that there is an increase risk that soil can dry on the device.

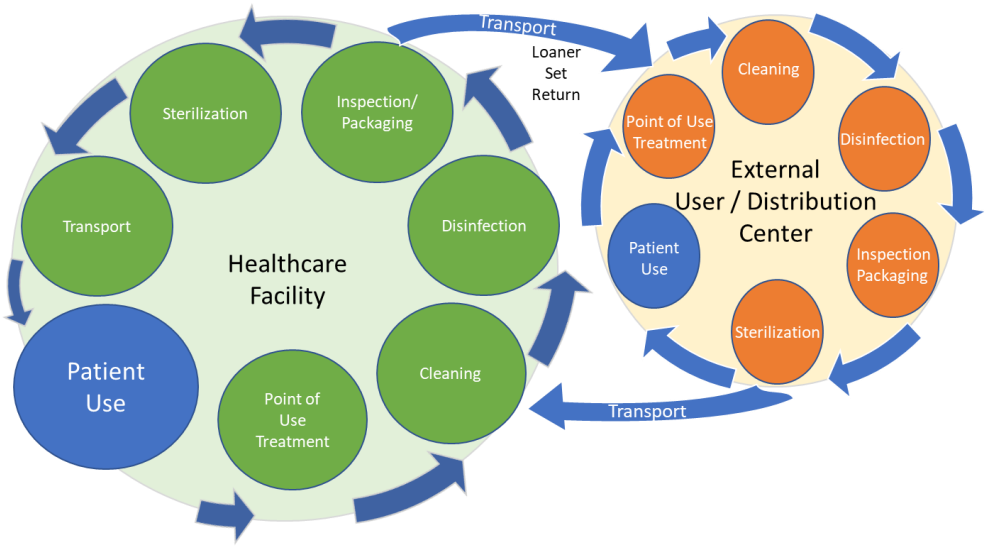


Figure 2.9: Device Processing Cycle External User / Distribution Center

The transport of the loaned instruments to the distribution center is cordinated by the inventory owner. Transport requirement are described in standards and guidance such as AAMI ST79 (Association for the Advancement of Medical Instrumentation, 2020). The transport of loaned inventory may occur in a variety of vehicles including custom transport vans to personal vehicles. The conditions in such vehicals can also be extreamly variable. Devices may experience extremes in temperature and humidity if conditions are not controlled during transport (Kremer, et al., 2023).

Once the instruments are received the responsibility for the device processing cycle shifts to another healthcare facility (external user) or an external centralized processing center (Distribution Center). The instruments are inspected and may have the decontaminatoin steps of cleaning and disinfection repeated if visible soil is present or the transport process is suspected to introduce contamination. As there is no standard that governs this process, the requirements of this process are defined by the distribution center so they can ensure the saftely of the processing personel handling the returned instruments. Just as decontamination processing technicans within the healthcare facility must wear appropriate personal

protective equipment (PPE) to protect agains blood borne pathogens, so too must the processing personel at the distribution center.

Following inspection/decontamination the instrument set will be replenished and if nessessary, instruments will be repaired or replaced. Once the instrument or set is in a state where it can be returned to a healthcare facility, it may or may not be sterilized. Some distribution centers will return devices to a healthcare facility in a state that is ready for patient use, but this is contract dependent. The inventory is then transported to a new healthcare facility where in most cases, it put through the full device processing cycle of decontamination, inspection, packaging and sterilization before it used on a patient. In some cases a similar loaner program is established internally within a healthcare system that encompass multiple facilities. In addition to general hospitals that require the use of reusable devices other examples of facilities may include examination offices, out-patient surgery centers or emergency care facilities. Depending on the size of the facility, it may not be practical to have Sterile Processing Department located within the building, so a healthcare system with multiple facilities may share a centralized Sterile Processing Department. In this situation, the healthcare system remains the inventory owner, but the use of the instrument or set is completed by an external user. Responsibility for the device processing cycle may be shared within the network similar to that of the Loaner Set Distribution Center were devices are transferred between facilities. Although standards such as AAMI ST79 may include general guidance for this type of arrangement, it is nessessary for the healthcare sytsem to have documented procedures for how the process occurs and which facility has the reponsibility for each processing step. Literature describing the management of loaner sets can be found in Table 2.15.

As indicated in Figures 2.9, the Sterile Processing Department within the healthcare facility where the device will be used will typically retain the end responsibility to ensure the device or instrument sets are ready for patient use. With so many transfers of responsibility during the loaner process, it is nessessary from an infection control perspective to have one department ultimately responsible for the microbiological quality and sterilty assurance of the devices regardless of inventory ownership.

Table 2.15: Literature review describing management of loaner sets

Title	Reference	Industry Impact
IAHCSMM Position Paper on the Management of Loaner Instrumentation	(Duro, 2011)	Healthcare Sterile Processing Association (HSPA), formally IAHCSMM, guidance to industry on the management of loaner sets. Guidance for the tracking and processing steps are included.
Loaner Instrumentation: Challenges, Risks and Strategies for Success	(Pyrek, 2013)	Opinion paper discussing the challenges of loaner sets including the major challenge that the responsibility for processing belongs to the institution that is using the set. This may lead to surgery delays due to processing time or increased risk of sets not being clean if the process is rushed.

2.8 External Processing Facilities

A further change to the device processing cycle is the addition of external processing facilities (e.g., 3rd party reprocessors). External reprocessors are independent companies that contract with healthcare facilities to replace or augment the duties performed by an inhouse Sterile Processing Department. These services are becoming more frequently used and are required to have the same standardization for performance or regulatory oversight activity as healthcare facilities. Such providers are advertising the complete replacement of an internal SPD, so directly after point of use treatment, the devices are transported to an external reprocessing site where they are processed through the full device processing cycle.

These external processing companies contract with the healthcare facility to process the owned or loaned inventory and return it to the healthcare facility in a state ready for use. Inventory oversight remains with the healthcare facility as does the legal responsibility for device microbiological quality and sterility assurance. This program is akin to how the manufacturer of a medical device may outsource a portion of the device manufacturing process like component molding or terminal sterilization. Although an external company performs the action, the governance of the process remains the responsibility of the device manufacturer. Therefore, healthcare facilities must have procedures and contracts in place for the successful completion of the device processing cycle. As with other programs, point-of-use treatment is expected to occur directly after patient use to reduce the residual soil left on the device after patient exposure. However, within this program, the devices are packaged before the decontamination process and transported to an external facility prior to any decontamination. There is currently no limitation for how far these devices may travel before processed, so the time for transport can be highly variable and like the loaner sets, conditions within the transport vehicle may vary.

The External Reprocessor is expected to process the device in accordance with the manufacturer's IFU as though they are an extension of the inventory owning healthcare facility. However, they also have efficiency expectations as a for profit business and will group devices to streamline the processing steps across as many devices as possible. Packaged and sterilized devices are returned to the owning healthcare facility where they are used without further processing. Figure 2.10 depicts the device processing cycle with the external reprocessor.

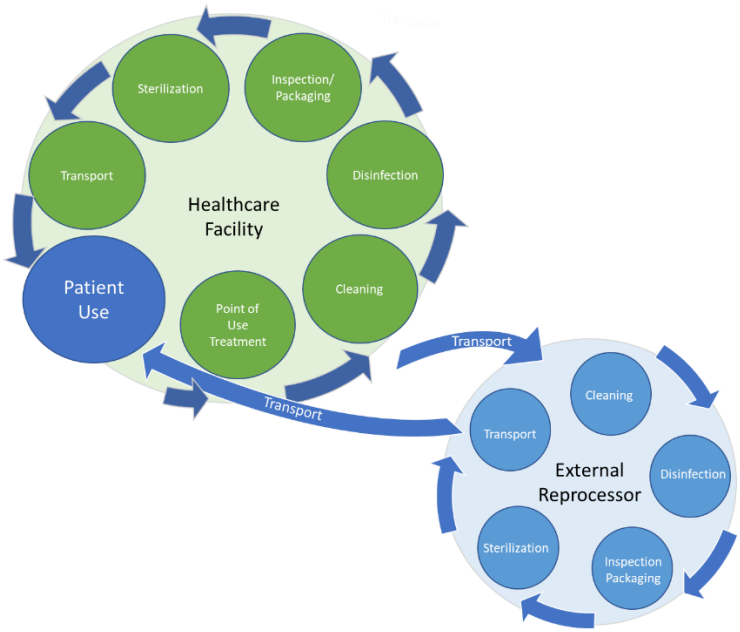


Figure 2.10: External Reprocessor Device Processing Cycle

2.9 Standardization

As depicted in Figure 2.4, the End-to-End device processing cycle is complicated with the transfer of responsibility for patient safety changing hands multiple times. That transfer is most successful when there are a set of rules that all parties agree to comply with. The Spaulding Classification provides a framework provided guiding criteria enabling medical device manufacturers to validate devices in healthcare facilities along with establishing standardized care to deliver the appropriate microbiological quality.

The Spaulding system continues to be used as the cornerstone for most industry standards concerning reusable medical devices, see Table 2.16. For example, in the early 2000's some washer-disinfectors intended for use in processing reusable medical devices became regulated as medical devices and as such required manufacturers to validate performance and clinical users to validate, monitor for routine control, and re-validate the equipment to effectively measure performance in the health care facility (International Organization for Standardization, 2006).

Table 2.16: Literature review describing cleaning verification

Title	Reference	Industry Impact
Verification of Cleaning Efficacy in Washer-Disinfector During Routine Operation	(Früh & Pfeifer, 2003)	Introduction of a process challenge device (PCD) to perform verification of an automated cleaning cycle.
Cleaning Efficacy of Medical Device Washers in North American Healthcare Facilities	(Alfa, et al., 2010)	Evaluation of the TOSI PCD with the conclusion that they are not representative of a worst-case challenge when cleaning complex devices in a washer-disinfector.
Thermostable Adenylate Kinase Technology: A New Process Indicator and	(Hesp, et al., 2010)	Demonstrated how the use of a thermostable enzyme could be used in performance monitoring in a washer-disinfector.

its Use as a Validation Tool for the Reprocessing of Surgical Instruments		
Quantitative Analysis of Residual Protein Contamination of Podiatry Instruments Reprocessed Through Local and Central Decontamination Units	(Smith, et al., 2011)	Study comparing the performance of a centralized Sterile Processing Department to local reprocessing. The study found reduced protein residual on the podiatry instruments processed in the central department.
Monitoring and Improving the Effectiveness of Cleaning Medical and Surgical Devices	(Alfa, 2013)	Evaluation of key issues when using rapid monitoring tests to measure cleaning effectiveness. This article highlights the limited connection of the units of measure to patient safety.
Comparison of Washer-Disinfector Cleaning Indicators: Impact of Temperature and Cleaning Cycle Parameters	(Alfa & Olson, 2014)	Performance characterization of cleaning PCDs available for the verification of automated cleaning cycles. Cleaning indicators provide a more sensitive tool than visual inspection alone to monitor cleanliness.
Current Issues Result in a Paradigm Shift in Reprocessing Medical and Surgical Instruments	(Alfa, 2016)	Regulation review intended to inform readers of new requirements for cleaning instruction validation, adequate staff training, ongoing competency, compliance with manufacturer's IFU, adequate facilities and equipment and process monitoring.
Adenosine Triphosphate Bioluminescence for Bacteriological Surveillance and Reprocessing Strategies for Minimizing Risk of Infection Transmission by Duodenoscopes	(Sethi, et al., 2018)	Demonstration that ATP can be used as an effective in processing monitoring tool for the decontamination of high-risk items like duodenoscopes.
Assessment of Test Methods for Evaluating Effectiveness of Cleaning Flexible Endoscopes	(Washburn & Pietsch, 2018)	This study provided evidence that protein concentration detection taken before a disinfection step has a high likelihood of being statistically similar to a measurement taken post disinfection.
The Value of Borescopes in Detecting Damage, Soil, Fluid and Foreign Objects in Flexible Endoscopes	(Ofstead & Hopkins, 2020)	Literature review of visual inspection results utilizing a borescope. Summary provides guidance for establishing a visual inspection program utilizing a borescope.

With the implementation of modern washer-disinfectors the cleaning process can become less variable; however, the 'most difficult-to-clean devices' will typically require a manual cleaning process in addition to the automated cleaning process. This will account for the inconsistent compliance with the processing instructions for use and human factors that contribute to probability of an unclean medical device. There is no requirement for an in-situ validation of the manual portion of the cleaning process at the healthcare facility using actual medical devices. Although the EU does have regulations and standards requiring in-

situ testing of the automated cleaning and sterilization process (World Health Organization, 2016), in the US it is only required to validate the equipment performance in lieu of device testing (Association for the Advancement of Medical Instrumentation, 2020).

Periodically, monitoring of devices to verify the effectiveness of the decontamination process has been encouraged, but not enforced. The Agency of Healthcare Research and Quality has identified the following monitoring strategies: visual inspection, microbiological methods, fluorescent markers, and adenosine triphosphate (ATP) (Rutala & Weber, 2019). ATP measured the organic debris using a relative light unit scale (RLU). There is conflicting literature regarding the reliability of this method as an indicator of microbial contamination (Rutala & Weber, 2019). The fluorescent marker is a method that includes the spraying an instrument or space with a marking solution that fluoresces when exposed to ultraviolet (UV) light. In the 4 studies reviewed by Rutala et. al the fluorescent marker was found to be the most useful tool in determining the decontamination of a surface and showed correlation to the microbial contamination levels (Rutala & Weber, 2019). APT measured the level of ATP in organic debris using a relative light unit scale (RLU). (Rutala & Weber, 2019) (Rutala & Weber, 2019) Both the ATP and Fluorescent marker methods are preferred by healthcare facilities as they offer fast and objective monitoring that allow personnel to make immediate corrections to the decontamination process and ensure immediate patient safety (Rutala & Weber, 2016). There is gap in the published literature as to how to perform monitoring testing in order to assess the probability of soil accumulation in the most difficult to clean device feature. Unlike monitoring programs designed for terminal sterilization modalities (e.g., moist heat) (International Organization for Standardization, 2006), cleaning monitoring is most often not used as an indicator that a cleaning process is ineffective, but that a particular device is not clean. Table 2.16 describes relevant published studies that focus on device cleaning verification.

2.10 End-to-End Device Processing Cycle

The typical representation of the device processing cycle only includes the processing steps within a health care facility. However, it is actually far more complicated when one considers the entirety of the responsibility for device processing. Device processing (including ensuring the patient safety from a microbiological quality and sterility assurance perspective) begins with the device manufacturer. The transfer of this responsibility is communicated through the device instructions for use (IFU) to the healthcare facility who takes ownership of the device. However, the ownership may continue to be shared with the manufacture in programs like the loaner set program when devices or instruments sets are transported between facilities where processing steps are carried out. The responsibility becomes more complicated when centralized Sterile Processing Departments are utilized within healthcare systems or external reprocessor. The inventory owner continues to hold the responsibility for the device and manages the performance of the other facilities or companies to execute the device manufacturer's IFU through contracts or procedures. A more accurate representation of the device processing cycle is depicted in Figure 2.11.

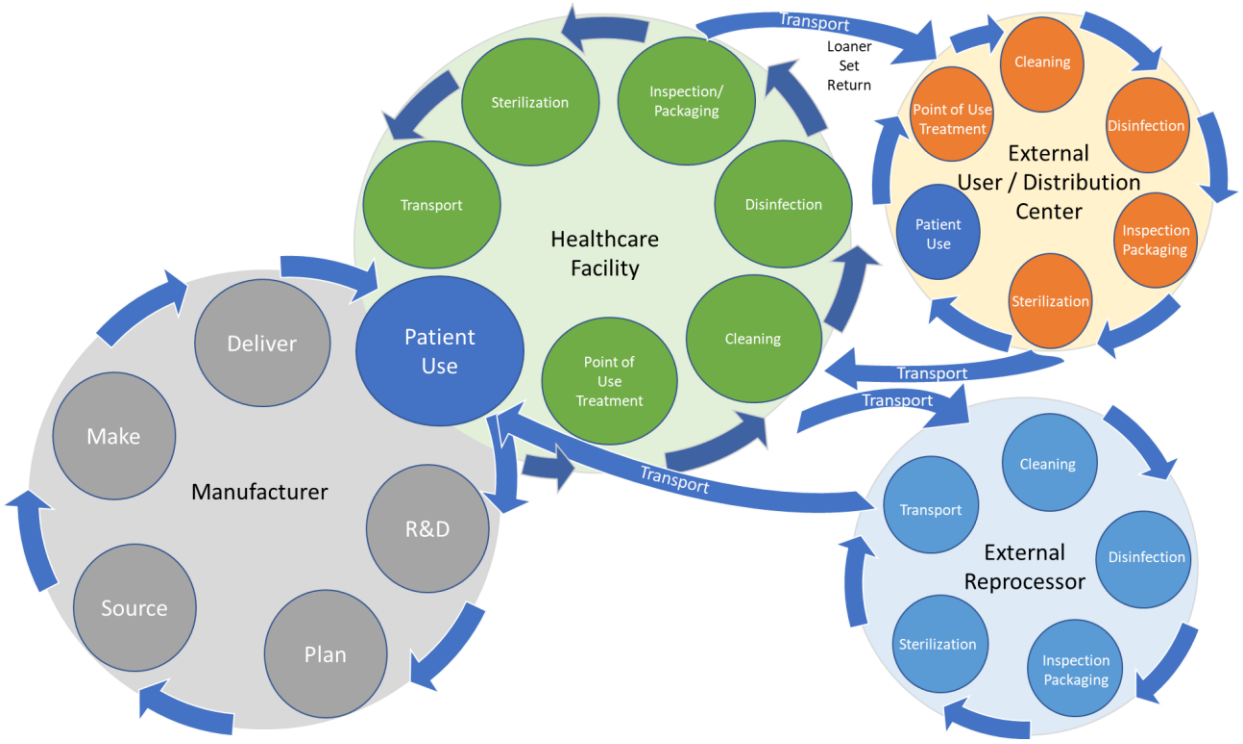


Figure 2.11: End-to-End Device Processing Cycle

As the device processing cycle increases in complexity it is becoming apparent that a paradigm shift is needed to truly focus on end-to-end microbiological quality for a reusable medical device. As described within this chapter, using the Spaulding Classification to establish cleaning processes has not been effective in preventing HAIs. Sterilization / disinfection use an overkill approach to establish requirements for microbial reduction; whereas, cleaning requirements have been established by evaluating the level of analytes after the cleaning process. Applying an overkill approach is not a practical solution for cleaning processes; thus, a new device classification system must be established so as to standardize the cleaning process. This must also ensure patient safety at a healthcare facility, external user / distribution center or external reprocessor.

Based on key developments and gaps in this literature review, the device feature is an important independent variable affecting the cleaning validation process. The research aim of this novel study is to characterize the difficult-to-clean device features and to establish a cleaning classification system that appropriately addresses the relationship between device feature and patient safety.

This new cleaning classification system will allow for transparency between how the manufacturer validated the device instructions for use and how the healthcare system adopts the device into their process, verifies process performance and monitors to ensure patient safety. New novel method(s) by which this new classification system will be established (as addressed in this thesis), will include an understanding of how residual soil on processed devices may affect patient risk including biocompatibility.

This risk can then be used to help inform and establish a new mathematical model to support an appropriate novel device cleaning classification system. Like the Spaulding Classification, this new cleaning classification can be used with the end-to-end device processing cycle to fully connect all the processes; thus, providing full transparency between cycles and reducing the risk of HAIs through standardization.

2.11 Cleaning Classification System

Despite the challenges with the Spaulding Classification for its intended use including microbiological reduction, newer requirements stipulated in standards (such as AAMI ST98) inform cleaning requirements for processed devices. For example, "*For critical and semi-critical medical devices according to the Spaulding classification, cleaning effectiveness shall be determined by visual examination of all components and surfaces in contact with soil, as well as detection of at least two quantitative, clinically relevant analytes using validated analytical method(s). For non-critical devices according to Spaulding classification, cleaning shall be assessed, at a minimum, by visual examination of all components and surfaces that could come in contact with soil. NOTE: Some non-critical can represent a higher level of risk for the presence of residuals below visually detectable levels, and in these cases the qualitative or quantitative detection of analytes can be appropriate*…" (Association for the Advancement of Medical Instrumentation, 2022).

Although this instruction may be appropriate to provide guidance to the medical device manufacturer during the cleaning validation, it is incorrectly assumed that the cleaning instructions for use are being followed exactly as written that presents an ever-present risk.

The overarching research aim of this novel project is to establish a new appropriate cleaning classification system for processed devices that will complement and potentially augment use of the Spaulding sterilization classification system. Such a new cleaning classification system that is evidence-based will inform appropriate and effective cleaning practices across the end-to-end Device Processing Supply chain and will mitigate patient safety risks (as identified in recent critical reflections of the Spaulding Classification.) As comprehensively addressed in this chapter, devices cannot be properly processed delivering the appropriate microbiological quality level if they are not clean. Moreover, by focusing on the devices by way of probability for harboring residual soil based on the complexity of the device feature itself along with detection of chemical analytes for cleanliness, a cleaning classification system can be derived meeting practical and appropriate needs. This approach complements established validation requirements for medical device manufacturers and also informs appropriate processing practices within the healthcare facility including tools to verify compliance and to ensure patient safety for every device processed.

As discussed above, there are a number of challenges facing the processing areas within a Healthcare facility that may increase the probability of non-effective (thorough) cleaning of devices. Point-of-use treatment is a critical step to prevent the drying of soil. If not performed appropriately, then this may

influence cleaning efficacy that includes addressing same in a timely manner. Even with the point-of-use treatment, soil should be prevented from drying if left on the device. Moreover, there is a gap in the literature addressing time and environmental conditions (experiences) for clinically-used medical devices prior to processing that will be addressed in this novel study.

Just as there was a technology boom in medical device innovation over the last 50 years leading to more complex device features, innovation within the supply chain is adding more complexity to an already difficult problem. A paradigm shift is needed to truly mitigate risk in the End-to-End Device Processing supply chain. Futureproofing for the processing of medical devices will be informed and enabled by the seamless convergence and holistic sharing of knowledge in real time, which in so doing, will harmonize each connected phase in the full process. It is appreciated that the processing cycle for reusable medical devices, is typically depicted as a standalone circle or loop encompassing point-of-use treatment, cleaning, disinfection, inspection / packaging, sterilization, storage, transport, and patient use. However, current complexities in device design and processing causes the medical community to work in restricted and unwanted silos promoting knowledge gaps leading to increased risk to patient safety (such as through failures in maintaining qualitative rigor in the end-to-end supply chain for reusable medical devices).

This timely novel research will address a new cleaning classification system that will connect each critical portion of the supply chain informing standardization thus providing a commensurate strategy for appropriately mitigating patient safety. By developing, simplifying and standardizing a new cleaning classification system that appropriately addresses all processing cycles using device feature approach will generate unique data informing future modelling, simulation and automation (such as use of artificial intelligence, machine learning, sensors, robotics, cobots). Additionally, such big data will also inform future Cloud-edge computing for automation. This constitutes the first study that considers key data generated from new cleaning device feature approach for ‘digital’ transformation that will advance medical device processing from a holistic perspective.

2.12 Systematic Literature Review

Systematic literature reviews were conducted to gather research for Chapter 2, covering the period from 2021 to 1956. Initially, a scoping study was undertaken by reviewing the abstracts of papers to identify key areas of interest. Subsequently, systematic searches were performed using the PubMed search engine on specific dates with relevant keywords. On September 29, 2021, the keywords "Device Processing Cycle" yielded 113 articles, of which 29 were deemed relevant. On October 6, 2021, searches for "Physical Cleaning Reusable Medical Device" and "Mechanical Cleaning Reusable Medical Device" resulted in 15 and 22 articles, respectively, with 8 and 9 being relevant. Additionally, a search on October 7, 2021, for "Automated Cleaning Versus Manual" produced 14 articles, 10 of which were relevant. On November 30, 2021, a search for "Loaner Instruments" yielded 20 articles, with 10 being relevant. Furthermore, a review of the bibliographies of relevant articles identified an additional 49 articles or standards pertinent to the chapter's topic. Additionally, during the course of the project from 2021 to 2024, additional relevant resources were added to the literature review where appropriate to ensure a comprehensive understanding of the subject matter.

Chapter 3: Materials & Methods

In Chapter 1, two of the four research objectives necessitated data generation to address the problem statement, with experimental designs commensurately crafted to present the most challenging cleaning scenarios for the test articles. These test variables, meticulously detailed in section 2.3.1, were chosen based on industry standards, available guidance, or preliminary research findings that were subsequently published. When establishing the test variables, if the literature lacked sufficient rationale for variable selection (e.g., soil composition, soil dry time, extraction method), additional experiments were conducted to identify the worst-case test condition. This chapter includes the experimental outline, results, and conclusions of these preliminary experiments to justify the selection of test variables for the experimental design supporting the research aims of this thesis.

Moreover, this chapter not only delineates the experimental design details but also elucidates the rationale behind method selection to meet the novel objectives. The methods chosen were purposefully designed to represent "worst-case" contamination scenarios based on best-published literature and standards; thus, effectively testing the most challenging aspects of cleaning reusable medical devices. The inclusion of these rationales within the chapter is vital for the dissertation's intended utility that serves several stakeholders needs. Strategically, this chapter serves as a bridge, providing explanatory context for stakeholders such as industry end-users (e.g., Johnson & Johnson), facilitating subsequent studies and development of industry standards, such as ISO 17664-3. While unconventional, certain results and discussions from later chapters will be brought forward to underscore the appropriate methodological approaches for addressing this unique opportunity, enhancing clarity and aiding readers' comprehension of the selected methods and their application. Results for each device feature can be found in the applicable appendices. Given the enormity of novel data generated, represented findings are presented in the main body of the thesis where the appendices serve as supporting resource for all data associated with each device feature. This was also done to facilitate readability and continuity; else the thesis would become too unyielding.

The content within this chapter will be separated into 3 inter-related sections.

(i) Variable Selection – This section outlines each constant variable employed to rigorously test the test articles (i.e., device feature representative of a reusable medical device) in each experiment. It will elucidate why these variables are deemed as worst-case scenarios, providing readers with the requisite assurance that the test articles were adequately challenged. Additionally, this information will inform the test design in Annex A of ISO 17664-3, thereby enhancing the quantitative cleaning classification strategy by incorporating additional device features.

(ii) Device Feature Approach Validation – This validation tackled cornerstone 2 outlined in Chapter 1, which aimed "To determine if device features could serve as a crucial novel independent variable in cleaning validations." By validating the device feature approach, it affirmed the theory of utilizing the most challenging-to-clean feature of the device as the foundation for a cleaning

classification. This was a verbose study constituting a requirement of 56,000 flushes to fully extract the 6 coupon types designed to represent the most challenging to clean feature.

(iii) 23 Device Features – The experiments performed on the 23 features systematically challenged each feature to the point of failure, ensuring a uniform comparison. Consequently, the data, 2,695 individual analyte measurements, was leveraged to address cornerstone 4, as delineated in Chapter 1: "To determine if these novel device feature(s) can be applied as an approach for device processing validations to generate data that can be future proofed for artificial intelligence and machine learning to include verification testing in the healthcare setting."

3.1 Variable Selection

Section 2.3.1 delineates the criteria for cleaning validations as per industry standards. The following aspects were addressed within this section to align with these criteria:

- Simulated Use Cycles: Test articles underwent conditioning using a standardized cleaning and sterilization method to replicate a used state.
- Test Article Preparation: Prior to study commencement, each test article underwent thorough cleaning to eliminate all residual analytes.
- Test Soil Selection / Volume / Application / Drying: Preliminary research was conducted to determine the appropriate soil recipe, application method, and drying technique, recognizing their significant impact on cleaning validation outcomes.
- Extraction Eluent / Volume / Container / Method: This section justifies the choice of extraction eluent and outlines the validation process for each extraction method to ensure complete removal and quantification of residual analytes.
- Experimental Controls: Controls were incorporated into each experiment to evaluate the experimental design's validity, an essential component of cleaning validations.
- Cleaning Agent: Given the absence of industry performance requirements for cleaning agents, the selection process for various experiments was discussed.
- Analyte Detection: The application of each analyte detection method was detailed in relation to the experimental designs and when necessary improved for increased precision and accuracy.

3.1.1 Simulated Use Cycles

Prior to validation, the samples and controls were conditioned, so they were in a used state to assess the potential of soil accumulation. Full cycling of the medical device's instructions for use includes the following: soiling, cleaning and/or disinfection, lubrication, sterilization if specified in the instructions for use. Simulated use cycling is used to condition the medical device into a used state and simulate ingress or conditioning of the test soil. For example, if actuation, cauterization, or heating occurs with the device during clinical use, these processes are replicated during the cycling to simulate the most difficult cleaning challenge.

Simulated use cycling replicates a device's used state. This is important for cleaning validations as new devices may not always adequately represent cleaning challenges due to surface changes over time. These changes, like microcracks and alterations, create intricate areas where residues accumulate, posing cleaning difficulties. Unlike new devices with smoother surfaces, used devices demand thorough validation to address residue buildup in these areas. Simulated use cycling mimics clinical conditions, allowing gradual residue accumulation assessment. This proactive approach ensures tailored cleaning validation to tackle specific challenges posed by blood residues, enhancing device reliability and safety in clinical use.

All test articles underwent conditioning through five simulated use cycles, employing the following processing steps:

1. Test articles were soiled using the immersion method with Defibrinated Blood Soil (DBLSO).
2. Test articles were allowed to dry for no less than 1 hr. under ambient conditions.
3. Test articles were rinsed under running cold tap water to remove gross soil. While rinsing, a lint-free cloth dampened with tap water was used to aid in the gross soil removal.
4. Test articles were soaked in a Valsure® Enzymatic detergent solution (Steris, Mentor, OH) prepared at 7.9mL/L of water with a temperature ≤ 40°C for 5 minutes.
5. While immersed, the test articles were blushed with a M-16 nylon bristle brush to thoroughly clean all traces of blood and debris from device surface. One minute was not exceeded.
6. During brushing, joints, handles, and other movable device features were actuated to expose all areas to the detergent solution.
7. All lumens and blind lumens were thoroughly brushed. The brush was pushed through the entire length of the lumen using a twisting motion to remove debris.
8. Test articles were mechanically washed in a washer disinfector with the following parameters:
    a. 2-minute pre-wash with cold tap water
    b. 1-minute wash with Valsure® Enzymatic (Steris, Mentor, OH) 1.9mL/L at a set-point of 43°C
    c. 5-minute wash with Valsure® Neutral Detergent (Steris, Mentor, OH) 1.9mL/L at a set-point of 66°C
    d. 2-minute rinse with hot tap water
    e. 1-minute thermal disinfection with Critical Water at a set point of 43°C
    f. 7-minute dry time with a set point of 115°C
9. Test articles were double wrapped in Halyard H600 sterilization wrap and sterilized in a moist heat sterilization cycle of 4 pulses, 4-minute exposure at 132°C with a 30-minute dry.
10. Following the five simulated use cycles, the test articles were cycled for a sixth time but following the manual cleaning process, the test articles were extracted and measured for residual protein and TOC residuals.

11. The test articles were cycled for a seventh time using the complete cleaning process, including mechanical cleaning but not sterilization. Protein and TOC analytes were again measured for the mechanical cleaning after an extraction.

Testing for all features resulted in values below 6.4µg/cm^2 for protein and 12 µg/cm^2 for TOC. These results are indicative that cleaning is effective and soil accumulation after wear on the feature presents negligible risk.

3.1.2 Test Article Preparation

Prior to each experiment, the test article (i.e., device feature) was cleaned to thoroughly remove residual analytes. The test articles were rinsed under RO/DI water for 1 minute and brushed until all visible soil was removed. The test article was placed in a container for an alkaline NeoDisher® MediClean forte (Dr. Weigert) cleaning agent soak prepared at a concentration of 10mL/L for 60 minutes. While soaking, the test articles were brushed until visibly clean, and the test article was actuated. Using a freshly prepared cleaning agent, the test articles were submersed in container until completely covered and sonicated for 15 minutes in a Branson 8800 Ultrasonic Cleaner or equivalent at 40kHz. Each test article was rinsed under running Critical Water (i.e., DI water). Lumens were rinsed by flushing lumen with 20mL of Critical Water 3 times using a 25mL syringe with 20G needle. The test articles were allowed to dry before being inspected for cleanliness using a borescope or 10X magnification.

3.1.2 Surface Area Calculation

The surface area is a critical measurement to evaluate the cleaning efficacy of the device. It is used to evaluate cleaning residuals against established acceptance criteria using the units of analyte µg/cm^2. The surface area is also critical to establishing the appropriate validation variables of test soil volume and extraction fluid volume. Whenever possible, the surface area of the device was calculated using the design drawing software (CAD).

3.1.3 Test Soil Selection

The ingredients within the selected test soil allow for the quantitative measurement of cleaning efficacy and provide reproducible results during cleaning efficacy studies. The devices within this study were challenged under testing conditions that represent the worst-case clinical use of medical devices. Therefore, it was assumed that the device contacts blood and other patient tissues during orthopedic, spinal or neurosurgery procedures. These surgery types present the most risk to the patient due to the possibility of transfer of residual soil and associated microorganisms that may not be inactivated during processing into the blood stream or spinal fluid. At the time of study design, insufficient information was available concerning test soil selection, so preliminary research was completed and subsequently published to justify the use of Modified Coagulated Blood Soil.

The relationship between device material, test soil and application method was investigated by testing 140 variable combinations including seven materials: (stainless steel, delrin, peek, nitinol, aluminum, titanium, and silicone), four test soils (defibrinated blood soil, coagulated blood, modified coagulated

blood and Miles test soil), and four soil application methods (pipetting, painting, handling with soiled gloves and immersion). The data collected using solubility testing indicates there may be a complex relationship for material adherence between device materials and test soil.

Prior to testing, the coupons were cleaned by soaking in a 4% v/v CIP100 cleaning agent (STERIS, Basingstoke, United Kingdom) for 60 minutes and brushed to remove all residual soil. Coupons were rinsed under running Critical Water and allowed to dry completely before weighing using an analytical balance. Coupons were soiled using the prescribed application method. A volume of 0.15mL of test soil was applied gravimetrically to the top surface of the coupon. Coupons were soiled and allowed to dry at 22°C ± 3°C / 50% RH for 19 hours. These conditions were selected to represent drying conditions that would be an appropriate solubility challenge [15]. A post dry coupon weight was taken.

A solubility test was performed using a gravimetric analysis on the soiled coupons and included placing soiled samples into preheated, 45°C/113°F extraction bag (Whirl-Pak) filled with 150mL of Critical Water as the extraction fluid, so they were completely submerged. Polypropylene extraction bags with samples were warmed in a water bath at 45°C/113°F for 60 minutes. Physical action has been shown to be effective for soil removal, so agitation of the samples (e.g., shaking or sonication) was not included in this experiment so to appropriately challenge the soil adhesion on the material surface. Samples were then removed from the extraction bags slowly so as not to shear the soil from the sample surface. The sample was placed on a polypropylene drying sheet and allowed to dry completely before a post extraction weight was taken. Using the weights of the pre-soiled coupon, post-soiled coupon and post extraction coupon, the percent soil remaining was calculated.

Modified Coagulated Blood Soil was established as the most difficult to remove test soil without overchallenging the test system by Kremer et. al (Kremer, et al., 2021). Modified Coagulated Blood Soil contains both water soluble protein complexes as well as water insoluble protein complexes, i.e., fibrin from the coagulated blood. Fibrinogen is a 45 nm-long and made up of 6 paired polypeptide chains $(A\alpha B\beta\gamma)_2$ held together by 29 disulfide bonds (Weisel & Litvinov, 2013). In instances where modified coagulated blood presented excessive difficulty for the feature, defibrinated blood soil (DBLSO) was employed as an alternative to emulate a less demanding cleaning challenge. The choice and application of test soils are vital factors in cleaning studies. The paper, *Material Adhesion Comparison of Test Soils for Reusable Device Cleaning* Efficacy by (Kremer, et al., 2021), compared four widely recognized test soils. The research highlights the importance of patient exposure and device use in selecting relevant test soils that provide a worst-case challenge for cleaning processes. The study found acceptable variability in all tested soils, except for Edinburgh soil, which exhibited a higher variation (Table 3.1). The impact of soil chemistry, drying, and heat application on soil properties is emphasized. Biomarker evaluation revealed that DBLSO and modified coagulated blood present appropriate challenges for all device types, while coagulated blood may be suitable for surgical devices but not orthopedic devices. The study emphasizes the need for clinically relevant test soils with appropriate viscosity, adhesion, analyte concentrations,

solubility, and reproducibility, concluding that DBLSO is an effective test soil for challenging surgical devices.

Table 3.1: Average percent soil remaining post solubility test (DBLSO = defibrinated blood soil, Coag = coagulated blood, Mod = modified coagulated blood, Mil= miles)

Coupon	Soil	Pipetting Neat	Pipetting Spreader	Paint Brush	Handling w/ Soiled Gloves	Immersion
Stainless Steel	DBLSO	1.5% ± 1%	1.7% ± 0%	3.5% ± 1%	2.1% ± 1%	1.5% ± 1%
	Coag	0% ± 1%	0% ± 1%	0% ± 2%	0% ± 1%	0% ± 3%
	Mod	2.3% ± 1%	2.9% ± 1%	3.7% ± 2%	2.6% ± 1%	2.2% ± 3%
	Mil	0% ± 0%	0% ± 1%	0% ± 0%	0% ± 0%	0% ± 0%
Nitinol	DBLSO	1.7% ± 1%	1.0% ± 1%	1.1% ± 1%	0.7% ± 0%	3.1% ± 2%
	Coag	2.4% ± 2%	5.5% ± 4%	2.7% ± 2%	0.6% ± 1%	1.8% ± 1%
	Mod	29.2% ± 8%	23.1% ± 2%	36.8% ± 3%	28.7% ± 15%	7.3% ± 4%
	Mil	0.2% ± 0%	0.2% ± 0%	0.2% ± 0%	0.1% ± 0%	0.1% ± 0%
Aluminum	DBLSO	0.5% ± 2%	0.8% ± 2%	1.4% ± 1%	0.5% ± 2%	0% ± 18%
	Coag	4.6% ± 2%	4.8% ± 2%	3.5% ± 1%	1.9% ± 0%	4.0% ± 2%
	Mod	31.1% ± 7%	26.0% ± 12%	23.9% ± 10%	19.5% ± 13%	16.4% ± 4%
	Mil	0.3% ± 0%	0.3% ± 0%	0.2% ± 0%	0.3% ± 0%	0.1% ± 0%
Titanium	DBLSO	0.7% ± 1%	2.0% ± 3%	0.8% ± 1%	0% ± 1%	4.2% ± 3%
	Coag	3.9% ± 1%	3.3% ± 2%	2.4% ± 2%	3.7% ± 2%	2.8% ± 2%
	Mod	0.3% ± 0%	0.8% ± 1%	1.0% ± 1%	8.4% ± 6%	3.8% ± 3%
	Mil	0.5% ± 0%	0% ± 2%	0.3% ± 0%	0.7% ± 0%	0.4% ± 0%
Delrin	DBLSO	7.3% ± 1%	8.3% ± 0%	8.3% ± 1%	7.1% ± 1%	5.6% ± 1%
	Coag	10.8% ± 2%	12.1% ± 2%	10.1% ± 1%	9.8% ± 1%	9.3% ± 1%
	Mod	14.0% ± 13%	12.2% ± 2%	5.9% ± 1%	6.1% ± 1%	5.8% ± 1%
	Mil	0% ± 0%	0% ± 2%	0% ± 0%	0% ± 1%	0% ± 0%
Peek	DBLSO	3.9% ± 0%	3.7% ± 1%	4.5% ± 1%	4.1% ± 0%	3.4% ± 1%
	Coag	7.7% ± 0%	8.3% ± 2%	5.6% ± 2%	6.0% ± 2%	6.6% ± 2%
	Mod	5.2% ± 1%	4.4% ± 2%	8.6% ± 3%	0% ± 14%	3.1% ± 2%
	Mil	0.2% ± 0%	0.1% ± 0%	0.2% ± 0%	0.1% ± 0%	0.1% ± 0%
Silicone	DBLSO	4.7% ± 0%	17.8% ± 1%	23.3% ± 4%	15.4% ± 3%	15.4% ± 10%
	Coag	67.0% ± 11%	58.5% ± 8%	54.5% ± 18%	51.2% ± 4%	42.5% ± 13%
	Mod	43.5% ± 26%	74.4% ± 6%	90.6% ± 18%	91.7% ± 2%	49.2% ± 6%
	Mil	0% ± 10%	0% ± 19%	0% ± 9%	0% ± 14%	0% ± 2%

The International Organization for Standardization (ISO) has recently revised the guidelines for testing soils in washer-disinfectors, emphasizing quantitative performance attributes over specific formulations. The paper, *A Standardized Method for Evaluating Test Soils used to Demonstrate Cleaning Efficacy* by Kremer et. al (Kremer, et al., 2022), details the quantitative assessment of test soils, including UK Test Soil,

Defibrinated Blood Soil (DBLSO), Blood Test Soil (BTS), Artificial Test Soil (ATS 2015), Modified Coagulated Blood Soil, Two Component Blood Test Soil, and Coagulated Blood, as outlined in ISO 15883-5:2020 Annex A. The preparation involved applying these soils to stainless steel test pieces and evaluating them using the Protein-based test soil performance assessment method in Annex B of ISO 15883-5:2020, employing UV-Vis spectrophotometry.

Test soils were applied onto standardized test pieces (STPs) (n=2) for each test condition made from 0.127mm thick annealed 316L stainless steel foil cut into 50mm^2 test pieces. STPs were pre-cleaned by submersing in a Decon 90® (Decon, East Sussex, England) detergent solution heated to 45-50°C for 10 minutes with mild rocking agitation, followed by rinsing twice with purified water and once with isopropanol to facilitate drying. Approximately 5mL of fresh test soil was applied to the STP and spread using a No. 5 Mayer rod to provide an even thickness. They were then dried for a minimum of 2 hours but not longer than 4 hours at ambient temperature (~25°C) and relative humidity (40-60%). The weight of the STPs post soiling was recorded using an analytical balance (defined as wt. 1).

Testing for soil removal was conducted in 400mL glass beakers containing a 25mm x 6mm magnetic stirring bar with a retort stand and clamp device designed to hold a PVC suction cap and placed on a magnetic stirrer hotplate (see ISO 15883-5 Annex B for diagram). The coated STP was attached to the suction cap and submersed in 100mL purified water, without touching the rotating (50rpm) stir bar. Testing was performed at 25°C ± 2°C and 75°C ± 2°C under 30 second and 90 second time intervals. Following immersion time, STPs were removed, and the water test solution was gently shaken off.

STPs were then extracted for protein residual analysis in triplicate by placing into a 500mL beaker filled with 10mL 1% sodium dodecyl sulfate and 2.0g of 200 – 400-m glass beads (Sigma-Aldrich, St. Louis, MO). The beaker was covered and agitated for 30 minutes on an orbital shaker at ambient temperature (~25°C). The STP was removed and dried before being weighed for gravimetric analysis (defined as wt. 2). The elution solution was transferred to a vial for protein concentration determination. Positive controls (2 STPs soiled and not exposed to test conditions but extracted) and negative controls (2 STPs not soiled but exposed to test conditions and extractions) were also prepared.

All extracts were analyzed for protein residuals using the ortho-phthalaldehyde (OPA) method and spectrophotometric analysis at 340nm in triplicate. Bovine Serum Albumin-Fraction V (BSA) (Sigma-Aldrich, St. Louis, MO) was used to generate a calibration curve ranging from 2.5 µg/mL to 800 µg/mL using 1% SDS as the diluent beginning with a stock solution of 1000 µg/mL. As controls, the absorbance of two reagent controls (A & B) were used to normalize the results from the curve points. Reagent A - included equal volumes of 1% SDS and SDS/borate solution and reagent B - with equal volumes of 1% SDS solution and the 0.16% w/v OPA reagent. The average normalized BSA concentrations (µg/mL) were plotted against the corrected normalized mean and used to calculate the slope (x) and graph intercept (c) for the calibration curve.

Each STP extract and controls were analyzed against the normalized BSA calibration curve. Each extract was mixed in the quartz cuvette with equal parts of the 0.16% w/v OPA reagent and Reagent A. The positive control was diluted 1:5 with 1% SDS solution to allow for an absorbance reading within the prepared curve. Each result was corrected for the normalized mean ($^{N}E_{n}$) using the same method as the calibration curve. The amount of protein (mg) was calculated using the following equation (($^{N}E_{n}$ – c)/x) ÷ 50 (International Organization for Standardization, 2021).

The STP coat weight was calculated gravimetrically (wt. 1 – wt. 2) and the fractional protein content per soil weight was calculated by dividing the mean positive control protein content by the mean positive control weight.

The percent soil remaining was then calculated using the following equations:

$$Theoretical\ Calculated\ Protein\ Content\ (mg) = STP\ coat\ weight\ x\ \frac{fractional\ protein\ content}{soil\ weight}$$

$$\%\ Protein\ Remaining = \frac{Protein}{Theoretical\ Calculated\ Protein\ Conent}\ x\ 100$$

The study demonstrates that all protein-based test soils meet the acceptance criteria in ISO 15883-5, confirming their validation. These test soils, exhibit satisfactory performance compared to historical test soils, adhering to the more rigorous standards introduced in ISO 15883-5:2021, thus ensuring their continued effectiveness (Table 3.2).

Table 3.2: Test soil performance results compared to ISO 15883-5 Annex B criteria

Mean % Soil Remaining					
Soil	**Condition 1 30 sec/25°C**	**Condition 2 90 sec/25°C**	**Condition 3 30 sec/75°C**	**Condition 4 90 sec/75°C**	**Pass/Fail**
Pass Criteria	≥12%	< condition 1 and ≥2%	≥ 12%	< condition 3 and ≥ 6%	
Artificial Test Soil (ATS 2015)	95.9%	77.3%	47.4%	23.7%	Pass
Blood Test Soil (BTS)	75.1%	53.1%	76.3%	66.8%	Pass
Coagulated Blood	20.2%	4.1%	18.4%	15.2%	Pass
Defibrinated Blood Soil (DBLSO)	77.2%	70.1%	76.4%	73.3%	Pass
Modified Coagulated Blood Soil	35.1%	30.2%	41.7%	35.6%	Pass
Two Component Blood Test Soil	28.0%	11.9%	70.6%	68.0%	Pass
UK Test Soil (Edinburgh)	70.4%	69.4%	70.0%	67.6%	Pass

3.1.4 Soil Volume

The amount of test soil used was demonstrated to be an appropriate challenge but also be representative of soil levels following clinical use (e.g., challenge protein concentration should be equivalent to clinical protein concentration levels and quantifiable via a validated protein residual test method). For surgical devices the following analyte levels are representative of clinical use (Cloutman-Green, et al., 2015):

- Protein - 244µg/cm^2
- Total Organic Carbon – 52µg/cm^2

Modified coagulated blood has an average protein concentration of 108,747 µg/mL. This value was determined by diluting the soil into to the concentration range of the protein BCA assay calibration curve and using the instrument value to extrapolate back to the original undiluted value. The average was calculated using a sample size of 10 instrument readings.

The following equation was used to calculate the minimum amount of soil that should be applied to a device:

$$Soil\ volume\ (mL) = \frac{protein\ analyte\ level\ (\frac{\mu g}{cm^2})\ x\ feature\ surface\ area\ (cm^2)}{soil\ protein\ concentration\ (\frac{\mu g}{mL})}$$

Inserting the constant values for the protein analyte level and soil protein concentration delivers the following equation where the surface area of the device is the only variable for the minimum soil volume.

$$Soil\ volume\ (mL) = \frac{244\ \frac{\mu g}{cm^2}\ x\ feature\ surface\ area\ (cm^2)}{108{,}747\ \frac{\mu g}{mL}}$$

3.1.5 Test Soil / Application

As described in ASTM F3293-18, Standard Guide for Application of Test Soils for the Validation of Cleaning Methods for Reusable Medical Devices, (ASTM International, 2018) there are a number of acceptable methods for applying the test soil to a device. Selection of the best application method has historically been the responsibility of the manufacturer to justify the soiling method to appropriately challenge the device in a manner that is representative of worst-case clinical use. Due to a gap in the literature comparing soiling application methods, an experiment was executed to determine the most challenging soil application method.

In the paper, *Test Soil Application Affinity for Reusable Device Cleaning Validations* by Kremer et. al (Kremer & Ratanski, 2023), the immersion method was demonstrated to be the most challenging soil application method (Table 3.1). With the other application methods, the desired volume or weight was confirmed during the delivery either by volume or by weight. With the immersion method, the coupon was submersed in the test soil face down and lifted out and weighed. Immersion left the coupon with an

even coating upon initial application, but the process of inverting the coupon for weighing and drying allowed the soil to migrate. However, even with migration, the soil was distributed in a way that did not result in cracking or flaking. For lumened devices and those with features that prevented soil penetration using immersion, direct pipetting was utilized to ensure the soil was deposited in the most challenging features of the device.

The articulation of the device during the soil application allowed for the migration of the soil into the complex features of the device. For example, when soiling a hemostatic clamp, articulating the box hinge by opening and closing the device allows for soil to migrate into the mated surfaces with small clearances that form when the device is in the closed position. The specifics of device articulation is dependent on the intended clinical use of the device and as appropriate was included in the test design for each device feature.

Depending on the experiment, either modified coagulated blood or DBLSO was prepared:

- Modified coagulated blood - 100 mL fresh egg yolk, 0.1 mL heparin, 100 mL sheep blood (Rockland Immunochemicals), and 2 g dehydrated hog mucin (Sigma Aldrich) were mixed using a blender. Soil was stored at 4–8°C and brought to room temperature prior to coagulation. The mixture was poured into a bowl, and 0.15 mL protamine sulphate (Thermo Scientific) was added to each 10 mL blood and mixed well using a magnetic stir bar. Soil was applied immediately before coagulation occurred.
- Defibrinated blood soil (DBLSO) - 100 mL fresh egg yolk, 100 mL defibrinated sheep blood (Rockland Immunochemicals), and 2 g dehydrated hog mucin (Sigma Aldrich, Burlington, MA) were mixed using a blender.

A polypropylene drying sheet was prepared with numbered sections to correspond to each test article. Each device feature was initially weighed using an analytical balance for the pre-soiling weight. Soil was deposited in lumens using a pipettor with 0.20mL (200µL) of soil using a pipettor, so soil was deposited through lumen when applicable, otherwise, the test article was immersed in 100mL of soil. The test article was rotated to coat all surfaces with soil. If applicable to the feature, the sample was actuated during the soiling process. The actions were repeated until the minimum applied soil volume was achieved and verified by weight. Test articles were weighed to verify minimum soil volume has been applied (Table 3.3 Device Feature Validation and Table 3.4 for 23 Features). Soiled test articles were placed on a polypropylene surface and allowed to dry as applicable to the experiment.

Table 3.3: Test soil volume per coupon type in device feature validation experiment

Lumen Depth	Feature Only Surface Area (cm^2)	Minimum Soil Volume Calculated (mL)	Minimum Applied Soil Volume (mL)
20mm	1.257	2.82	3.00
30mm	1.885	4.23	5.00
40mm	2.513	5.64	6.00

Table 3.4: Test soil volume per device feature in 23 Device Features Experiment

Device Feature	Feature Only Surface Area (cm^2)	Minimum Soil Volume Calculated (mL)	Minimum Applied Soil Volume (mL)
Ball Detent / Ball Bearing	163.87	0.36	0.40
Ball Seal Springs	6.31	0.014	0.02
Blind Slot	89.80	0.20	0.25
Button w/ Spring	37.15	0.08	0.10
Buttons - Exposed Springs	82.31	0.18	0.20
Captured Screw	62.88	0.14	0.15
Hinges, Joints, Pivot Points	9.93	0.02	0.03
Leaf Springs	73.85	0.16	0.20
Mated Surfaces	215.35	0.48	0.50
Mated Surfaces Small Clearance	211.63	0.47	0.50
O-rings - External O-ring	13.53	0.03	0.04
O-rings - Internal O-ring	18.48	0.04	0.05
Rough Surface	60.22	0.135	0.15
Screws Threaded Rod / Threaded Thru Hole	52.42	0.12	0.20
Screws Threaded Rod/ Threaded Blind Hole	67.72	0.15	0.20
Sliding Shaft Short	21.10	0.05	0.10
Sliding Shafts Long	72.13	0.16	0.20
Smooth Blind Lumen	4.57	0.01	0.02
Smooth Through Lumen	54.36	0.122	0.25
Snap Rings	10.6	0.02	0.03
Spring Internal	151.78	0.34	0.34
Threads Blind Hole	31.71	0.07	0.10
Through Slot	95.33	0.214	0.25

3.1.6 Soil Conditioning / Drying

Guidance provided to health care facilities highlight that the amount of time between use and decontamination should be minimized to prevent soil from drying. Little information was available in literature at the time of this project's initiation but did suggest that drying of soil increased the challenge to cleaning. To understand the impact of time, temperature, and humidity on the soil solubility rate experimentation was completed and subsequently published in the article, *Effects of Time, Temperature, and Humidity on Soil Drying on Medical Devices* by Kremer et. al (Kremer, et al., 2023). This research demonstrated a statistically significance (coupon p_{value} = 0.041 and surrogate p_{value} = 0.066) difference after 8 hours with the most soil retention at the 72-hour dry time point (Figures 3.1, 3.2, and 3.3). The optimal temperature and humidity for soil drying was demonstrated to be 22°C and 50% relative humidity (RH).

Within each experiment, a stainless-steel test coupon with the dimensions of 25mm x 50mm x 6mm and surface area of 34cm^2 was used to evaluate material adsorption, while a hemostatic clamp (VWR #10807-438) with a surface area of 159cm^2 was tested to evaluate the impact of device features as a surrogate device. Prior to testing, the coupons and surrogate device samples were cleaned by soaking in a cleaning agent (Steris CIP100) for 60 minutes and brushing to remove all residual soil. Coupons were rinsed under running critical water and allowed to dry completely before a pre-soiling weight was taken.

The test soil, Modified Coagulated Blood Soil, contained constituents equivalent to the worst-case clinical exposure and was prepared by blending 100mL of fresh egg yolk (Eggland's Best), 100mL of heparinized sheep blood (Rockland) and 2g dehydrated hog mucin (Sigma-Aldrich). To reverse the anticoagulant, 0.05mg of protamine sulphate (Thermo Scientific) was added to each 5mL of soil, mixed and used in the test system within 10 minutes.

The average protein concentration of Modified Coagulated Blood was determined to be approximately 560,214µg/mL, using the BCA Protein Residual Test, and the maximum protein found on highest soiled devices was determined by Cloutman-Green et. al to be 117,758µg/cm^2 (Cloutman-Green, et al., 2015). The appropriate soil volume calculated for the coupon was 82,042µg, delivered gravimetrically as 0.22g. Due to the complexity of the surrogate device, the soil was applied evenly over one side but not delivered gravimetrically. For both the coupon and surrogate device samples, weights were taken post soiling to ensure all samples received an equivalent soil amount. The soil was applied using a foam-tipped paint brush with the necessary coats to reach the desired soil weight.

A solubility test was performed using a gravimetric analysis for three of the four experiments. Solubility included placing soiled samples into a preheated, 45°C/113°F extraction bag (Whirl-Pak) filled with the appropriate volume of extraction fluid [150mL of Critical Water for the coupons and 500mL of Critical Water for the surrogate devices, so they were completely submerged. Extraction bags with samples were warmed in a water bath at 45°C/113°F for 60 minutes. Samples were then removed from the extraction bags slowly to avoid shearing soil from the sample surface. The sample was placed on a polypropylene drying sheet and allowed to dry completely before a post-extraction weight was taken.

Visibly Dry

Coupons and surrogate devices (n=25) were soiled and allowed to dry at ambient laboratory conditions (~22°C/71.6°F, 50%RH). At the following time points, 0.5hr, 1 hour, 2 hours, 3 hours, 4 hours, 6 hours, 19 hours, 24 hours, and 36 hours, samples were weighed using an analytical balance (Mettler Toledo) capable of measuring to 0.0001g.

How Time Affects Soil Solubility

For each time point tested (1 hour, 2 hours, 4 hours, 6 hours, 19 hours, 24 hours, 48 hours, and 72 hours), a sample size of n=12 for both coupons and surrogate devices was soiled and allowed to dry in an

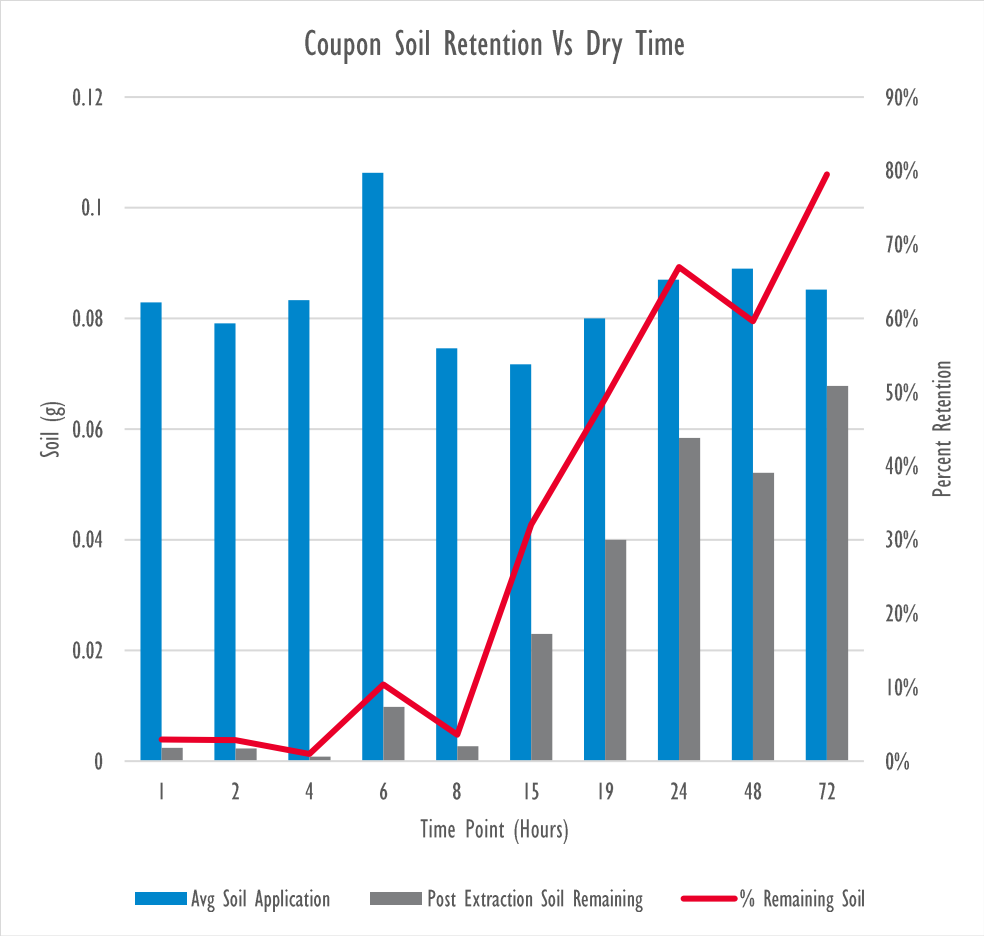
environmental chamber (Thermo Scientific) set to 22°C/71.6°F, 50%RH. After drying for the allotted time, samples were tested for solubility.

How Temperature Affects Soil Solubility

A sample size of n=25 for each temperature point (4°C/39.2°F, 11°C/51.8°F, 22°C/71.6°F, 35°C/ 95°F, 45°C/113°F and 55°C/131°F) was soiled and dried for 24 hours at the specified temperature and 50% relative humidity (RH) in an environmental chamber (Thermo Scientific). After drying for the allotted time, samples were tested for solubility.

How Humidity Affects Soil Solubility

A sample size of n=25 for each humidity point (30%RH, 50%RH, 80%RH and 100%RH) was soiled and dried for 24 hours at 45°C/113°F with the specified RH in an environmental chamber (Thermo Scientific). After drying for the allotted time, samples were tested for solubility.



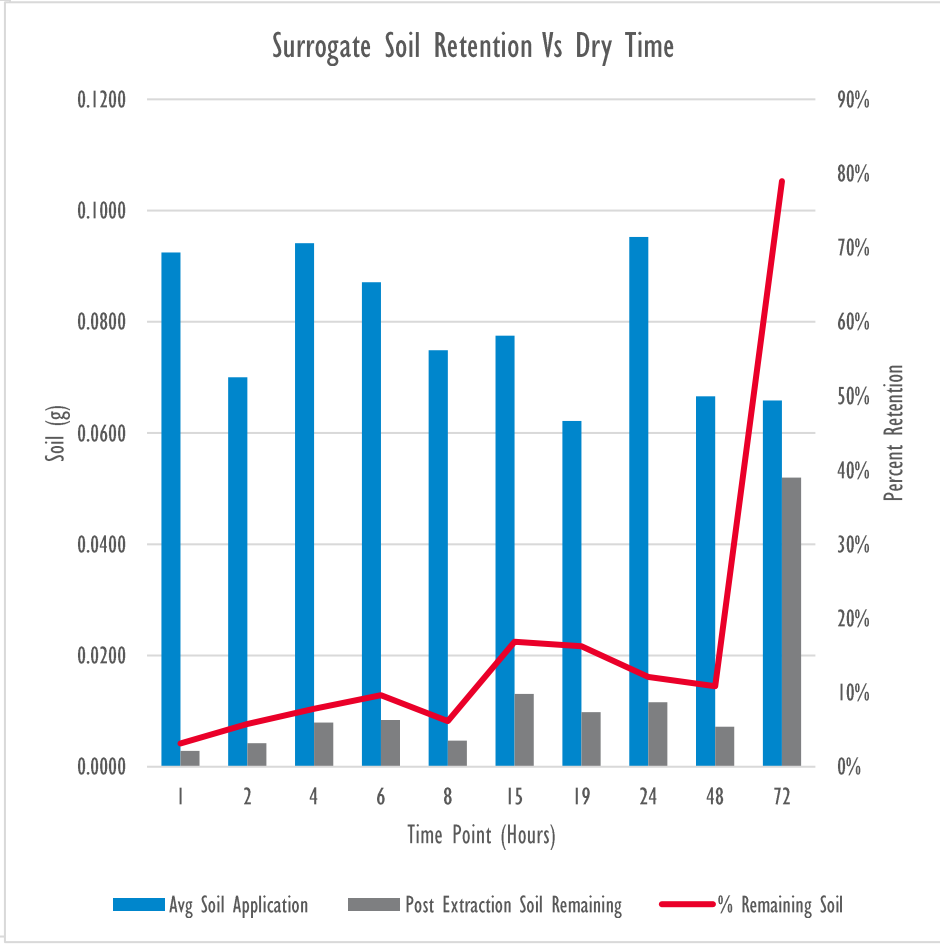


Figure 3.1: Results for Time Impact on Soil Solubility

With both the surrogate devices and coupons, the percentage of soil retention increased with time, but it was not significantly statistically different until the 8–hour time point when compared to the 1–hour time point using an ANOVA analysis (coupon p_{value} = 0.041 and surrogate p_{value} = 0.066). The soil retention continued to increase over time but more slowly with the surrogate device. The most soil retention was observed at the 72–hour dry time point.

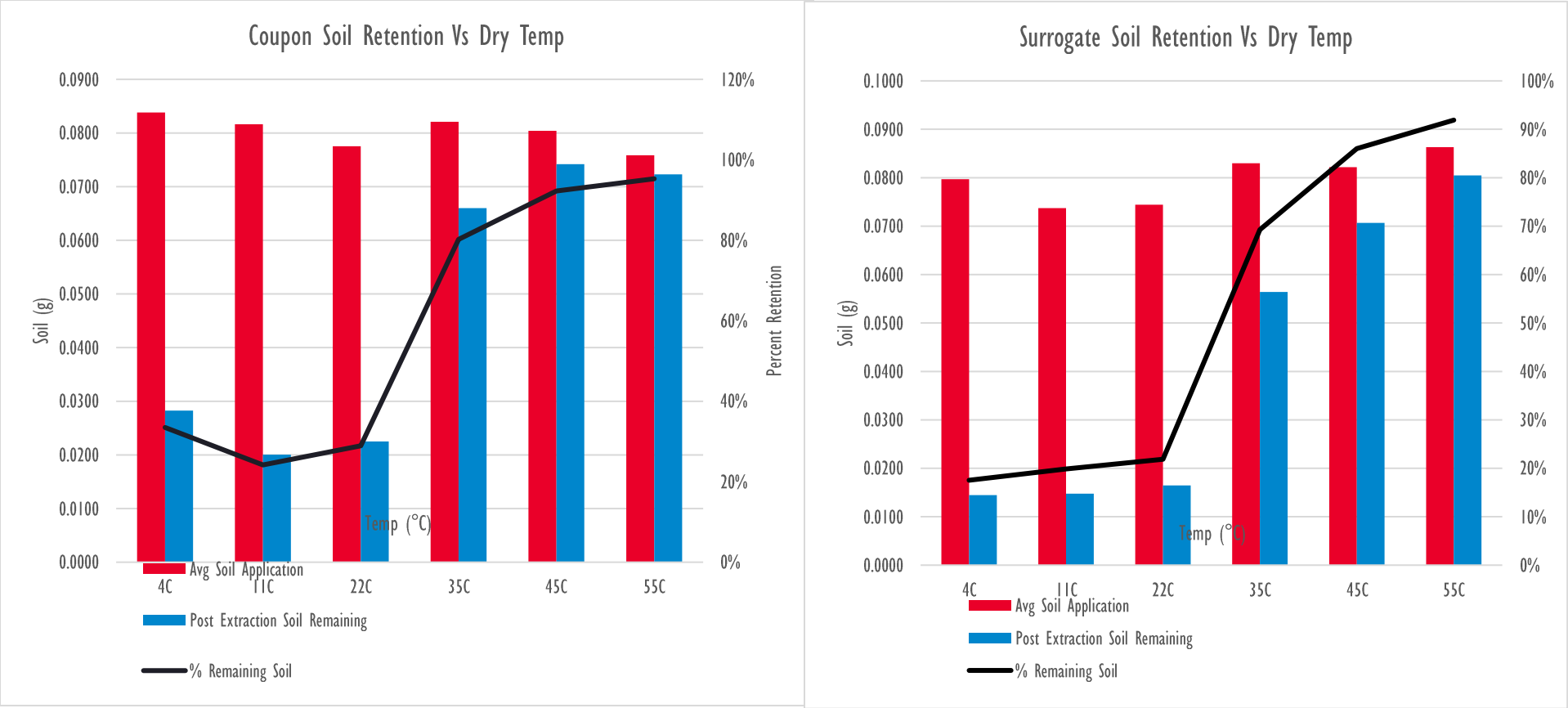


Figure 3.2: Results for Temperature Impact on Soil Solubility

Using coupons, the results show that with increased temperatures, the time required for the solubility rate to shift decreases. Results for the impact of temperature on drying showed a positive correlation after the 22°C temperature between increased temperature and soil retention. An ANOVA analysis of the average soil retention data showed that for the 4°C, 11°C, and 22°C temperatures were statistically similar in soil retention (coupon p_{value} of 0.215 and surrogate p_{value} of 0.214). The results for temperatures higher than 22°C had a p-value of 0 demonstrating no statistical similarity (22°C compared to higher temperatures). This demonstrated that as temperatures increased, soil retention increased, and soil solubility decreased.

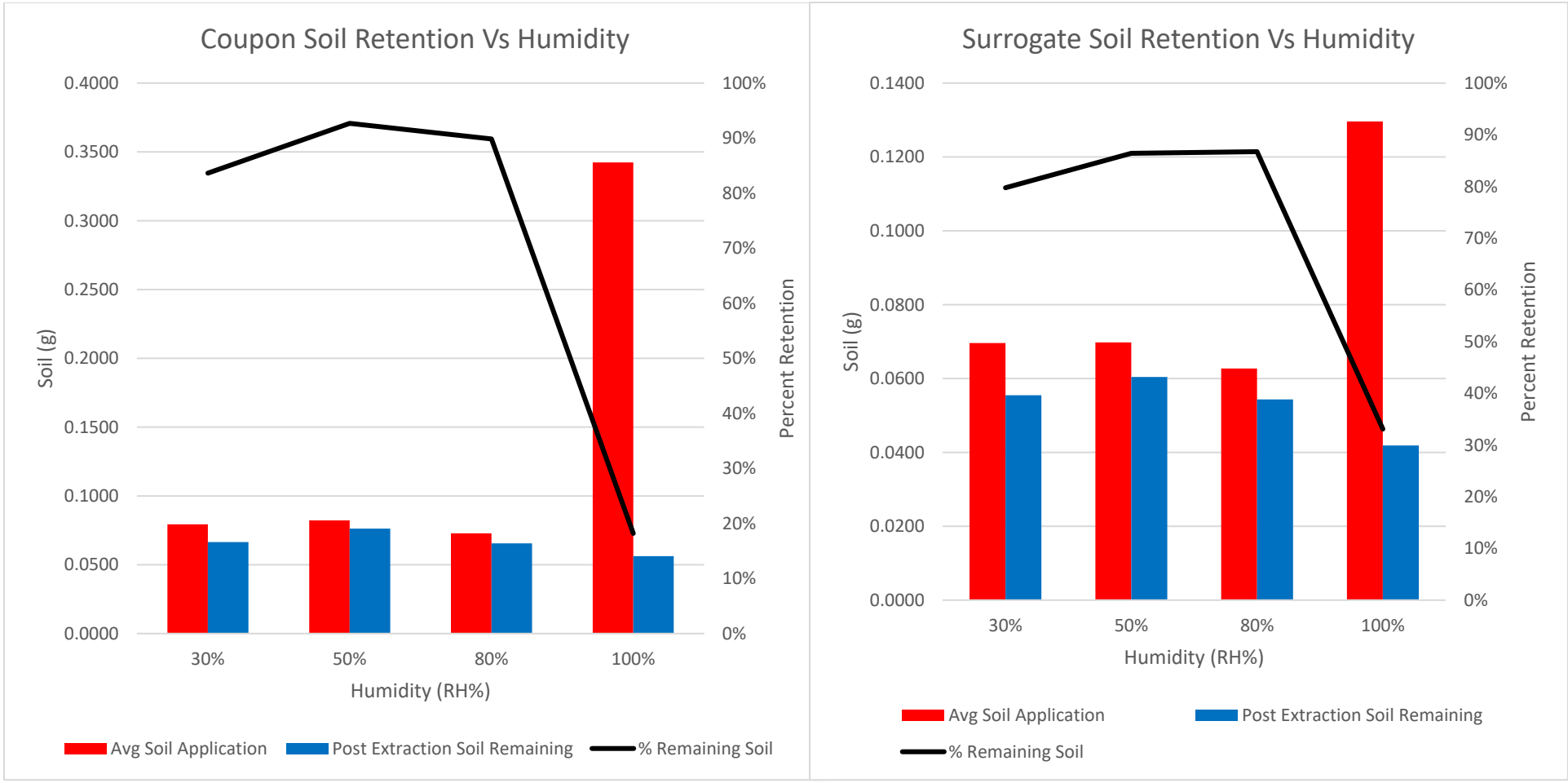


Figure 3.3: Results for Humidity Impact on Soil Solubility

In the humidity experiment, soiled devices exposed to 100% humidity did not visibly dry and, therefore, did not present the same challenge as the other humidity conditions. The humidity points of 30%, 50% and 80% RH were statistically different (p_{value} of 0 compared to 100% RH), with 50% RH showing the most soil retention for both the coupon and surrogate device.

Successive to the drying condition experiment, an additional study was executed and subsequently published to gain a deeper understanding of the protein chemistry changes occurring during the drying process in the paper, *Chemical Changes Over Time Associated with Protein Drying* by Kimble et. al (Kimble, et al., 2023). The albumin matrix creates a water insoluble barrier that may present an increased cleaning challenge to remove residual proteins. As albumin is a primary protein in clinical soils the risk of allowing soil to dry following clinical use should be mitigated. For re-solubilization following drying, water (or the cleaning solution) must integrate into the spaces between the molecules and aggregates to lessen the attractive and cohesive forces holding them together. As water is removed by drying, polarity changes resulting from tertiary structural changes in the proteins can reduce the wetting effectiveness of water and make rehydration and re-solubilization more difficult. Introducing water into a dried proteinaceous soil initially leads to wetting, followed by swelling of the proteins and aggregates followed by dissolution facilitating their removal (Figure 3.4). However, water alone may not be sufficient to facilitate removal of dried protein and other bodily soils (Kimble, et al., 2023).

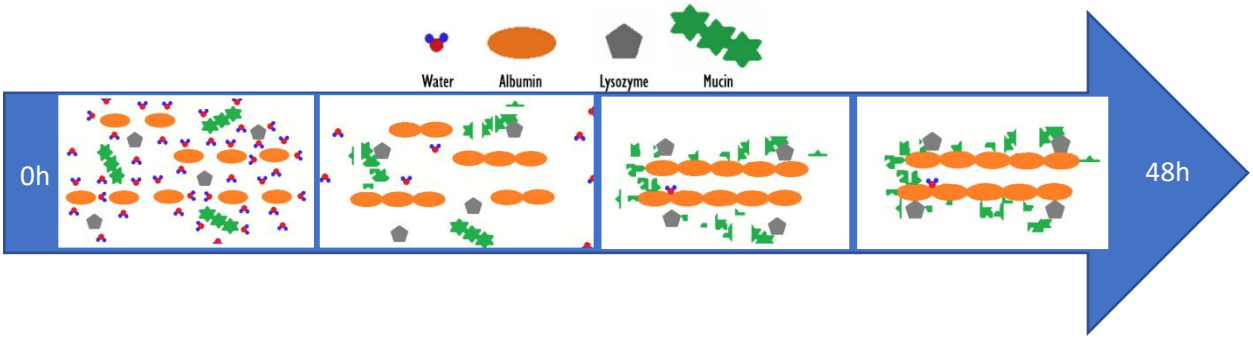


Figure 3.4: Illustration of soil component interactions and changes with increased drying time

3.1.7 Extraction Eluent Preparation

Purified water (TOC < 50ppb), produced by a ELGA filtration system or purchased ACS grade water (Ricca, Arlington, TX), was used as the extraction eluent for water soluble analytes (i.e., TOC, Protein). 2% Alkaline Sodium Dodecyl Sulfate (SDS), pH 10, was used as an extraction eluent for the water insoluble proteins. The Alkaline SDS solution is a more aggressive extraction eluent and is selected if devices are exposed to heat or extended dry times after soiling. SDS is only compatible with the BCA protein assay, so it was not used with the other test analytes. With the additive extraction, devices were first extracted using water and then followed by the 2% alkaline SDS extraction for total removal of protein.

Alkaline SDS was prepared by measuring an amount of 20.00g of sodium dodecyl sulfate (Thermo Scientific) using an analytical balance into a 1000mL volumetric flask. The flask was filled to the line with purified water, and the solution was mixed until the SDS was fully dissolved using a magnetic stir bar and stirring plate. The pH was adjusted using a pH Meter. 1.00N Sodium Hydroxide dropwise was added, stirring in between, until solution reached a pH of 10±0.9.

3.1.8 Extraction Volume

ISO 10993-12 provides guidance in section 10.3.3 for the determination of extraction volumes for surface residual and biocompatibility testing. The recommended extraction ratio for molded items (e.g., medical devices) is 3cm^2:1ml. This ratio is meant to be used when the device will be "cut into small pieces before extraction to enhance submersion in the extract media." (International Organization for Standardization, 2012) ANSI/AAMI ST98 states, "It is recommended that an optimal extraction volume be established. This should be large enough to allow for effective extraction. Similarly, it should be small enough to ensure that the resulting extract is not excessively diluted and, therefore, can be assayed using the test method." (Association for the Advancement of Medical Instrumentation, 2022) ASTM F2314 recommends to flush internal sites (lumens) "with a volume of elution fluid equal to at least three times the void volume of the lumen" recommends repeating this procedure 3 times to ensure an efficient extraction has been performed (ASTM International, 2014).

Although the extraction ratio of 3cm^2:1ml has been established as the preferred method for calculating maximum extraction volume when testing deconstructed devices, an alternate calculation method is required when a device cannot be deconstructed for the extraction process. When utilizing extraction methods of sonication, the device is fully submersed in the extraction fluid for the process to be effective. Using the 3cm^2:1ml method was not result in sufficient volume of extraction eluent to sufficiently extract the device. Using extraction fluid to flush a lumen requires a sufficient volume to ensure all remaining soil has effectively been removed from the device. This rationale will demonstrate an alternate method for calculating the maximum extraction volume that accurately measures TOC and protein residuals. Minimum volume needed for TOC (wet oxidation method) and protein (BCA method) residual testing is not less than 20mL. The following equation is used to calculate the maximum extraction volume:

$$Device\ Maximum\ Extraction\ Volume\ (mL) = \frac{Device\ Surface\ Area\ (cm^2)\ x\ Method\ Acceptance\ Criteria\ (\frac{\mu g}{cm^2})}{Method\ LOQ\ (\frac{\mu g}{mL})}$$

The equation variables were calculated using the following rationales:

- Device Surface Area (cm^2) – The surface area of the device was calculated by finding the area of the device that will come in contact with the extraction fluid. If the device is not disassembled the surface area may not include the inner surfaces of the device if fluid ingress is not expected (e.g., intact seals which prevent ingress of soil or extraction fluid).
- Method Acceptance Criteria (µg/cm^2)- Cleaning endpoints are defined in ANSI/AMMI ST98 as 12.0 µg /cm^2 TOC and 6.4 µg/cm^2 protein. These cleaning endpoints are defined in terms of mass-per-area (µg /cm^2) to normalize the residual results in a manner where they can be evaluated for patient safety. Within a cleaning validation, any amount of analyte above these values would fail the acceptance criteria for the test sample.
- Method LOQ (µg/mL) –The lowest accurate value that an assay can measure is at or slightly above the LOQ. Within the method validation the LOQ is determined using a fixed volume of extraction

fluid and is not dependent on the device surface area. However, the extraction volume used for a device may directly impact the method's ability to detect the analyte concentration (e.g., a large extraction volume may dilute the analyte concentration resulting in the measurement falling below the LOQ and resulting in a false negative result). In addition, if the concentration of the analyte is too high for the extraction volume, the measurement may fall outside of the calibration curve and result in data imprecision. Understanding the optimum extraction volume for the assay is a critical aspect of a cleaning validation.

$$too\ dilute \leq LoQ \leq accurate\ assay\ result \leq LoQ \leq too\ concentrated$$

Both the TOC and the protein assays measure values in units of concentration – that is mass-per-volume (µg/mL). The device maximum extraction volume defines maximum amount of extraction fluid used on a device without the analyte being over-diluted (e.g., possible false negative). The maximum extraction ratio is calculated using the following equation:

$$Maximum\ extraction\ volume\ ratio\ \left(\frac{mL}{cm^2}\right) = \frac{Method\ Acceptance\ Criteria\ ug/cm^2}{LOQ\ (ug/mL)}$$

The equation solves the maximum theoretical extraction ratio appropriate to the method. Any ratio greater than this value would result in extractions too dilute for the assay. The Protein LOQ for the standard addition water method is 2.5 µg/mL and 1 µg/mL for the standard addition alkaline pH 10 method for the Spectramax Plus Spectrophotometer. The Total organic carbon LOQ for water is 0.125 µg/mL for the wet oxidation method for the Shimadzu TOC analyzer. The maximum extraction volume ratio was calculated using the analyte method with the largest LOQ (i.e., 2.5 µg/mL for standard addition water protein) yielding maximum extraction ratio of 2.56 mL/cm^2. The ratio was then multiplied by the surface area to calculate the maximum extraction volume per each feature. The maximum extraction volume was calculated and is reported in the applicable device appendix.

3.1.9 Extraction Container

The extraction containers needed for testing must not impede the extraction process nor contribute to an analyte for proper evaluation of extraction performance. Thin-walled polyethylene was determined to be an acceptable material for extraction as it does not contribute analyte as demonstrated through the negative sample control and allows for cavitation to take place during ultrasonic cleaning. Whirl-Pak polyethylene bags were used as extraction containers and when necessary were modified using a heat sealer to allow for complete submersion of the device with the lowest extraction volume. The bag modification specifications are specific to the device and are reported in each applicable device appendix.

3.1.10 Extraction Method

Soil extraction methods are described by ASTM F3321-19, *Standard Guide for Methods of Extraction of Test Soils for the Validation of Cleaning Methods for Reusable Medical Devices* (ASTM International, 2019). The following extraction methods were selected and validated as preferred for optimal extraction and when appropriate will be used on the device.

- Sonication - Each device was placed inside an extraction container, where it was fully immersed in the extraction fluid and sonicated for 15 minutes. Partial immersion is not effective with sonication.
- Orbital Shaking –Following sonication the extraction container was placed on an orbital shaker for 30 minutes at 150 rotations per minute (RPM).
- Flushing – When applicable, the device was flushed with extraction fluid to remove soil from lumens or hard to reach locations of the device. To facilitate the removal of soil from the inside of lumens, the total extraction volume will be divided into thirds. Each third is flushed and then followed by a 15-minute sonication. The sequence is the following: flush, sonicate, flush, sonicate, flush, sonicate, dry inside lumen with air wash until no droplets elute from distal end of lumen.

An extraction container was prepared. For device features containing lumens or areas requiring flushing, a third of the extraction volume was flushed through the device, otherwise, the entirety of the extraction eluent was added to the extraction container with the device feature (Table 3.5). The extraction container was placed into a Branson 8800 Ultrasonic Cleaner for sonication at 40 kHz for 15 minutes. The bag was opened, and if applicable, the lumens were flushed with extraction fluid using a syringe to ensure that the entire width of lumen had been flushed. The sealed extraction bags were removed from ultrasonic cleaner and placed onto an orbital shaker lying flat. They were shaken at 150 rotations per minute (RPM) for 30 minutes. The bag was opened again, and if applicable, the lumens were flushed with an additional volume of extraction fluid using a syringe to ensure that the entire width of lumen had been flushed. The test article was removed from the extraction fluid. The exact extraction method for the device feature is described in the applicable annex.

Table 3.5: Extraction volume per device feature in 23 Device Features Experiments

Device Feature	Total Extraction Volume (mL)	Required Flushing	Flushing Volume (mL)
Ball Detent / Ball Bearing	400mL	Yes	200mL
Ball Seal Springs	15mL	No	NA
Blind Slot	200mL	Yes	25mL
Button w/ Spring	90mL	No	NA
Buttons - Exposed Springs	200mL	No	NA
Captured Screw	150mL	No	NA
Hinges, Joints, Pivot Points	20mL	No	NA
Leaf Springs	180mL	No	NA
Mated Surfaces	500mL	No	NA
Mated Surfaces Small Clearance	500mL	No	NA
O-rings - External O-ring	30mL	No	NA
O-rings - Internal O-ring	45mL	No	NA
Rough Surface	150mL	No	NA
Screws Threaded Rod / Threaded Thru Hole	130mL	No	NA
Screws Threaded Rod/ Threaded Blind Hole	150mL	Yes	25mL
Sliding Shaft Short	50mL	No	NA
Sliding Shafts Long	180mL	No	NA
Smooth Blind Lumen	11mL	Yes	3mL
Smooth Through Lumen	130mL	Yes	15mL
Snap Rings	25mL	No	NA
Spring Internal	350mL	No	NA
Threads Blind Hole	80mL	Yes	20mL
Through Slot	200mL	Yes	25mL

3.1.11 Extraction Method Validation

Before a device can be evaluated for cleaning efficacy the extraction method must be validated to demonstrate the method of soil application and extraction is an effective challenge for the device. AAMI ST98, *Cleaning validation of health care products – Requirements for development and validation of a cleaning process for medical devices*, requires the extraction method validation be performed using a minimum of three (3) replicates (Association for the Advancement of Medical Instrumentation, 2022). The method validation also includes the following samples as described by ANSI/AAMI ST98, *Cleaning validation of health care products – Requirements for development and validation of a cleaning process for medical devices* and ASTM F3321-19, *Standard Guide for Methods of Extraction of Test Soils for the Validation or Cleaning Methods for Reusable Medical Devices*:

- Extraction Method Test Samples - A minimum of three (3) test samples should be used to calculate the test soil recovery efficiency.
- Positive Sample Control – The extraction fluid volume is spiked with a known amount of test soil that is close to the Limit of Quantitation of the assay and undergoes the same extraction

conditions as the test sample. This control assesses whether the extraction conditions decrease in the amount of measured soil in the sample.

- Positive Device Control – The medical device is spiked with a known amount of test soil that is close to the Limit of Quantitation for the assay. The device is extracted in the same manner as the test samples and a percent recovery is calculated by the ratio of the amount of recovered analyte to the total amount of test analyte applied times by 100.
- Negative Sample Control – The extraction fluid alone undergoes the same extraction conditions as the test sample. This control assesses the introduction of the test analyte from the testing material and is considered a "blank". This value is subtracted from the test sample and other controls during the analysis.
- Negative Device Control – This control assesses the contribution of the analyte from the device. For example, this control will determine there are residual manufacturing materials left on the device after manufacturing.

The extraction efficiency of a process directly influences the likelihood that all analytes present on the device will be effectively removed and accurately quantified. Extraction efficiency refers to the ability of a method or technique to extract target analytes from a sample. When the extraction efficiency is high, it signifies that a greater proportion of the analytes of interest have been successfully isolated from the sample matrix. Consequently, a high extraction efficiency increases the probability that all relevant analytes have been captured, allowing for more comprehensive analysis and quantification.

The test articles were cleaned using the applicable cleaning process, soiled and dried (see applicable device feature appendix). An extraction container as specified with the extraction fluid volume was prepared and the soiled test article added. The extraction was performed a total of 5 time in this order: 1X water, 1X SDS, 3X water. The test articles were removed from the extraction container and dried overnight until completely dry. A 1:20 dilution was performed on the first extraction by pipetting 2mL of the extraction eluent to 38mL of the diluent (e.g., water, SDS, Alkaline SDS) appropriate to the extraction and vortexing until fully mixed. Water and SDS extractions were tested for protein residuals using the standard addition method using the Thermo Fisher Micro BCA Colorimetric Protein Assay. The water extraction was tested for Total Organic Carbon (TOC) using the Wet Oxidation Method. The samples are first acidified with hydrochloric acid and purged of total inorganic carbon (TIC). Following this, sodium persulfate (an oxidant) is added to release the organic carbon. The organic carbon is purged from the solution and quantified by a non-dispersive infrared (NDIR) detector calibrated to directly display the mass of carbon dioxide detected. This mass is proportional to the TOC in the sample.

The instrument concentration was corrected by subtracting the negative sample control concentration for the appropriate eluent from each concentration result to determine the "Corrected Instrument Concentration". If the instrument result for the negative sample control resulted in a negative number, then the method LOD was substituted in the calculation.

The first and second extraction were corrected for the dilution factor (1:20) by multiplying the corrected concentration by 20 to calculate “Corrected Dilution Concentration” for the protein. The first extraction for TOC was corrected for the dilution factor (1:40) by multiplying the corrected concentration by 40 to calculate “Corrected Dilution Concentration”. Unless otherwise diluted, the remaining extractions are multiplied by 1. The additive extraction exhaustive extraction efficiency for the protein testing was calculated using the following equation: The 1:20 extraction (water) plus the second extraction (SDS) was divided by the sum of all the extractions multiplied by 100.

$$\%Extraction\ Efficiency = \left(\frac{Extraction\ \#1 + Extraction\ \#2}{\sum All\ Extractions}\right) x\ 100$$

The compendial exhaustive extraction efficiency with water only was calculated by dividing the 1:20 extraction (water) by the sum of all the extractions the water extractions multiplied by 100. The compendial exhaustive extraction efficiency with SDS extraction was calculated by dividing the 1:20 extraction (water) by the sum of all the extractions the water extractions multiplied by 100. The exhaustive extraction efficiency for the TOC was calculated by dividing the 1:40 extraction (water) by the sum extractions 1:20, 3, 4, 5 multiplied by 100.

$$\%\ Extraction\ Efficiency = \left(\frac{Extraction\ \#1}{\sum All\ Extractions}\right) x\ 100$$

Using MiniTab® 19, a one-sample t-test was used to calculate the 99% confidence intervals of the sample set. Utilizing the lower value of the 99% confidence interval as the worst-case result to evaluate the sample set against the acceptance criteria serves as a robust strategy to mitigate the risk of error in statistical analyses and decision-making processes. The extraction method validation was first challenged using a 72hr soil dry at 22°C and 50%HR. If the low value of the 99% confidence interval fell below the acceptance criteria of 70% then the method validation was repeated with a soil dry time of 2hr at 22°C and 50% RH. The method validation specific to each device feature can be found in the applicable appendix.

3.1.12 Experimental Controls

Positive Sample Control - An extraction container was prepared with a total extraction volume. A volume of 5.0µL of test soil for each 100mL of extraction fluid was added and vortexed until fully mixed. The extraction method was performed. A positive sample control was prepared for each extraction eluent (i.e., water and SDS).

Negative Sample Control - An extraction container was prepared with a total extraction volume. The extraction method was performed. A negative sample control was prepared for each extraction eluent (i.e., water and SDS).

Negative Device Control - A device feature was cleaned using the applicable experimental procedure before being extracted. A negative device control was prepared for each extraction eluent (i.e., water and SDS).

Positive Device Control - The device feature was weighed using an analytical balance prior to soiling. The soil was dried as applicable to the experiment. Post drying, the device feature was weighed before extracting. The extract was diluted 1:20 for the protein assay and 1:40 for the TOC assay. A positive device control was prepared for each extraction eluent (i.e., water and SDS).

3.1.13 Cleaning Agent (i.e., Detergent) Selection

The selection of a cleaning agent for the experimental design focused on utilizing a neutral enzymatic detergent (Valsure) to effectively challenge the cleaning process. This choice aimed to ensure compatibility with device materials while leveraging enzymes to catalyze soil breakdown. Enzymatic detergents, typically operating within a pH range of 6-9, offer targeted breakdown of specific soil components such as proteins or lipids. Optimal enzyme performance occurs within specific temperature ranges, generally between 40-60°C, with lower temperatures potentially compromising efficacy and higher temperatures risking enzyme denaturation. Concentration also plays a critical role, with adherence to detergent label instructions necessary to maintain cleaning effectiveness while mitigating residual risks.

Additionally, an alkaline cleaning agent was employed specifically for the semi-automated experiment to introduce a more aggressive soil removal step. Alkaline detergents utilize saponification and degradation mechanisms to remove residual soil, and the more alkaline the detergent the more aggressively it will clean the device.

3.1.14 Analyte Detection

AAMI ST98, *Cleaning validation of health care products – Requirements for development and validation of a cleaning process for medical devices* requires the testing of two quantitative analytes in addition to visual inspection when evaluating cleaning efficacy. The following analytes were used to measure cleaning efficacy:

- Bicinchoninic acid (BCA) Protein Residual Assay - The Bicinchoninic acid (BCA) assay is a biochemical assay for determining the total concentration of protein in a solution. The assay is based on protein-copper chelation and secondary detection of the reduced copper. The assay relies on two reactions: Firstly, the peptide bonds in the protein sample reduce Cu2+ ions, in a temperature dependent reaction, from the copper solution to Cu+. The amount of Cu2+ reduced is proportional to the amount of protein present in solution. Next, two molecules of BCA chelate with each Cu+ ion, forming a purple-colored product.
- Total Organic Carbon – Determination of Total Organic Carbon (TOC) is performed by wet oxidation. The samples are first acidified with hydrochloric acid and purged of total inorganic carbon (TIC). Sodium persulfate (an oxidant) is then added to release the organic carbon. The organic carbon is purged from the solution and quantified by a non-dispersive infrared (NDIR)

detector calibrated to directly display the mass of carbon dioxide detected. This mass is proportional to the TOC in the sample.

- Adenosine Triphosphate (ATP) - The testing of Adenosine Triphosphate (ATP) with a luminometer involves a process called bioluminescence detection. ATP is an energy-carrying molecule found in all living cells, and its presence indicates the potential existence of organic residues on surfaces. The luminometer (Hygiena) is a device that measures the light produced during the enzymatic reaction between ATP and a luciferin-luciferase enzyme system. In the presence of ATP, the enzyme catalyzes the oxidation of luciferin, resulting in the emission of light. The test results are typically expressed in Relative Light Units (RLUs), with higher RLUs indicating higher ATP concentrations.

3.1.14.1 Protein Analyte Analysis

A calibration curve was prepared for both water and SDS. For the best possible fit, a third order polynomial equation was used for the line. The protein determination for the test extracts were established using the applicable curve to the extraction eluent. The standard addition method was used (Kremer, et al., 2023).

The preparation of the 10 µg/mL BSA stock solution involved measuring 500mg of powdered bovine serum albumin (BSA) on an analytical balance. The measured BSA was then transferred to a 500mL volumetric flask and filled to the line with ACS reagent grade water to deliver a concentration of 1000µg/mL albumin solution. The 10 µg/mL was prepared by using an Eppendorf pipette to transfer 10mL of the 1000 µg/mL albumin solution into a 1000mL volumetric flask, which was then filled to the line with ACS reagent grade water. The preparation of a 2% SDS Solution with an alkaline pH of 10 involved transferring 10mL of the 1000µg/mL albumin solution into the volumetric flask. Sodium dodecyl sulfate (20.00g) was measured using an analytical balance and transferred to a 1000mL volumetric flask, which was filled with ACS reagent grade water. The stock solution was mixed until SDS was fully dissolved, and a desired amount was poured into a 250ml beaker. Using a pH meter, 1.00N Sodium Hydroxide was added dropwise, stirring in between, until the solution reached a pH of 10±0.9.

The preparation of the Bovine Serum Albumin (BSA) Calibration Curve involved following the Thermo Fisher Micro BCA Protein Assay Kit Instructions to prepare BSA standards in a serial dilution series using the appropriate eluent (Table 3.6). A minimum of eight standards, including a blank, were prepared to obtain a third-order polynomial calibration curve. Pyrogenic-free conical 15 mL vials labeled A through I were prepared, indicating the eluent being used. The preparation of Standard Solution A, the highest concentration solution referred to as "Solution A," involved using an Eppendorf Automatic Pipette and pipetting 0.5mL of Bovine Serum Albumin (BSA) Standard, 2 mg/mL, Product #: 23209/23210 or equivalent to tube A. Tube A then received 4.5mL of diluent (extraction eluent). The vial was capped, and the solution was thoroughly mixed using a mini vortexer for 30 seconds to ensure homogeneity. It was essential to allow the mixed solution to settle before removing aliquots for each dilution to avoid transferring bubbles, as this could decrease accuracy. Solution A (200 µg/mL) was prepared to create a series of standard

solutions with concentrations in the range of 0.5-40 µg/mL. Importantly, Solution A was not used as part of the analysis for the Micro BCA Protein Assay.

Table 3.6: BCA calibration curve dilution scheme

Standard Solution	Standard Addition Method: Concentration (µg/mL)	Vol. and Source of BSA (mL)	Vol. Diluent (mL)
A	210	0.5 mL from BSA Stock	4.5
B	50	2.0 mL Solution A	8.0
C	30	4.0 mL Solution B	4.0
D	20	4.0 mL Solution C	4.0
E	15	4.0 mL Solution D	4.0
F	12.5	4.0 mL Solution E	4.0
G	11.0	3.2 mL Solution F	4.8
H	10.5	4.0 mL Solution G	4.0
I	10.0	0	8.0

The preparation of the Working Reagent (WR) involved determining the total volume of the WR and the volume of each individual component based on the number of wells to be analyzed in the 96-well plate. The plate map was used to calculate the number of wells requiring analysis, with each well needing 187µL of WR. The total amount of WR was then calculated by multiplying the number of wells by 200µL. The preparation ratio was set as follows: Reagent A (50 parts), Reagent B (48 parts), and Reagent C (2 parts). It was noted that the WR could be utilized within 24 hours of preparation and must be discarded if stored for more than 24 hours after initial preparation. Using an Eppendorf automatic pipette and appropriate disposable tip, the predetermined amounts of each reagent were measured to the nearest 0.10mL. The three Micro BCA reagents were placed in a labeled sterile/pyrogenic-free conical 50 mL vial designated for the WR. Stirring for 30 seconds occurred upon adding each reagent to ensure a homogeneous distribution of each component. The reagents were added in the sequence of Reagent A, Reagent B, and Reagent C. It was emphasized to touch the tip to the side of the vial for complete liquid delivery and to change the tip for each reagent aliquot to prevent contamination of stock reagent solutions. Micro BCA Reagent C exhibited a light blue color, turning light, bright green upon addition to Reagents A and B.

The control analysis using the standard addition method involved pipetting 1mL of extraction eluent into a new conical bottom tube for each extraction. Each collected diluent volume (1mL) was spiked with a

10µL volume of the 1000µg/mL Albumin solution using a sterile pipette tip, followed by vortexing. The Positive Reference Control included two eluents: Purified Water Eluent and 2% SDS Eluent. For the Purified Water Eluent, 50 µL of BSA from a fresh ampule was transferred into a 15 mL conical bottom tube, and 9.95 mL of ACS grade water was added to the same tube. Similarly, for the 2% SDS Eluent, 50 µL of BSA from a fresh ampule was transferred into a 15 mL conical bottom tube, and 9.95 mL of 2% SDS was added to the same tube.

The Microplate Procedure involved using an Eppendorf automatic pipette and an appropriate disposable tip to place 150 µL of each standard, control, or unknown solution in its respective well based on the plate map. Subsequently, 187.5 µL of WR was delivered into each well using the same pipette, with the tip changed for each transfer to prevent contamination. It was noted that the reaction between the sample and WR was time-sensitive, emphasizing the need for efficiency in delivering the WR to all wells, so a multi-channel pipettor was used. Finally, the 96-well plate was inserted into the microplate drawer with well A1 in the top left corner, and the workflow was initiated by opening the workflow tab in the Softmax Pro software. The plate was placed in the Spectramax Plus384 (Molecular Devices) where it was incubated at 37°C for 2 hours. The plate was then read by the instrument at an absorbance of 562nm.

A standard quadratic curve was prepared from the albumin standard concentrations by the Spectramax Plus 384. The unknown samples and control protein concentration were then determined from the standard curve. The blank (10 µg/mL for the standard addition method) was subtracted from the controls and unknown samples for a corrected value.

3.1.14.2 Total Organic Carbon Analysis

In the calibration process, a calibration curve, comprising two concentration ranges, was obtained by accurately preparing solutions with given quantities containing Total Organic Carbon (TOC). The validity of the calibration curve for sample analysis extended for one week (7 days) after its generation. Commercially available standards (Sigma-Aldrich) of High (10 mg/L or ppm) and Low (1 mg/L or ppm) were used.

TOC vials were prepared by pouring stock solution into a 40mL TOC vial, ensuring that the liquid did not exceed the point at which the vial started to invert. Each vial was acidified by adding 2 drops of ACS grade 37% HCl, and pH measurements were avoided to prevent sample contamination.

To generate the calibration curve, the procedures in the operation manual for the Shimadzu Total Organic Carbon TOC-L Analyzer were followed. The mean area of each point, slope, intercept, and R^2 value were recorded. An acceptable calibration curve for unknown sample analysis was defined as having an R^2 value ≥0.99.

The preparation of the 1 ppm standard check involved pipetting 10.0 mL of the 10ppm stock solution into a 100 mL volumetric flask, bringing it to volume with sterile water, and vortexing the solution. Subsequently, the 1 ppm solution was poured into a 40 mL TOC vial, ensuring that the liquid did not exceed

the point at which the vial started to invert. Each vial was acidified by adding 2 drops of ACS grade 37% HCl using a disposable pipette directly to the TOC vial and then vortexing. It was emphasized that pH measurements were not to be taken as probes or strips could contaminate the sample. The standard check was deemed to pass if the response fell within 80-120% of the response of the prepared concentration.

For the blanks, sterile box water was poured into a 40 mL TOC vial, ensuring that the liquid did not exceed the point at which the vial started to invert. Each vial was then acidified by adding 2 drops of ACS grade 37% HCl using a disposable pipette directly to the TOC vial, followed by vortexing.

During sample preparation, all aliquots were transferred into a 40 mL TOC vial, and precautions were taken to ensure that the liquid did not exceed the point at which the vial started to invert. Similar to the standard check, each vial was acidified by adding 2 drops of ACS grade 37% HCl using a disposable pipette directly to the TOC vial, followed by vortexing.

The setup for the TOC evaluation included 50 injections for each sample. Three blank control samples were run after every 10 unknown samples, and a 1 ppm Control Sample was included at the beginning, middle, and end of the run.

3.1.14.3 ATP Analysis

ATP testing was completed after protein analysis on the remaining eluent. Each sample was vortexed until all precipitates were in solution. The Aquasnap Free ATP test devices were allowed to equilibrate to room temperature (21-25°C) before use. To prepare the device, a downward flicking motion was used to shake the liquid extractant from the collection dipper to the bottom of the tube, facilitating accurate ATP extraction and ensuring a consistent sample. Holding the swab tube firmly, the collection device was twisted and pulled out of the tube. The sample dipper was then submerged in the extraction sample for 1-2 seconds, lifted vertically, and reinserted into the test tube. A gentle shake for 1-2 seconds released excess sample from the collection tip and mixed it with the extractant at the bottom of the test tube.

The Aquasnap device was activated by breaking the Snap Valve with a thumb and forefinger, squeezing the bulb twice to expel all liquid into the tube. The sample was shaken for 3-5 seconds to thoroughly mix it. The entire Aquasnap device was inserted into the Hygiena luminometer, and the lid was closed and "OK" was pressed to initiate measurements within 15 seconds of activation. The luminometer was held upright, and for the positive device control, the diluted sample was tested instead of the original extraction to avoid potential inhibition of the bioluminescence reaction by large quantities of certain soil types. If the instrument calibration was unsuccessful, it was ensured that the lid was closed properly, and precautions were taken to prevent any materials or liquids from entering the luminometer's test chamber. The lid was always closed when not in use, and the outside of the test device was dried before inserting tests for reading. Results for each extraction were recorded.

3.2 The Device Feature Approach Validation

Two (2) types of coupons were evaluated during these experiments, a single feature coupon and a multi-feature coupon.

- Single Feature – The single feature consists of a 300 series stainless steel block (6mm x 6mm x 50mm) with a 2mm diameter hole drilled in the top center (Figure 3.5).

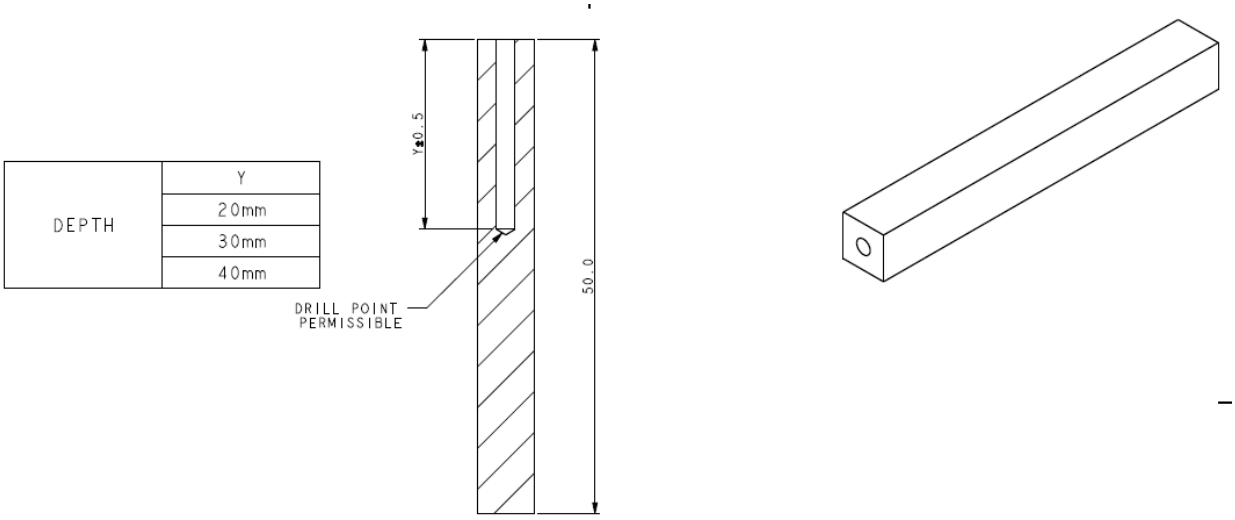
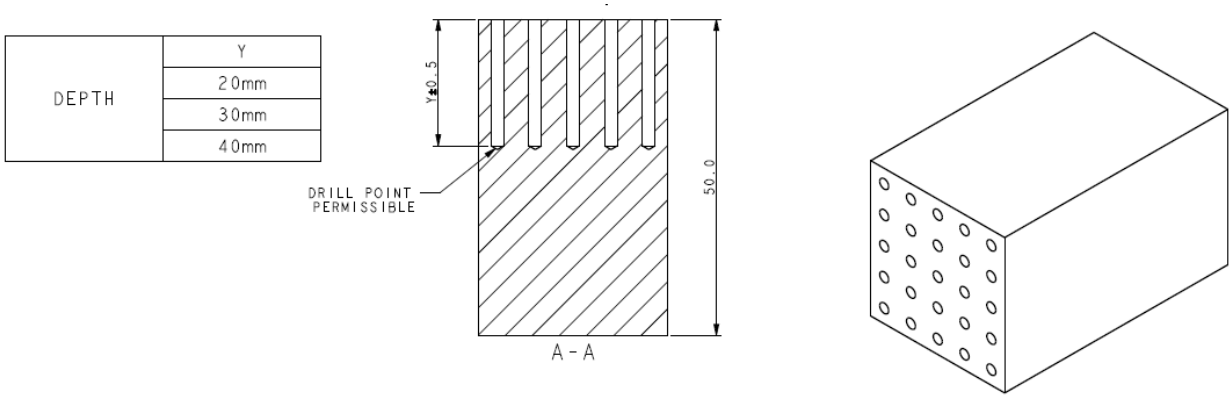


Figure 3.5: Schematic of Single Feature Coupon

- Multiple Feature – The single feature will consist of a 300 series stainless steel block (30mm x 30mm x 50mm) with twenty-five 2mm holes drilled in the top (Figure 3.6).



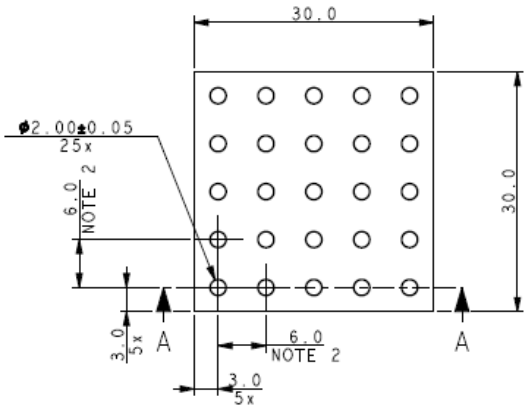


Figure 3.6: Schematic of Multiple Feature Coupon

To challenge the flow velocity of the lumen, each coupon type had three (3) lumen lengths, for a total of six (6) coupon types for testing.

- Single Feature – 20mm length x 2mm diameter lumen
- Single Feature – 30mm length x 2mm diameter lumen
- Single Feature – 40mm length x 2mm diameter lumen
- Multiple Feature – 20mm length x 2mm diameter lumen
- Multiple Feature – 30mm length x 2mm diameter lumen
- Multiple Feature – 40mm length x 2mm diameter lumen

A full description of the test method used for the device feature approach validation can be found in Appendix 2, but the following summary described the materials and methods of the experiment.

In preparation for soiling, the devices were rinsed under running critical water for 1 minute while brushing the lumen with a 2.2 mm × 12-inch lumen brush (Key Surgical). Devices then were immersed in a 10 mL/L concentration of alkaline cleaning agent (NeoDisher), and each lumen was flushed with the cleaning agent solution using a 16.5-G needle and 3-mL syringe. Following a 60-minute soak, the lumens were again flushed with 10 mL of the detergent solution and sonicated for 15 minutes at 45 kHz in a fresh batch of the alkaline cleaning agent. Then, they were rinsed under running critical water and each lumen was flushed two times. The lumens were dried using medical grade compressed air and inspected for cleanliness using a FIS-007 Flexible Inspection borescope (Heatlhmark).

Modified Coagulated Blood soil was prepared and applied using a pipette within 10 minutes of preparation (i.e., before coagulation). The volume of test soil applicable to each coupon type is detailed in Table 18. When depositing the test soil, the pipette tip was inserted as far as it would go into the lumen. The coupon was gently tapped on the counter to promote the migration of the test soil to the dead end of the lumen. The devices then were dried under the most challenging conditions (72 hours at 22°C/50% relative humidity).

The cleaning procedure began with a prerinse, where each lumen was flushed with 10 mL water and bushed five times using a 2.2 mm × 12-inch lumen brush with a twisting motion. The devices then were immersed in a 4-mL/L concentration-neutral pH cleaning agent solution (Valsure Neutral; STERIS) at less than 40°C for 5 minutes. While immersed, a 2.2 mm × 12-inch lumen brush was used to clean all traces of test soil from the lumen and exterior surface using a twisting motion five times for a minimum of 1 minute. To rinse, the devices were immersed in critical water (<40°C) for a minimum of 1 minute. An ultrasonic bath was prepared with the neutral pH cleaning agent at a concentration of 4 mL/L. The lumens were flushed with the cleaning agent solution using a 50-mL syringe before being sonicated for 5 minutes at 40 kHz. The devices then were immersed in critical water (<40°C) for a minimum of 1 minute while the lumens were flushed with 50 mL water. The lumens in the devices were dried by flushing the lumen with air using a 16.5-G needle until no droplets exited the lumen, and the outside of the device was dried using a lint-free cloth.

To account for the water-soluble and -insoluble protein present in the test soil post drying, an additive extraction was validated. This was performed by first extracting with high-purity water (<50 ppb total organic carbon), followed by a second extraction of 2% alkaline sodium dodecyl sulfate (SDS) at a pH 10. Cleaned devices were extracted using the validated method of flushing three times; thus, the extraction volume was divided by three to deliver the flush volume per lumen per flush (Table 3). The device was inserted into the Whirl-Pak extraction bag (Nasco) with the lumen to the side of the bag. Using a 16.5-G needle and 3-mL syringe, the lumen(s) were flushed. The device was oriented so that the lumen opening was completely covered by the extraction fluid, then the bag was closed and sonicated for 15 minutes at 40 kHz. Following sonication, the devices were placed so that the lumen was oriented to the bag opening and an additional flush was completed. The bag was again sealed and sonicated for an additional 15 minutes at 40 kHz. After the second sonication, the device was again flushed with extraction fluid. The extraction fluid was measured for protein residuals using the standard addition micro-BCA protein assay18 using a Spectra-Max Plus 384 UV-VIS Spectrophotometer and the Pierce BCA Protein Assay Kit (Thermo Scientific). Testing was completed with a sample size of 30 coupons.

3.3 23 Device Features

The device features within this experimental design were identified as common reusable medical device features assumed to be worst case (contact with the open wound). For device features where engineering specifications could be established (knurling, lumens, mated surfaces, hole shaft arrangements, etc.), the most challenging features (i.e., geometries) were determined. These features are not necessarily represented on a single device, for example: one device may possess the smallest diameter lumen while another device may possess the longest length. Test articles (i.e., master products) were manufactured representing or exceeding the most challenging features represented on each device for each device feature that poses a challenge for the cleaning process.

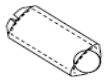
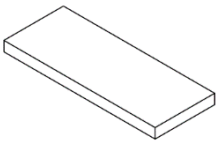
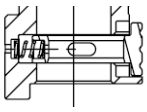
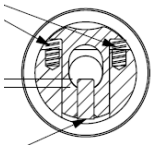
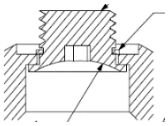
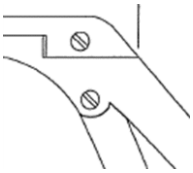
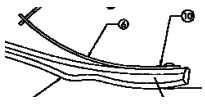
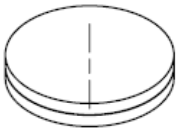
Other device features that are more complex (hinges, quick connects, ratcheting mechanisms, ball detents, etc.) were grouped together based on their designs. The design which poses the greatest

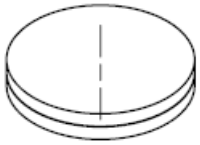
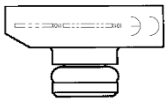
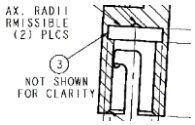
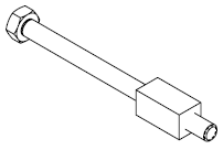
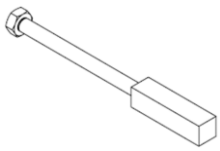
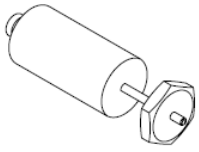
challenge to cleaning was selected for each feature. Factors contributing to a cleaning challenge are limited access to brushing/flushing, ability to collect and retain blood soil, ease of visual inspection.

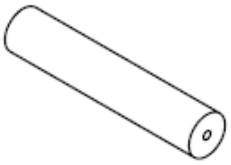
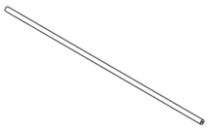
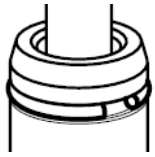
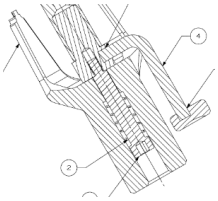
Surface material of the device can impact the soil adhesion and cleaning efficacy. Although most controlled laboratory studies within the literature use stainless steel devices as the test sample (Gettens & Gilbert, 2007) (Gonzalez, et al., 2017) (Lappalainen, et al., 2009) at the time of project initiation, no information in the literature was available on the comparison of medical device material with soil adhesion. An experiment was performed and subsequently published in the paper, *Test Soil Application Affinity for Reusable Device Cleaning Validations* by Kremer et. al (Kremer & Ratanski, 2023). Seven materials (stainless steel, delrin, peek, nitinol, aluminum, titanium, and silicone) were tested against challenging test soils including Modified Coagulated Blood. Stainless steel was the only material that showed consistent soil application in a thickness (at ~6µL/cm^2) that fully covered the test surface without some element of pooling, cracking, flaking or soil migration with all test soils and application methods (Table 3.1). The results of this study demonstrate the stainless steel is the most challenging material of those tested for test soil adherence and can be used as a master challenge during device evaluation using the family grouping approach. Therefore, stainless steel was used as the material for each device tested within this experiment. The grade of stainless steel is reported in the applicable device annex with 304 stainless-steel preferred as the most challenging to clean.

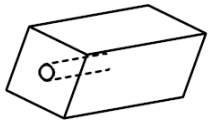
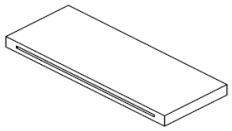
It is not possible to test every device on the market and control all variables to establish these catagories, however, by using a design feature approach the work we can establish a controllled experiment to isolate this variable and evaluate it within the cleaning process the likelihood of soil retention (Table 3.7).

Table 3.7: 23 Device Features

Feature Name	Most Challenging Geometry	Coupon Image	Rationale
Ball Detent/Ball Bearing	Ball Diameter = ≥1.1 mm		The critical dimensions of a ball detent are the smallest diameter. This increases the difficulty of accessing blood soil with a brush and high impingement water.
Ball Seal Springs	External Ball Seal Spring		The ball seal spring is fits tightly around a small diameter cylinder. The coil is small and very fine. This can trap blood soil and become difficult to remove.
Blind Slot	Depth = 24.9 mm Width = 1.0 mm		The critical dimensions of a blind slot are the smallest width and the greatest depth. The extremes of these two dimensions increase the difficulty of accessing blood soil with a brush and high impingement water.
Button w/ Spring	Deep internal springs		The springs are located at the distal end of the button mechanism. This increases the difficulty of accessing blood soil with a brush and high impingement water.
Buttons - Exposed Springs	Exposed Springs		The springs are located in the middle, at the side of the button mechanism. This increases the exposure to blood soil.
Captured Screw	Captured screw held in place with an external snap ring		The captured screw spans multiple diameters and is held in place on the other side by a snap ring. This increases the difficulty of accessing blood soil with a brush and high impingement water.
Hinges, Joints, Pivot points	Rongeur Hinge		The Rongeur hinge is more complex than a scissor hinge as it has one component "sandwiched" in between two sides of another component connected by a hinge pin. This type of hinge is more difficult to access for cleaning than a standard scissor hinge.
Leaf Springs	The challenging geometry is observed among all leaf springs.		The spring creates a mated surface configuration as it meets the device handle. This can trap blood soil and become difficult to remove.
Mated Surfaces	Zero Clearance		The critical dimension of a mated surface is the smallest clearance. The zero clearance test coupon accounts for those designs where two materials come into planar contact, but the interface is not sealed, potentially allowing ingress of blood soil or residuals.

Feature Name	Most Challenging Geometry	Coupon Image	Rationale
Mated Surfaces – Small Clearance	Minimal Clearance = 0.10 mm		The critical dimension of a mated surface is the smallest clearance. This increases the difficulty of accessing blood soil with a brush and high impingement water. There were no mated surfaces identified in the evaluation that had a minimal clearances less than 0.25 mm, therefore, the master challenge device will have a minimal clearance of 0.10 mm.
O-rings, External O-ring	External O-ring		The O-ring fits tight against the external surface of the device. This increases the difficulty of accessing blood soil with a brush and high impingement water.
O-rings, Internal O-ring	Internal O-ring		The O-ring fits tight against the internal surface of the device. This increases the difficulty of accessing blood soil with a brush and high impingement water.
Rough Surface	Medium Knurl (21 Pitch)		All evaluated devices containing knurling are designed with medium (21 pitch) knurling.
Screws-Threaded Rod/Threaded Thru Hole	Minor Diameter: 6.912 mm Thread Length – 108.32 mm Mated Length – 25.4 mm		The longest threaded length was 108.32 mm. There were no other devices found to have a mated length over 25.4 mm.
Screws-Threaded Rod/Threaded Blind Hole	Minor diameter - 6.912 mm Thread Length – 108.32 mm Thread Depth – 25.4 mm Hole Depth – 35.0 mm		The longest threaded length was 108.32 mm. There were no other devices found to have a thread depth over 25.4 mm.
Sliding Shaft - Short	Sliding Length = 25.4 mm Minimal Clearance = 0.02 mm (diametrical clearance of 0.04mm)		The critical dimensions of a sliding shaft are the greatest length and the smallest clearance. The extremes of these two dimensions increase the difficulty of accessing blood soil with a brush and high impingement water.

Feature Name	Most Challenging Geometry	Coupon Image	Rationale
Sliding Shafts - Long	Sliding Length = 127.0 mm Minimal Clearance = 0.0381 mm (diametrical clearance of 0.0762mm)		The critical dimensions of a sliding shaft are the greatest length and the smallest clearance. The extremes of these two dimensions increase the difficulty of accessing blood soil with a brush and high impingement water.
Smooth Blind Lumen	Depth = 6.0 mm Diameter = 1.0 mm		The critical dimensions of a blind lumen are the smallest diameter and the greatest depth. The extremes of these two dimensions increase the difficulty of accessing blood soil with a brush and high impingement water. These two geometries can be expressed in terms of a ratio. As the diameter increases, so may the depth and vice versa. There were no true blind holes identified in the evaluation that had a diameter less than 1.5 mm and no device that resulted in a diameter to depth ratio greater than 4. Therefore, the master challenge device will have a diameter of 1.0 mm and depth ≥4.0 mm.
Smooth Through Lumen	Length = 277.2mm Diameter = 1.2 mm		The critical dimensions of a through lumen are the smallest diameter and the greatest length. The extremes of these two dimensions increase the difficulty of accessing blood soil with a brush and high impingement water.
Snap Rings	External Snap Ring		The snap ring fits semi-tightly against the external surface of the device. This increases the difficulty of accessing blood soil with a brush and high impingement water.
Spring - Internal	Internal Springs		The spring is located internally. This increases the difficulty of accessing blood soil with a brush and high impingement water.

Feature Name	Most Challenging Geometry	Coupon Image	Rationale
Threads – Blind Hole	Internal thread – Minor diameter - 2.599 mm Thread Depth – 25.4 mm Hole Depth – 35.0 mm		The critical dimensions of internal threads are diameter and thread length. Internal threads are more challenging to clean than external threads due to accessibility. This increases the difficulty of accessing blood soil with a brush and high impingement water. There were no internal threads identified in the evaluation that had a minimum diameter of less 2.8 mm and a thread depth greater than 25.4 mm. Therefore, the master challenge device will blind lumen representing this geometry.
Through Slot	Depth = 30.0 mm Width = 1.0 mm		The critical dimensions of a through slot are the smallest width and the greatest depth. The extremes of these two dimensions increase the difficulty of accessing blood soil with a brush and high impingement water.

Within this experimental design, the device feature was challenged under a variety of test conditions to understand the point of failure. Within each experimental design, only one variable was changed at a time. Otherwise, the most challenging test conditions were applied.

2hr Soil Dry – Soil was allowed to dry for a minimum of 2 hours at 22°C and 50%RH followed by manual cleaning.

2hr Dry No Brush/Flush – If protein values were > 3.2µg/cm^2, the brushing/flushing phase of the manual cleaning was omitted from the cleaning procedure following the 2hr soil dry.

1hr Soil Dry – If protein values were > 3.2µg/cm^2 from the 2hr soil dry experiment, an additional experiment with a 1hr dry was completed. Soil was dried for 1hr at 22°C and 50%RH.

1hr Soil Dry DBLSO – If protein values were > 3.2µg/cm^2 from the 1hr soil dry experiment, the soil recipe was changed to defibrinated blood soil (DBLSO) (ASTM, 2020) (Kremer, et al., 2022).

No Dry DBLSO - If protein values were > 3.2µg/cm^2 from the 1hr soil dry DBLSO experiment, the soil was prevented from drying by covering with a sopping wet OR towel and conditioned at 22°C and 50%RH for 2 hours.

72hr Soil Dry – Soil was allowed to dry for a minimum of 72 hours at 22°C and 50% RH followed by manual cleaning.

Extended Soak – If protein residual values fell below the alert level specified in ISO 15883-5 of 3.2µg/cm^2 (International Organization for Standardization, 2021), a pre-soak of 15 minutes in an alkaline cleaning agent was performed prior to the manual cleaning.

NOTE: The extended soak protocol failed with all tested device features to achieve protein residual values > 3.2µg/cm^2, so the protocol was canceled for some features.

Semi-Automated – Soil was applied but was prevented from drying by covering with a sopping wet OR towel and conditioned at 22°C and 50%RH for 2 hours. A semi-automated cleaning method was employed without any manual brushing or flushing after point of use treatment.

The decision tree for the test conditions is depicted in Figure 3.7:

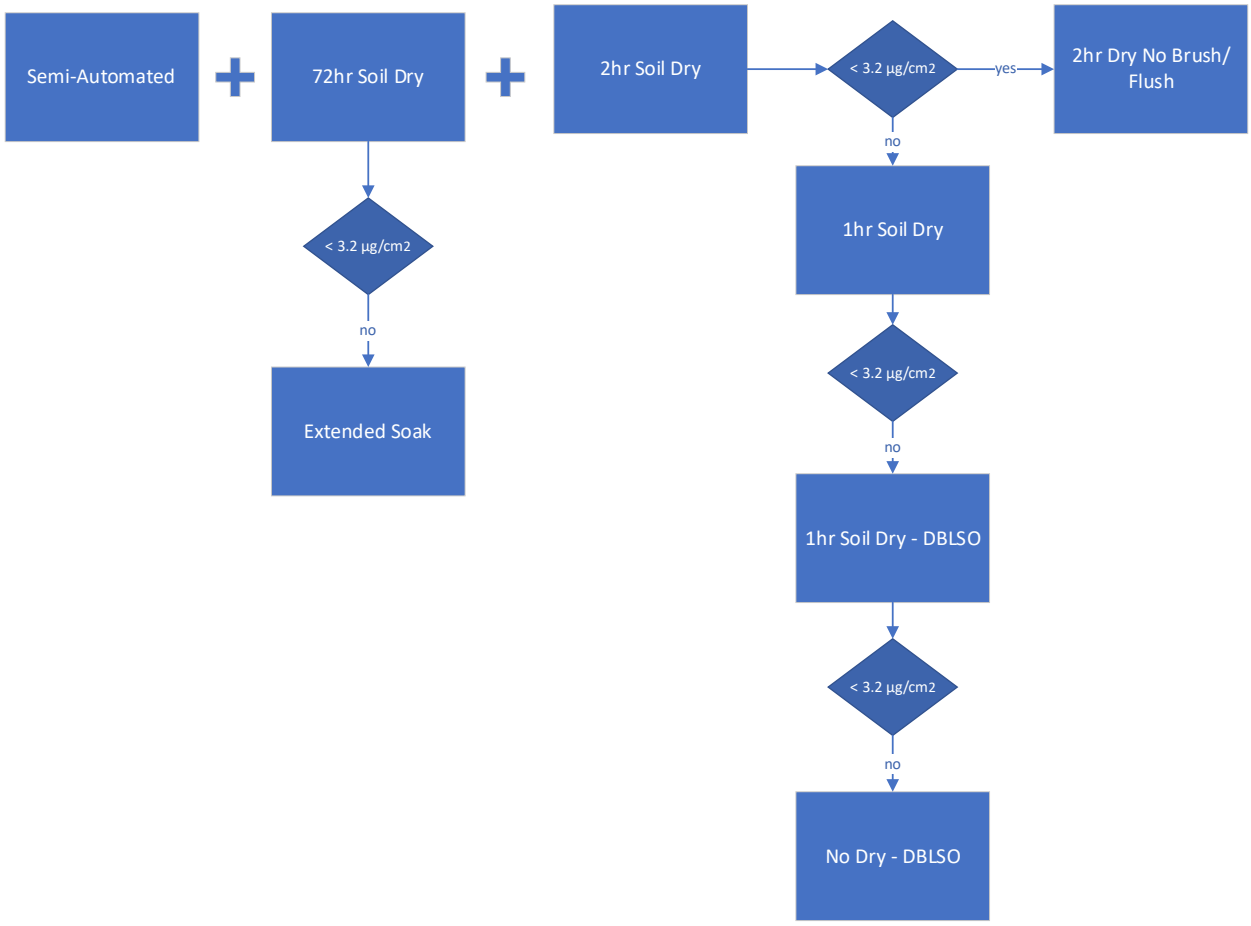


Figure 3.7: Testing Decision Tree

Each test condition was given a unique identifier starting with TV-VAL. The specific test conditions for each feature can be found in the applicable appendix.

Table 3.8: 23 Device Feature appendix list

Device Feature	Appendix #
Ball Detent / Ball Bearing	4
Ball Seal Springs	5
Blind Slot	6
Button w/ Spring	7
Buttons - Exposed Springs	8
Captured Screw	9
Hinges, Joints, Pivot Points	10
Leaf Springs	11
Mated Surfaces	12
Mated Surfaces Small Clearance	13
O-rings - External O-ring	14
O-rings - Internal O-ring	15
Rough Surface	16
Screws Threaded Rod / Threaded Thru Hole	17
Screws Threaded Rod/ Threaded Blind Hole	18
Sliding Shaft Short	19
Sliding Shafts Long	20
Smooth Blind Lumen	21
Smooth Through Lumen	22
Snap Rings	23
Spring Internal	24
Threads Blind Hole	25
Through Slot	26

3.3.1 2hr Soil Dry Experiment

Test articles were tested in sets of 5. Device features were soiled with modified coagulated blood. Soil was dried for a minimum of 2 hours at 22°C and 50% RH. Test articles were allowed to dry for 30 min on polypropylene sheet and then flipped to dry for an additional 1.5 hours at 22°C and 50% RH.

Test articles were cleaned using the following process:

1. Pre-rinse (point of use treatment) – Device feature was manually brushed to remove debris using a soft bristled brush (Sklar) and a lumen brush (Key Surgical). The feature was flushed with water with manual flushing of lumens and tight crevices using a syringe.
2. Manual Cleaning – Device features were soaked in Valsure® Enzymatic cleaning agent (Steris) at a concentration of 7.9mL cleaning agent to 1L of critical water and a temperature of ~20°C for 5 minutes. While immersed, a soft non-metallic bristle brush (Sklar) was used to thoroughly clean all traces of blood and debris from all device surfaces. During brushing, movable device features were actuated to expose all areas to the cleaning agent solution. All lumens were thoroughly brushed (Key Surgical) by pushing the brush through the entire length of the lumen using a

twisting motion to remove debris from both ends. A syringe to was used flush small clearances, moving, and intricate parts of the test articles in the open and closed position.

3. Ultrasonic Cleaning – Device features were ultrasonically cleaned for 10 minutes at 40kHz in Valsure® Enzymatic cleaning agent (Steris) prepared in accordance with the manufacturer's instructions at 3.9mL per 1L of ~25°C water. All lumens, blind holes, small clearances, and moving / intricate parts were flushed with cleaning agent solution to minimize the formation of air pockets or bubbles.
4. Intermediate Rinse - Device features were rinsed by immersion in ambient, <40°C, tap water for a minimum of one minute and until evidence of debris, soil, and cleaning solution were visually removed. Flushing was completed using a large syringe (50ml), filled to capacity with tap water, to thoroughly flush lumens, blind holes, small clearances, and moving and intricate parts. Actuation of joints, handles and other moveable device features was completed to rinse thoroughly.
5. Final Rinse – A repeated rinse was performed using ambient, < 40°C critical water for 15 seconds.
6. Drying – Device features were dried using a clean, soft, lint-free cloth or clean compressed air. All lumens and articulated areas were dried using compressed air. Device's moving parts were actuated during drying paying special attending to any device threads, ratchets and hinges or areas where fluid can accumulate.

Post cleaning, the device feature was extracted in water followed by SDS. The water extract was tested for protein, TOC, and ATP residuals. The SDS extract was tested for protein residuals only.

3.3.2 1hr Soil Dry Experiment

Test articles were tested in sets of 5. Device features were soiled with modified coagulated blood. Soil was dried for a minimum of 1hr at 22°C and 50% RH. Test articles were allowed to dry for 30 min on polypropylene sheet and then flipped to dry for an additional 30 minutes at 22°C and 50% RH.

Test articles were cleaned using the following process:

1. Pre-rinse (point of use treatment) – Device feature was manually brushed to remove debris using a soft bristled brush (Sklar) and a lumen brush (Key Surgical). The feature was flushed with water with manual flushing of lumens and tight crevices using a syringe.
2. Manual Cleaning – Device features were soaked in Valsure® Enzymatic cleaning agent (Steris) at a concentration of 7.9mL cleaning agent to 1L of critical water and a temperature of ~20°C for 5 minutes. While immersed, a soft non-metallic bristle brush (Sklar) was used to thoroughly clean all traces of blood and debris from all device surfaces. During brushing, movable device features were actuated to expose all areas to the cleaning agent solution. All lumens were thoroughly brushed (Key Surgical) by pushing the brush through the entire length of the lumen using a twisting motion to remove debris from both ends. A syringe to was used flush small clearances, moving, and intricate parts of the test articles in the open and closed position.

3. Ultrasonic Cleaning – Device features were ultrasonically cleaned for 10 minutes at 40kHz in Valsure® Enzymatic cleaning agent (Steris) prepared in accordance with the manufacturer's instructions at 3.9mL per 1L of ~25°C water. All lumens, blind holes, small clearances, and moving / intricate parts were flushed with cleaning agent solution to minimize the formation of air pockets or bubbles.
4. Intermediate Rinse - Device features were rinsed by immersion in ambient, <40°C, tap water for a minimum of one minute and until evidence of debris, soil, and cleaning solution were visually removed. Flushing was completed using a large syringe (50ml), filled to capacity with tap water, to thoroughly flush lumens, blind holes, small clearances, and moving and intricate parts. Actuation of joints, handles and other moveable device features was completed to rinse thoroughly.
5. Final Rinse – A repeated rinse was performed using ambient, < 40°C critical water for 15 seconds.
6. Drying – Device features were dried using a clean, soft, lint-free cloth or clean compressed air. All lumens and articulated areas were dried using compressed air. Device's moving parts were actuated during drying paying special attending to any device threads, ratchets and hinges or areas where fluid can accumulate.

Post cleaning, the device feature was extracted in water followed by SDS. The water extract was tested for protein, TOC, and ATP residuals. The SDS extract was tested for protein residuals only.

3.3.3 1hr Soil Dry DBLSO Experiment

Test articles were tested in sets of 5. Device features were soiled with DBLSO. Soil was dried for a minimum of 1hr at 22°C and 50% RH. Test articles were allowed to dry for 30 min on polypropylene sheet and then flipped to dry for an additional 30 minutes at 22°C and 50% RH.

Test articles were cleaned using the following process:

1. Pre-rinse (point of use treatment) – Device feature was manually brushed to remove debris using a soft bristled brush (Sklar) and a lumen brush (Key Surgical). The feature was flushed with water with manual flushing of lumens and tight crevices using a syringe.
2. Manual Cleaning – Device features were soaked in Valsure® Enzymatic cleaning agent (Steris) at a concentration of 7.9mL cleaning agent to 1L of critical water and a temperature of ~20°C for 5 minutes. While immersed, a soft non-metallic bristle brush (Sklar) was used to thoroughly clean all traces of blood and debris from all device surfaces. During brushing, movable device features were actuated to expose all areas to the cleaning agent solution. All lumens were thoroughly brushed (Key Surgical) by pushing the brush through the entire length of the lumen using a twisting motion to remove debris from both ends. A syringe to was used flush small clearances, moving, and intricate parts of the test articles in the open and closed position.
3. Ultrasonic Cleaning – Device features were ultrasonically cleaned for 10 minutes at 40kHz in Valsure® Enzymatic cleaning agent (Steris) prepared in accordance with the manufacturer's

instructions at 3.9mL per 1L of ~25°C water. All lumens, blind holes, small clearances, and moving / intricate parts were flushed with cleaning agent solution to minimize the formation of air pockets or bubbles.

4. Intermediate Rinse - Device features were rinsed by immersion in ambient, <40°C, tap water for a minimum of one minute and until evidence of debris, soil, and cleaning solution were visually removed. Flushing was completed using a large syringe (50ml), filled to capacity with tap water, to thoroughly flush lumens, blind holes, small clearances, and moving and intricate parts. Actuation of joints, handles and other moveable device features was completed to rinse thoroughly.
5. Final Rinse – A repeated rinse was performed using ambient, < 40°C critical water for 15 seconds.
6. Drying – Device features were dried using a clean, soft, lint-free cloth or clean compressed air. All lumens and articulated areas were dried using compressed air. Device's moving parts were actuated during drying paying special attending to any device threads, ratchets and hinges or areas where fluid can accumulate.

Post cleaning, the device feature was extracted in water followed by SDS. The water extract was tested for protein, TOC, and ATP residuals. The SDS extract was tested for protein residuals only.

3.3.4 No Dry DBLSO Experiment

Test articles were tested in sets of 5. Device features were soiled with DBLSO. Before placed to dry, the features were placed in a polypropylene bin and covered with a sopping wet operating room towel. Soil was dried for a minimum of 1hr at 22°C and 50% RH. Test articles were allowed to dry for 30 min on polypropylene sheet and then flipped to dry for an additional 30 minutes at 22°C and 50% RH.

Test articles were cleaned using the following process:

1. Pre-rinse (point of use treatment) – Device feature was manually brushed to remove debris using a soft bristled brush (Sklar) and a lumen brush (Key Surgical). The feature was flushed with water with manual flushing of lumens and tight crevices using a syringe.
2. Manual Cleaning – Device features were soaked in Valsure® Enzymatic cleaning agent (Steris) at a concentration of 7.9mL cleaning agent to 1L of critical water and a temperature of ~20°C for 5 minutes. While immersed, a soft non-metallic bristle brush (Sklar) was used to thoroughly clean all traces of blood and debris from all device surfaces. During brushing, movable device features were actuated to expose all areas to the cleaning agent solution. All lumens were thoroughly brushed (Key Surgical) by pushing the brush through the entire length of the lumen using a twisting motion to remove debris from both ends. A syringe to was used flush small clearances, moving, and intricate parts of the test articles in the open and closed position.
3. Ultrasonic Cleaning – Device features were ultrasonically cleaned for 10 minutes at 40kHz in Valsure® Enzymatic cleaning agent (Steris) prepared in accordance with the manufacturer's instructions at 3.9mL per 1L of ~25°C water. All lumens, blind holes, small clearances, and moving

/ intricate parts were flushed with cleaning agent solution to minimize the formation of air pockets or bubbles.

4. Intermediate Rinse - Device features were rinsed by immersion in ambient, <40°C, tap water for a minimum of one minute and until evidence of debris, soil, and cleaning solution were visually removed. Flushing was completed using a large syringe (50ml), filled to capacity with tap water, to thoroughly flush lumens, blind holes, small clearances, and moving and intricate parts. Actuation of joints, handles and other moveable device features was completed to rinse thoroughly.
5. Final Rinse – A repeated rinse was performed using ambient, < 40°C critical water for 15 seconds.
6. Drying – Device features were dried using a clean, soft, lint-free cloth or clean compressed air. All lumens and articulated areas were dried using compressed air. Device's moving parts were actuated during drying paying special attending to any device threads, ratchets and hinges or areas where fluid can accumulate.

Post cleaning, the device feature was extracted in water followed by SDS. The water extract was tested for protein, TOC, and ATP residuals. The SDS extract was tested for protein residuals only.

3.3.5 2hr Dry No Brush/Flush Experiment

Test articles were tested in sets of 5. Device features were soiled with modified coagulated blood. Soil was dried for a minimum of 2 hours at 22°C and 50% RH. Test articles were allowed to dry for 30 min on polypropylene sheet and then flipped to dry for an additional 1.5 hours at 22°C and 50% RH.

Test articles were cleaned using the following process:

1. Pre-rinse (point of use treatment) – Device feature was manually brushed to remove debris using a soft bristled brush (Sklar) and a lumen brush (Key Surgical). The feature was flushed with water with manual flushing of lumens and tight crevices using a syringe.
2. Manual Cleaning – Device features were soaked in Valsure® Enzymatic cleaning agent (Steris) at a concentration of 7.9mL cleaning agent to 1L of critical water and a temperature of ~20°C for 5 minutes.
3. Ultrasonic Cleaning – Device features were ultrasonically cleaned for 10 minutes at 40kHz in Valsure® Enzymatic cleaning agent (Steris) prepared in accordance with the manufacturer's instructions at 3.9mL per 1L of ~25°C water. All lumens, blind holes, small clearances, and moving / intricate parts were flushed with cleaning agent solution to minimize the formation of air pockets or bubbles.
4. Intermediate Rinse - Device features were rinsed by immersion in ambient, <40°C, tap water for a minimum of one minute and until evidence of debris, soil, and cleaning solution were visually removed. Flushing was completed using a large syringe (50ml), filled to capacity with tap water, to thoroughly flush lumens, blind holes, small clearances, and moving and intricate parts.

Actuation of joints, handles and other moveable device features was completed to rinse thoroughly.

5. Final Rinse – A repeated rinse was performed using ambient, < 40°C critical water for 15 seconds.
6. Drying – Device features were dried using a clean, soft, lint-free cloth or clean compressed air. All lumens and articulated areas were dried using compressed air. Device's moving parts were actuated during drying paying special attending to any device threads, ratchets and hinges or areas where fluid can accumulate.

Post cleaning, the device feature was extracted in water followed by SDS. The water extract was tested for protein, TOC, and ATP residuals. The SDS extract was tested for protein residuals only.

3.3.6 72hr Dry Experiment

Test articles were tested in sets of 5. Device features were soiled with modified coagulated blood. Soil was dried for a minimum of 72 hours at 22°C and 50% RH. Test articles were allowed to dry for 2 hours on polypropylene sheet and then flipped to dry for an additional 70 hours at 22°C and 50% RH.

Test articles were cleaned using the following process:

1. Pre-rinse (point of use treatment) – Device feature was manually brushed to remove debris using a soft bristled brush (Sklar) and a lumen brush (Key Surgical). The feature was flushed with water with manual flushing of lumens and tight crevices using a syringe.
2. Manual Cleaning – Device features were soaked in Valsure® Enzymatic cleaning agent (Steris) at a concentration of 7.9mL cleaning agent to 1L of critical water and a temperature of ~20°C for 5 minutes. While immersed, a soft non-metallic bristle brush (Sklar) was used to thoroughly clean all traces of blood and debris from all device surfaces. During brushing, movable device features were actuated to expose all areas to the cleaning agent solution. All lumens were thoroughly brushed (Key Surgical) by pushing the brush through the entire length of the lumen using a twisting motion to remove debris from both ends. A syringe to was used flush small clearances, moving, and intricate parts of the test articles in the open and closed position.
3. Ultrasonic Cleaning – Device features were ultrasonically cleaned for 10 minutes at 40kHz in Valsure® Enzymatic cleaning agent (Steris) prepared in accordance with the manufacturer's instructions at 3.9mL per 1L of ~25°C water. All lumens, blind holes, small clearances, and moving / intricate parts were flushed with cleaning agent solution to minimize the formation of air pockets or bubbles.
4. Intermediate Rinse - Device features were rinsed by immersion in ambient, <40°C, tap water for a minimum of one minute and until evidence of debris, soil, and cleaning solution were visually removed. Flushing was completed using a large syringe (50ml), filled to capacity with tap water, to thoroughly flush lumens, blind holes, small clearances, and moving and intricate parts. Actuation of joints, handles and other moveable device features was completed to rinse thoroughly.

5. Final Rinse – A repeated rinse was performed using ambient, < 40°C critical water for 15 seconds.
6. Drying – Device features were dried using a clean, soft, lint-free cloth or clean compressed air. All lumens and articulated areas were dried using compressed air. Device's moving parts were actuated during drying paying special attending to any device threads, ratchets and hinges or areas where fluid can accumulate.

Post cleaning, the device feature was extracted in water followed by SDS. The water extract was tested for protein, TOC, and ATP residuals. The SDS extract was tested for protein residuals only.

3.3.7 Extended Soak Experiment

Test articles were tested in sets of 5. Device features were soiled with modified coagulated blood. Soil was dried for a minimum of 72 hours at 22°C and 50% RH. Test articles were allowed to dry for 2 hours on polypropylene sheet and then flipped to dry for an additional 70 hours at 22°C and 50% RH.

Test articles were cleaned using the following process:

1. Pre-soak – The device feature was soaked in an alkaline cleaning agent of Neodisher® MediClean Forte (Dr. Weigert) prepared at a concentration of 10mL per 1L of water and warmed to be between 32°C and 49°C for 15 minutes.
2. Pre-rinse (point of use treatment) – The device feature was manually brushed to remove debris using a soft bristled brush (Sklar) and a lumen brush (Key Surgical). The feature was flushed with water with manual flushing of lumens and tight crevices using a syringe.
3. Manual Cleaning – Device features were soaked in Valsure® Enzymatic cleaning agent (Steris) at a concentration of 7.9mL cleaning agent to 1L of critical water and a temperature of ~20°C for 5 minutes. While immersed, a soft non-metallic bristle brush (Sklar) was used to thoroughly clean all traces of blood and debris from all device surfaces. During brushing, movable device features were actuated to expose all areas to the cleaning agent solution. All lumens were thoroughly brushed (Key Surgical) by pushing the brush through the entire length of the lumen using a twisting motion to remove debris from both ends. A syringe to was used flush small clearances, moving, and intricate parts of the test articles in the open and closed position.
4. Ultrasonic Cleaning – Device features were ultrasonically cleaned for 10 minutes at 40kHz in Valsure® Enzymatic cleaning agent (Steris) prepared in accordance with the manufacturer's instructions at 3.9mL per 1L of ~25°C water. All lumens, blind holes, small clearances, and moving / intricate parts were flushed with cleaning agent solution to minimize the formation of air pockets or bubbles.
5. Intermediate Rinse - Device features were rinsed by immersion in ambient, <40°C, tap water for a minimum of one minute and until evidence of debris, soil, and cleaning solution were visually removed. Flushing was completed using a large syringe (50ml), filled to capacity with tap water, to thoroughly flush lumens, blind holes, small clearances, and moving and intricate parts.

Actuation of joints, handles and other moveable device features was completed to rinse thoroughly.

6. Final Rinse – A repeated rinse was performed using ambient, < 40°C critical water for 15 seconds.
7. Drying – Device features were dried using a clean, soft, lint-free cloth or clean compressed air. All lumens and articulated areas were dried using compressed air. Device's moving parts were actuated during drying paying special attending to any device threads, ratchets and hinges or areas where fluid can accumulate.

Post cleaning, the device feature was extracted in water followed by SDS. The water extract was tested for protein, TOC, and ATP residuals. The SDS extract was tested for protein residuals only.

3.3.8 Semi-Automated Experiment

Test articles were tested in sets of 5. Device features were soiled with modified coagulated blood. Before placed to dry, the features were placed in a polypropylene bin and covered with a sopping wet operating room towel. Soil was dried for a minimum of 2 hours at 22°C and 50% RH. Test articles were allowed to dry for 30 min on polypropylene sheet and then flipped to dry for an additional 1.5 hours at 22°C and 50% RH.

Test articles were cleaned using the following process:

1. Pre-rinse (point of use treatment) – Device feature was rinsed under cold running water to remove gross soil.
2. Manual Cleaning – Device features were soaked in Valsure® Enzymatic cleaning agent (Steris) at a concentration of 7.9mL cleaning agent to 1L of critical water and a temperature of ~20°C for 15 minutes.
3. Rinse – Device feature was rinsed by submersing in Critical water 3 times.
4. Ultrasonic Cleaning – Device features were ultrasonically cleaned for 15 minutes at 40kHz in an alkaline cleaning agent of Neodisher MediClean Forte prepared at a concentration of 10mL per 1L of water and warmed to be between 32°C and 49°C.
5. Rinse – Device feature was rinsed by submersing in Critical water 3 times.
6. Automated Cleaning – Features were loaded into the Getinge washer disinfector model 8668 so that each basket was accessible to the washer arms. Features with hinges were actuated into the open position. Empty spaces within the rack were filled with dunnage baskets filled with hemostatic clamps to simulate a full washer-disinfector loading pattern. Figure 3.8 is an example loading pattern.

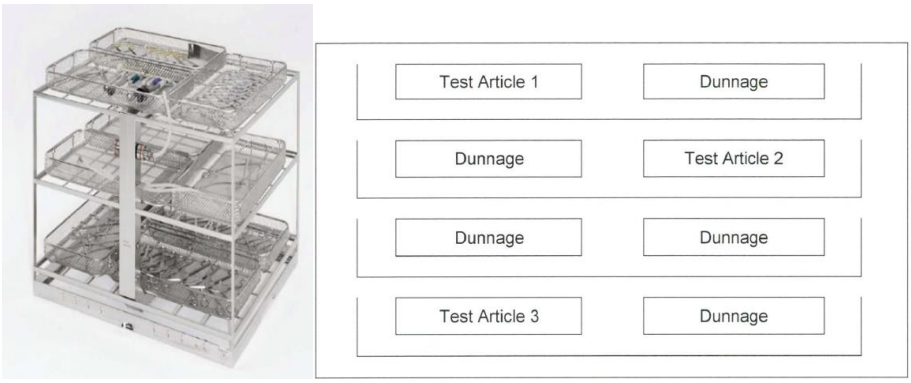


Figure 3.8: Washer-disinfector Loading Pattern

The following washer-disinfector cycle was programed:

Phase	Recirculation Time (min)	Temperature	Detergent Type and Concentration (if applicable)
Pre-wash 1	02:00	Cold tap water	N/A
Wash 1	01:00	43°C (109°F)	Valsure Enzymatic 1.9mL/L
Wash 2	05:00	66°C (151°F) (Set Point)	Valsure Neutral 1.9mL/L
Rinse 1	02:00	Hot tap water	N/A
Rinse 2	00:15	Ambient DI	N/A
Thermal Disinfection	01:00	43°C (109°F) (Critical Water)	N/A
Dry Time	07:00	90°C	N/A

Post cleaning, the device feature was extracted in water followed by SDS. The water extract was tested for protein, TOC, and ATP residuals. The SDS extract was tested for protein residuals only.

3.4 Analyte Calculations

Negative Sample Control – No Calculation was performed, but if the instrument result was a negative number, then the method LOD, 0.023µg/mL for protein and 0.0035 µg/mL for TOC, was substituted in calculations using the negative sample control.

Positive Sample Control – The corrected instrument concentration was calculated by subtracting the negative sample control from the instrument result for the positive sample control. The total residual amount / extraction volume was calculated by multiplying the corrected positive sample control by the extraction volume.

Negative Device Control - The corrected instrument concentration was calculated by subtracting the negative sample control from the instrument result for the negative device control. The total residual amount / extraction volume was calculated by multiplying the corrected instrument concentration by the

extraction volume. The total residual amount / surface area, i.e., residual concentration, was calculated by multiplying the corrected instrument concentration by the by the extraction volume divided by the feature surface.

$$Residual\ Concentration\ \left(\frac{\mu g}{cm^2}\right) = \frac{Corrected\ Concentration\ \left(\frac{\mu g}{mL}\right) x\ Extraction\ Volume\ (mL)}{Surface\ Area\ cm^2}$$

Positive Device Control - The corrected instrument concentration was calculated by subtracting the negative sample control from the instrument result for the positive device control and applying a correction for the dilution. For Protein, the result was multiplied by 20 to correct for the 1:20 dilution factor. For TOC, the result was multiplied by 40 to correct for the 1:40 dilution factor. The total residual amount / extraction volume was calculated by multiplying the corrected instrument concentration by the extraction volume. The total residual amount / surface area, i.e., residual concentration, was calculated by multiplying the corrected instrument concentration by the by the extraction volume divided by the feature surface.

$$Residual\ Concentration\ \left(\frac{\mu g}{cm^2}\right) = \frac{Corrected\ Concentration\ \left(\frac{\mu g}{mL}\right) x\ Extraction\ Volume\ (mL)}{Surface\ Area\ cm^2}$$

Test Articles - The corrected instrument concentration, i.e., corrected extraction was calculated by subtracting the negative sample control from the instrument result for the test article. The residual concentration was calculated by multiplying the corrected extraction by the extraction volume divided by the feature surface area. The corrected residual concentration was calculated by applying the method validation correction factor for each analyte as applicable to the calculation.

- Protein Water Extraction - The corrected residual concentration compendial extraction was calculated by multiplying the residual concentration for the water by the compendial extraction efficiency / SDS correction factor.
- Protein Additive Extraction – The residual concentrations for water and SDS were added for a total concentration. The corrected residual concentration additive extraction was calculated by multiplying the total concentration by the additive extraction efficiency correction factor.
- TOC extraction – The residual concentration was multiplied by the extraction efficiency correction factor.

A one-sample t-test was used to calculate the 99% confidence intervals of the sample set specific to each test scenario. Utilizing the upper value of the 99% confidence interval as the worst-case result to evaluate the sample set against the acceptance criteria serves as a robust strategy to mitigate the risk of error in statistical analyses and decision-making processes.

3.5 Analyte Acceptance Criteria

Each of the test articles and the high value of the 99% confidence interval must be less than acceptance criteria (Table 3.9) to be considered passing.

Table 3.9: Analyte acceptance criteria

Analyte	Acceptance Criteria
Protein	≤ 6.4 µg/ cm^2
TOC	≤ 12 µg/ cm^2

Chapter 4: Results & Discussion

4.1 The Device Feature Approach Validation

The method validation and cleaning efficacy results for the Device Feature Approach Validation are detailed in Appendix 2 and 3. However, a summary of the findings is provided within this chapter.

Using the exhaustive recovery extraction efficiency method, recovery efficiencies and correction factors were calculated for each coupon (Table 4.1).

Table 4.1: Method validation results for Device Feature Approach Validation

Coupon Type	Total Extraction Volume	Average Extraction Efficiency	Correction Factor
Single Feature – 20mm	2.4mL	93.418%	1.0658
Single Feature – 30mm	4.5mL	87.837%	1.1216
Single Feature – 40mm	6.0mL	88.214%	1.1179
Multiple Feature – 20mm	60mL	98.039%	1.0196
Multiple Feature – 30mm	112.5mL	96.477%	1.0352
Multiple Feature – 40mm	150mL	93.282%	1.0672

The results for the controls for each of the test coupons demonstrated the test system was in a state of control and that the test coupons were appropriately challenged. Because the results of the single feature test coupon were compared to the multiple feature test coupon, the protein residual results for the single lumen and multiple lumen coupons post cleaning are shown together in the tables below organized by lumen length. Table 4.2 reflect the results from the 20mm coupons, Table 4.3 reflects the 30mm coupons and Table 4.4 reflects the 40mm coupons. Table 4.5 is a summary of the ATP results that were recorded. Due to the soil used, ATP results indicate it was not an appropriate cleaning marker (very low values).

The cleaning method for a reusable medical device can be validated using the actual medical device, test coupons, or process control devices that are representative of the individual features found on the device (International Standard Organization, 2021). Cleaning efficacy recommended test methods and acceptance criteria commonly used by medical device manufactures are provided in ANSI/AAMI ST98:2022 *Cleaning validation of healthcare products – Requirements for development and validation of a cleaning process for medical devices* (Association for the Advancement of Medical Instrumentation, 2022) and ISO *15883-1, Washer-disinfectors Part 1: General requirements, terms and definitions and tests* (International Organization for Standardization, 2021) and *ISO 15883-5, Washer-disinfectors Part 5: Performance requirements and test method criteria for demonstrating cleaning efficacy* (International Organization for Standardization, 2021). These criteria are based on the surface area of the device, but also consider the cumulative effects on the whole device.

The device feature approach focuses the validation exclusively on the device features that pose a known challenge to cleaning without including the surface area from other exposed parts of the actual device

that are not a challenge for cleaning. The results of the device feature testing can then be directly applied during the evaluation of actual devices to validate by equivalency. The results can also be applied to devices that contain multiple features. For example, a device that contains multiple lumens without any other challenging features can be validated by equivalency using the results from one lumen feature validation if the lumen feature is more challenging than the lumens found on the actual device. As the number of lumens increase, so does the surface area, keeping the amount of analyte (e.g., protein) per cm^2 the same. Furthermore, the amount of analyte per cm^2 is likely to decrease given the addition of any smooth surface area that is not a significant challenge for cleaning.

To validate the device feature approach the most challenging to clean feature was challenged to demonstrate that the device feature approach is a more conservative method of calculating the presence of residual analyte remaining on a device post cleaning. A full report for the method validation can be found in Appendix 2 and the cleaning efficacy results are reported in Appendix 3.

The feature selected, the dead-end lumen. The dead-end lumen requires a backflow of the flush once it hits the dead-end for the soil to be removed from the feature. This requires competing pressure gradients in the lumen and can limit the sheer force of the liquid over the surface resulting in ineffective soil removal. The longer the lumen and the smaller the diameter the more challenging this feature becomes. As the diameter narrows, the competing flow of the liquid increases. The length of the lumen will require more force for the liquid to reach the dead-end with enough flow velocity for the liquid to exit the lumen.

Table 4.2: Protein cleaning efficacy results for 20mm coupons

Coupon #	20mm Single Lumen		20mm Multiple Lumen	
	Total Lumen Concentration µg/cm²	Total Device Concentration µg/cm²	Total Lumen Concentration µg/cm²	Total Device Concentration µg/cm²
1	36.111	3.248	38.629	11.091
2	25.490	2.292	20.714	5.947
3	31.158	2.802	24.805	7.122
4	29.981	2.696	34.263	9.838
5	28.477	2.561	32.269	9.265
6	34.251	3.080	30.524	8.764
7	32.997	2.968	13.729	3.942
8	23.685	2.130	19.633	5.637
9	57.183	5.143	15.693	4.506
10	44.759	4.025	20.234	5.810
11	52.413	4.714	21.596	6.201
12	42.055	3.782	24.310	6.980
13	16.699	1.502	17.039	4.892
14	17.792	1.600	13.806	3.964
15	12.658	1.138	22.480	6.454
16	16.127	1.450	17.056	4.897
17	15.995	1.438	18.597	5.340
18	13.948	1.254	13.972	4.012
19	26.259	2.362	31.729	9.110
20	25.852	2.325	32.051	9.203
21	26.137	2.351	37.821	10.859
22	21.534	1.937	36.711	10.541
23	25.089	2.256	25.718	7.384
24	21.355	1.921	37.123	10.659
25	28.300	2.545	29.636	8.509
26	27.293	2.455	32.419	9.308
27	18.781	1.689	28.051	8.054
28	19.420	1.746	25.798	7.407
29	22.889	2.059	32.849	9.432
30	25.795	2.320	25.609	7.353
Average	**27.350**	**2.460**	**25.829**	**7.416**

Table 4.3: Protein cleaning efficacy results for 30mm coupons

Coupon #	30mm Single Lumen		30mm Multiple Lumen	
	Total Lumen Concentration µg/cm²	Total Device Concentration µg/cm²	Total Lumen Concentration µg/cm²	Total Device Concentration µg/cm²
1	20.733	2.676	22.322	8.407
2	24.605	3.176	26.911	10.135
3	19.383	2.502	27.102	10.207
4	21.293	2.748	27.994	10.543
5	22.152	2.859	25.970	9.781
6	32.996	4.259	26.593	10.015
7	18.438	2.380	44.305	16.686
8	22.854	2.950	24.475	9.218
9	21.084	2.721	22.878	8.616
10	16.002	2.065	24.302	9.152
11	22.171	2.861	26.832	10.106
12	17.579	2.269	23.451	8.832
13	20.821	2.687	19.040	7.171
14	23.625	3.049	19.161	7.216
15	34.394	4.439	21.924	8.257
16	26.902	3.472	20.950	7.890
17	20.714	2.673	23.540	8.866
18	30.394	3.923	25.648	9.660
19	23.868	3.081	20.903	7.873
20	22.995	2.968	19.087	7.188
21	23.277	3.004	19.969	7.521
22	25.649	3.310	21.919	8.255
23	23.046	2.974	24.381	9.182
24	21.145	2.729	18.195	6.852
25	31.845	4.110	24.638	9.279
26	24.198	3.123	23.187	8.733
27	21.097	2.723	24.242	9.130
28	30.316	3.913	17.881	6.734
29	21.309	2.750	26.098	9.829
30	26.415	3.409	21.205	7.986
Average	**23.710**	**3.060**	**23.837**	**8.977**

Table 4.4: Protein cleaning efficacy results for 40mm coupons

Coupon #	40mm Single Lumen		40mm Multiple Lumen	
	Total Lumen Concentration µg/cm²	Total Device Concentration µg/cm²	Total Lumen Concentration µg/cm²	Total Device Concentration µg/cm²
1	16.278	2.685	13.391	5.974
2	19.110	3.153	17.067	7.614
3	16.198	2.672	17.747	7.918
4	14.204	2.343	15.633	6.974
5	13.129	2.166	20.754	9.259
6	15.056	2.484	17.617	7.860
7	23.826	3.931	19.182	8.558
8	15.859	2.616	16.010	7.143
9	14.637	2.415	18.703	8.344
10	11.341	1.871	18.292	8.161
11	17.914	2.955	20.430	9.115
12	15.104	2.492	19.317	8.618
13	47.972	7.914	54.972	24.526
14	30.410	5.017	77.547	34.597
15	19.742	3.257	38.432	17.146
16	34.868	5.752	17.661	7.879
17	29.850	4.924	37.250	16.619
18	43.929	7.247	55.079	24.573
19	30.424	5.019	10.193	4.548
20	32.903	5.428	1.144	0.510
21	28.265	4.663	6.978	3.113
22	43.763	7.220	6.224	2.777
23	34.467	5.686	10.356	4.620
24	34.304	5.659	7.742	3.454
25	19.529	3.222	7.337	3.274
26	32.183	5.309	4.945	2.206
27	24.365	4.020	5.032	2.245
28	18.170	2.998	1.890	0.843
29	23.775	3.922	7.982	3.561
30	24.523	4.046	5.743	2.562
Average	**24.870**	**4.103**	**19.022**	**8.486**

Table 4.5: ATP cleaning efficacy results

ATP Results						
Coupon #	**20mm Single Lumen**	**30mm Single Lumen**	**40mm Single Lumen**	**20mm Multiple Lumen**	**30mm Multiple Lumen**	**40mm Multiple Lumen**
1	0	1	8	0	6	2
2	1	5	1	0	7	1
3	1	1	3	0	1	1
4	5	3	5	0	4	1
5	1	3	1	0	16	3
6	2	3	2	0	18	3
7	0	3	*	10	81	6
8	8	3	*	11	2	6
9	4	3	*	3	9	3
10	1	7	*	3	15	2
11	0	1	*	12	14	3
12	3	6	*	12	9	7
13	4	12	3	37	0	2
14	1	2	7	19	0	3
15	3	1	1	1	0	1
16	4	1	0	2	2	0
17	4	2	5	20	1	2
18	3	5	1	28	1	5
19	3	2	2	3	1	1
20	2	0	0	5	5	2
21	1	5	2	8	10	0
22	0	1	1	16	5	1
23	5	1	1	8	2	1
24	2	2	2	20	14	4
25	2	13	4	7	0	*
26	8	6	8	9	0	*
27	14	1	9	7	0	*
28	22	2	6	11	1	*
29	10	1	2	7	2	*
30	1	3	2	28	3	*
Average	**4**	**3**	**3**	**10**	**8**	**3**

*Not measured

The cleaning efficacy results from the device feature samples demonstrate the design feature approach to be a more conservative approach when estimating protein residual concentration. The design feature approach isolates the most difficult to clean feature of the device for the cleaning challenge whereas the compendial method challenges the entirety of the device. In the design feature approach, only the surface area from the individual difficult to clean features are used to calculate the extraction volume and soil application amount in the test system. The surface area is then used to calculate the residual protein level per cm^2. The compendial method uses the entirety of the device, including easy to clean features and surfaces of the device. As such, the whole surface area of the device is included in the calculations and underestimate the impact of the cleaning challenge in the more challenging features.

Comparing the residual protein concentrations between the design feature approach and the compendial method, it is evident that the concentration of protein is diluted when using the surface area of the entire device (see Appendix 3). This dilution of the analyte can result in passing results when the most challenging device feature still harbors residual soil in a concentration above the acceptable level.

For example, if a medical device is used to flush a solution into the patient, the lumened portion of the device is the highest risk feature as the fluid pathway of the lumen will deposit fluid into the patient whereas the rest of the device is only external communicating. Although other features within the device may be difficult to clean, if the surface of the lumen will have direct contact with the fluid being flushed through it, remaining soil in the lumen is of the highest risk to the patient. Once fluid flows through the lumen, any residual soil solubilized in the fluid pathway and inserted into the patient becomes a major concern to patient safety.

When employing the recommended techniques for assessing cleaning effectiveness, it is customary to utilize the entire surface area of the device (or, in certain instances, each side of the device) for calculating residual concentration in relation to the surface area (Association for the Advancement of Medical Instrumentation, 2022). This approach may result in an underestimated concentration of residual contaminants in the most challenging-to-clean parts of the device, potentially diluting the analyte below the limit of detection for the test method (refer to Figure 4.1). In contrast, the device feature approach involves scrutinizing the most challenging area of the medical device for cleanability and comparing the results against established acceptance criteria. Hence, the device feature approach emerges as the most suitable and conservative method for conducting a risk assessment.

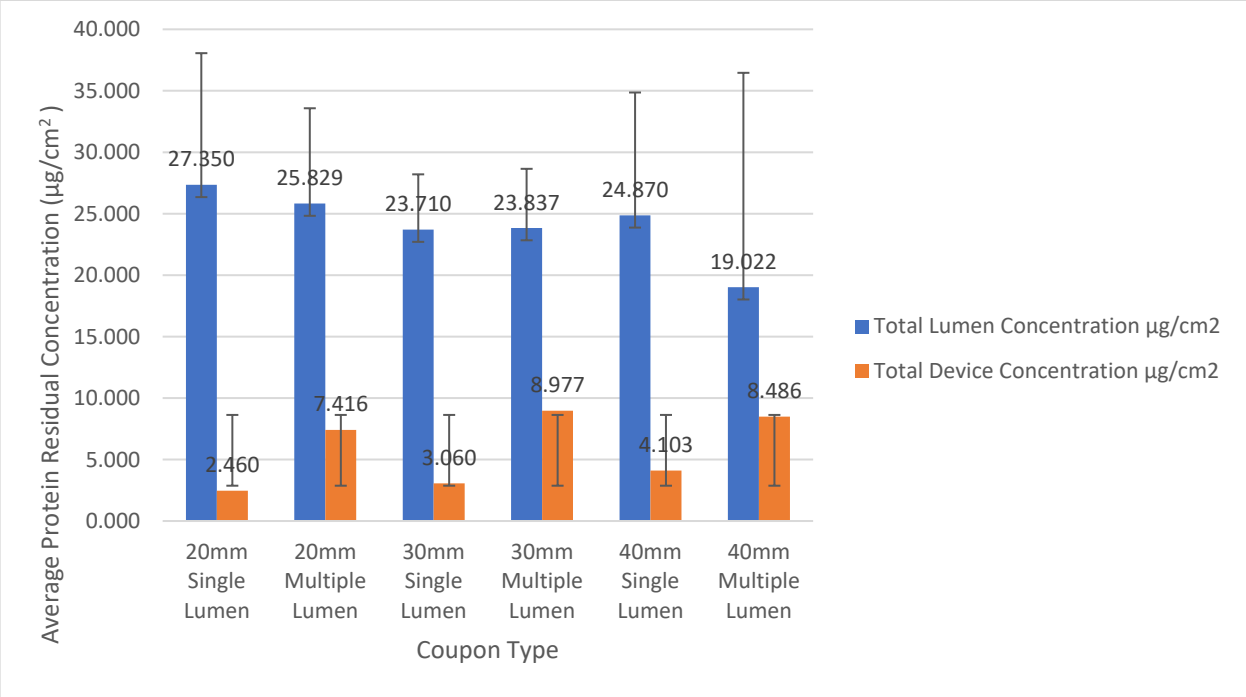


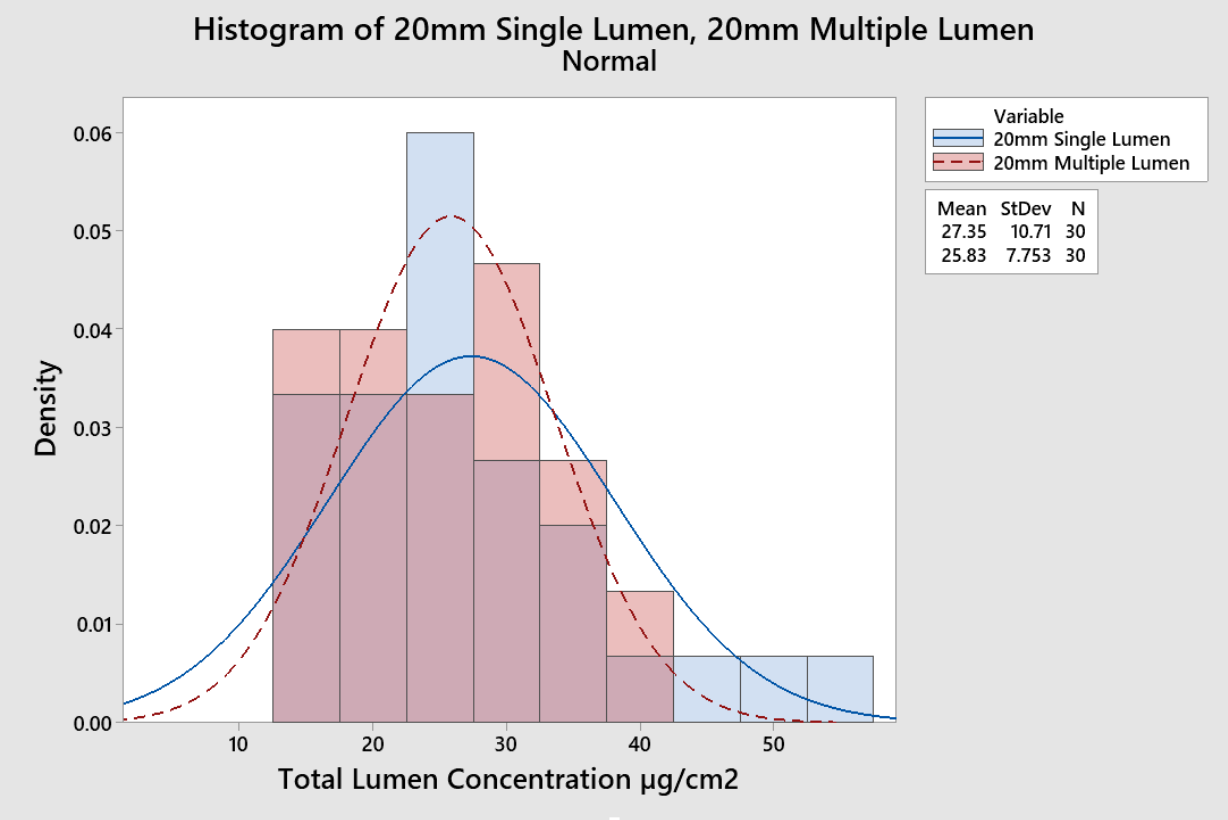
Figure 4.1: Protein Residual Concentration for Design Feature Approach vs Compendial Method

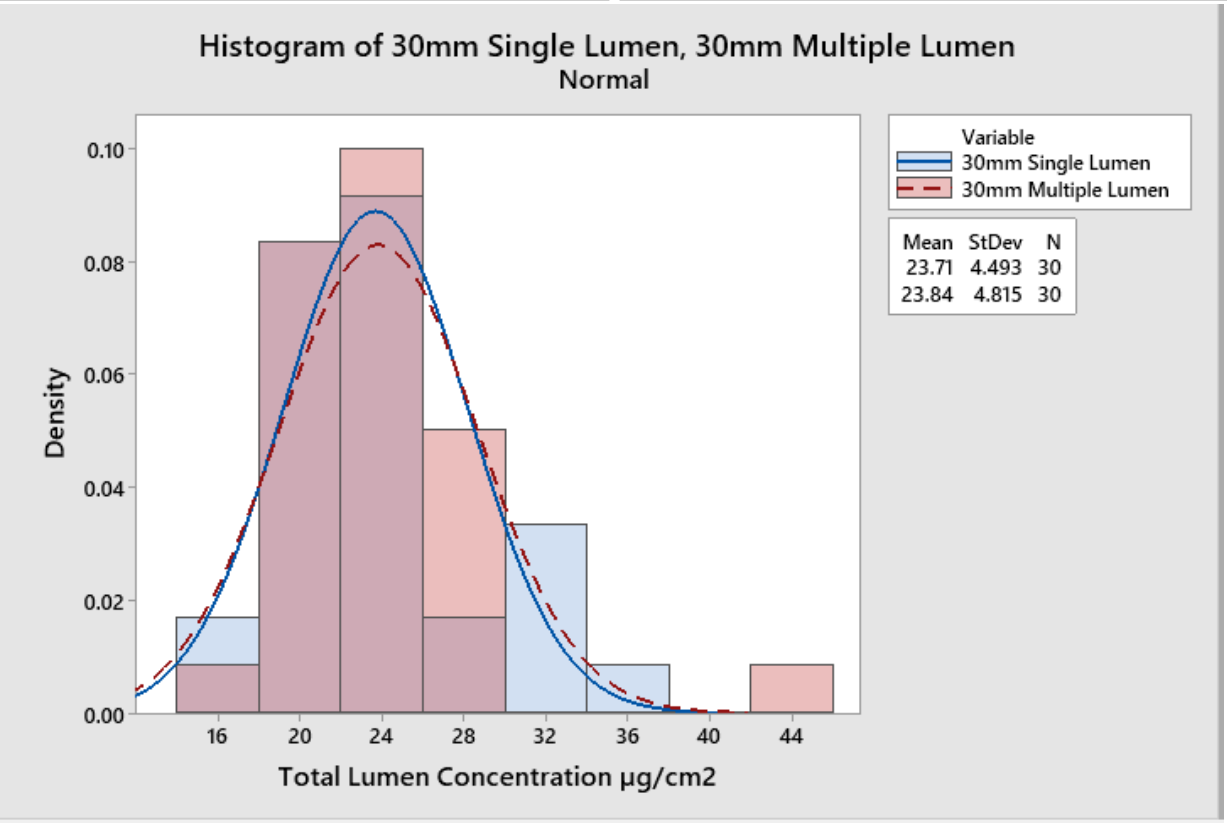
The data from both coupon types at 20, 30, and 40mm was normally distributed as demonstrated by the probability density of results. When comparing the cleaning efficacy of the devices with a 20, 30, and 40mm single lumen to the results from the devices with twenty-five 20, 30, and 40mm lumens, it is evident that by using the associated surface areas they were statistically similar with the p-value of 0.534 for the 20mm devices, 0.925 for the 30mm devices, and 0.079 for the 40mm devices. All means, except for the 40mm multiple features were statistically similar with a p-value of 0.368.

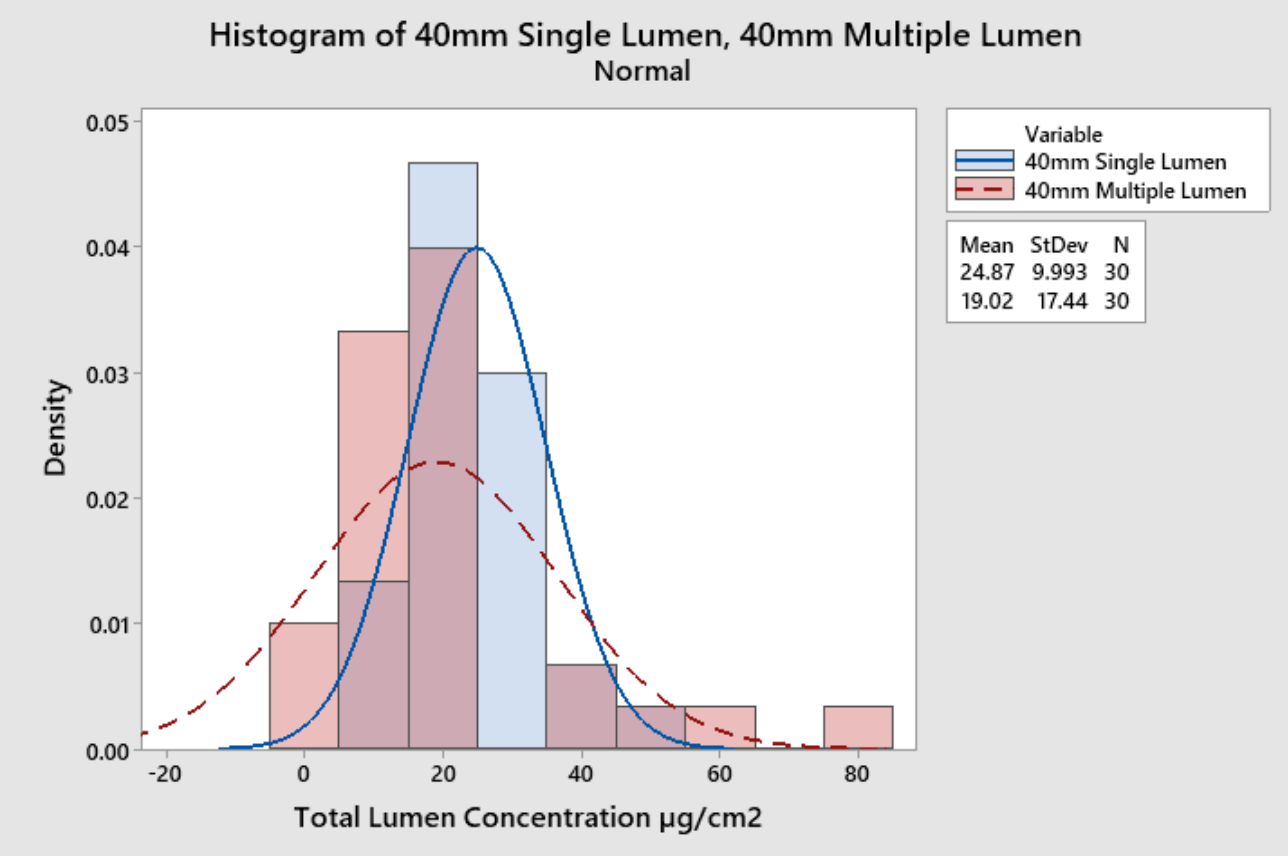
Results for the multiple features to the single feature demonstrated statistical equivalence (see Appendix 3). This highlights that by isolating the worst-case feature from a device and challenging it against the worst-case cleaning conditions, all features on the device will be assessed for cleaning efficacy. In simple terms, if the cleaning instructions can clean the hardest to clean location of the device, the remaining features, which are easier to clean, will also meet the cleaning requirement. Results from each of the three coupon types that both test designs, single and multiple lumens, had normally distributed data and statistically equivalent data when the surface area was applied to deliver the result in µg/cm^2 (Figure 4.2). The device feature validation confirmed that a process challenge feature can effectively evaluate the risk associated with a larger device or family of devices, thereby supporting subsequent experiments focused solely on the 23 device features to establish a cleaning classification. This achievement aligns with the following research aim outlined in Chapter 1:

(ii) To ascertain if device features can be used as an important novel independent variable within cleaning validations.

Figure 4.2: Histograms of Single vs Multiple Lumen Results Demonstrating Normal Distribution and Statistical Equivalence







The device feature approach validation also demonstrated that when a sufficient sample size is tested for cleaning efficacy, the resulting values are normally distributed. The value of having normally distributed data lies in its statistical advantages and the convenience it offers in various analyses. The normal distribution, often referred to as the bell curve, is characterized by a symmetrical shape, with the majority of data points clustering around the mean. This distribution facilitates the application of many statistical methods, such as the calculation of probabilities and confidence intervals. Additionally, in inferential statistics, normality simplifies hypothesis testing and enables the use of parametric tests, which are generally more powerful than their non-parametric counterparts. Normal distribution is a common assumption in many statistical models and allows for the generalization of findings to a broader population. Overall, the prevalence of normally distributed data enhances the reliability and interpretability of statistical analyses. Because the data was demonstrated in the device feature validation to be normally distributed (see Appendix 3), statistical evaluations for the 23 features will be completed accordingly.

Full experimental design and research results for the device feature approach validation are described in appendices 2 and 3 and have been subsequently published (Kremer, et al., 2023). The device feature approach can be used to develop a design feature database that can be used to design and validate device cleanliness. It can also be used to commensurately develop a quantitative cleaning classification system that will augment and innovate the effectiveness of the Spaulding Classification for microbial risk

reduction. This report constitutes a study that investigates this validation approach to verify the efficacy of device cleaning procedures and mitigate patient risk. This feature categorization approach will help to close the existing patient safety gap at the important interface between device manufacturers and healthcare facilities for the effective and reliable processing of reusable medical devices. A total of 56,000 extractions of the device features were conducted in this study and highlights the rigor associated with the validation. Generating information from design features as a critical control point for cleaning and microbiological quality will inform future digital transformation of the medical device industry and healthcare delivery, including automation.

4.2 23 Features Extraction Method Validation

The extraction efficiency was calculated in two ways (Table 4.6 and Table 4.7), the compendial exhaustive extraction efficiency and the additive exhaustive extraction efficiency. For both methods, the instrument concentration was corrected by subtracting the negative sample control concentration for the appropriate eluent from each concentration result to determine the "Corrected Instrument Concentration". If the instrument result for the negative sample control resulted in a negative number, then the method LOD was substituted in the calculation. The first extraction result was corrected for the dilution factor (1:20) by multiplying the corrected concentration by 20 to calculate "Corrected Dilution Concentration". Unless otherwise diluted, the remaining extractions were multiplied by 1.

The compendial exhaustive extraction efficiency with water only was calculated by dividing the 1:20 extraction (water) by the sum of all the extractions the water extractions multiplied by 100.
The compendial exhaustive extraction efficiency with SDS extraction was calculated by dividing the 1:20 extraction (water) by the sum of all the extractions multiplied by 100.

The additive extraction exhaustive extraction efficiency for the protein testing was calculated by adding the 1:20 extraction (water) plus the second extraction (SDS) and dividing by the sum of all the extractions multiplied by 100.

Table 4.6: 72hr soil dry method validation extraction efficiency results

Device Feature – 72hr Dry	Protein Additive Extraction – Ave Exhaustive Extraction Efficiency	Protein Compendial – Ave Exhaustive Extraction Efficiency w/ SDS	TOC Ave Extraction Efficiency
Ball Detent / Ball Bearing	98.095%	75.163%	96.3465%
Ball Seal Springs	95.407%	81.585%	96.5754%
Blind Slot	86.247%	79.407%	90.4004%
Button w/ Spring	97.716%	58.705%	95.0360%
Buttons - Exposed Springs	97.716%	58.705%	95.0360%
Captured Screw	99.623%	57.323%	96.4285%
Hinges, Joints, Pivot Points	89.988%	88.601%	90.1588%
Leaf Springs	99.977%	96.660%	99.2599%
Mated Surfaces	95.756%	91.850%	95.8175%
Mated Surfaces Small Clearance	95.957%	93.594%	96.6463%
O-rings - External O-ring	98.635%	92.223%	95.0237%
O-rings - Internal O-ring	95.906%	81.056%	91.0928%
Rough Surface	99.783%	98.097%	99.2233%
Screws Threaded Rod / Threaded Thru Hole	99.775%	91.966%	96.0339%
Screws Threaded Rod/ Threaded Blind Hole	99.320%	98.030%	96.7571%
Sliding Shaft Short	99.128%	96.452%	98.3958%
Sliding Shafts Long	99.863%	97.844%	54.7300%
Smooth Blind Lumen	98.251%	94.691%	98.7278%
Smooth Through Lumen	98.099%	97.099%	93.1769%
Snap Rings	98.700%	88.635%	93.5469%
Spring Internal	98.070%	87.980%	93.0076%
Threads Blind Hole	98.332%	96.186%	95.9214%
Through Slot	87.847%	58.520%	92.7300%

Table 4.7: 2hr soil dry method validation extraction efficiency results

Device Feature – 2hr Dry	Protein Additive Extraction – Exhaustive Extraction Efficiency	Protein Compendial – Exhaustive Extraction Efficiency w/ SDS	TOC Extraction Efficiency
Ball Detent / Ball Bearing	98.588%	96.389%	98.0507%
Ball Seal Springs	99.863%	98.812%	98.3921%
Blind Slot	99.876%	99.869%	95.1537%
Button w/ Spring	99.275%	97.079%	96.4398%
Buttons - Exposed Springs	99.275%	97.079%	96.4398%
Captured Screw	99.538%	92.218%	96.9506%
Hinges, Joints, Pivot Points	NA	NA	NA
Leaf Springs	NA	NA	NA
Mated Surfaces	NA	NA	NA
Mated Surfaces Small Clearance	NA	NA	NA
O-rings - External O-ring	NA	NA	NA
O-rings - Internal O-ring	99.911%	99.882%	91.2950%
Rough Surface	NA	NA	NA
Screws Threaded Rod / Threaded Thru Hole	NA	NA	NA
Screws Threaded Rod/ Threaded Blind Hole	NA	NA	NA
Sliding Shaft Short	NA	NA	NA
Sliding Shafts Long	94.451%	93.057%	61.1100%
Smooth Blind Lumen	NA	NA	NA
Smooth Through Lumen	NA	NA	NA
Snap Rings	NA	NA	NA
Spring Internal	94.305%	87.409%	96.5969%
Threads Blind Hole	NA	NA	NA
Through Slot	99.984%	99.979%	98.5430%

(NA) = Not applicable as 72hr results were acceptable. 72hr correction factor will be used for all experimental soil drying times.

The correction factor derived from extraction efficiency is used to mitigate the risk of not quantifying all analytes accurately (Table 4.8 and Table 4.9). Extraction efficiency represents the effectiveness of isolating analytes from a sample, and if it is not 100%, a correction factor is applied to compensate for potential losses. By calculating this correction factor, an adjustment to the measured concentration is made to account for any inefficiencies in the extraction process. This correction factor acts as a multiplier, scaling up the obtained results to reflect the likely total amount of analytes present in the original sample. Utilizing such correction factors enhances the accuracy and completeness of quantitative analyses, reducing the risk of underestimating analyte concentrations. The correction factor was calculated using the average extraction efficiency result for each analyte with the following equation:

$$Correction\ Factor = 1 + \frac{(100 - \%\ Extraction\ Efficiency)}{100}$$

Table 4.8: 72hr soil dry correction factor results

Device Feature – 72hr Dry	Protein Additive Extraction – Correction Factor	Protein Compendial – Correction Factor	TOC Correction Factor
Ball Detent / Ball Bearing	1.019	1.248	1.037
Ball Seal Springs	1.046	1.184	1.034
Blind Slot	1.138	1.206	1.096
Button w/ Spring	1.014	1.209	1.044
Buttons - Exposed Springs	1.023	1.413	1.050
Captured Screw	1.004	1.427	1.036
Hinges, Joints, Pivot Points	1.100	1.114	1.098
Leaf Springs	1.000	1.033	1.007
Mated Surfaces	1.042	1.082	1.042
Mated Surfaces Small Clearance	1.040	1.064	1.034
O-rings - External O-ring	1.014	1.078	1.050
O-rings - Internal O-ring	1.041	1.189	1.089
Rough Surface	1.002	1.019	1.008
Screws Threaded Rod / Threaded Thru Hole	1.002	1.080	1.080
Screws Threaded Rod/ Threaded Blind Hole	1.007	1.020	1.032
Sliding Shaft Short	1.009	1.035	1.016
Sliding Shafts Long	1.001	1.022	1.453
Smooth Blind Lumen	1.017	1.053	1.013
Smooth Through Lumen	1.019	1.029	1.068
Snap Rings	1.013	1.114	1.065
Spring Internal	1.019	1.120	1.070
Threads Blind Hole	1.017	1.038	1.041
Through Slot	1.122	1.415	1.073

Table 4.9: 2hr soil dry correction factor results

Device Feature – 2hr Dry	Protein Additive Extraction – Correction Factor	Protein Compendial – Correction Factor	TOC Correction Factor
Ball Detent / Ball Bearing	1.014	1.036	1.019
Ball Seal Springs	1.001	1.012	1.016
Blind Slot	1.001	1.001	1.048
Button w/ Spring	1.001	1.005	1.016
Buttons - Exposed Springs	1.007	0.029	1.036
Captured Screw	1.005	1.038	1.030
Hinges, Joints, Pivot Points	1.000	1.033	1.007
Leaf Springs	1.042	1.082	1.042
Mated Surfaces	1.042	1.082	1.042
Mated Surfaces Small Clearance	1.040	1.064	1.034
O-rings - External O-ring	1.014	1.078	1.050
O-rings - Internal O-ring	1.001	1.001	1.087
Rough Surface	1.002	1.019	1.008
Screws Threaded Rod / Threaded Thru Hole	1.002	1.080	1.080
Screws Threaded Rod/ Threaded Blind Hole	1.007	1.020	1.032
Sliding Shaft Short	1.009	1.035	1.016
Sliding Shafts Long	1.055	1.069	1.359
Smooth Blind Lumen	1.017	1.053	1.013
Smooth Through Lumen	1.019	1.029	1.068
Snap Rings	1.013	1.114	1.065
Spring Internal	1.057	1.126	1.034
Threads Blind Hole	1.017	1.039	1.041
Through Slot	1.000	1.000	1.015

The assessment results indicate that the additive extraction method provides a more stringent measure for determining extraction efficiency. Across all features, the additive extraction efficiency calculation exceeded the acceptance threshold of 70% during the 72-hour dry period. However, when employing the compendial calculation for exhaustive extraction efficiency, results varied depending on the device feature. Utilizing the additive extraction method for protein residuals offers a more conservative approach to determining total protein residual concentration during a cleaning validation.

The extraction efficiency performance of the 23 features is influenced by the soil's ability to fully dry and the extraction fluid's capacity to access and remove dried soil. This was evident in the comparison of features with internal components (e.g., ball detent/ball bearing, ball seal springs, O-rings – Internal O-ring, etc.) to those with exposed complex features. For instance, both external and internal O-rings were assessed. While the external O-ring met the method validation acceptance criteria for all calculations, the

internal O-ring required a less rigorous 2-hour soil dry period to meet the criteria. This disparity underscores the importance of considering the fluid dynamics of the feature when evaluating risk.

4.3 23 Device Features

A full report of the cleaning validation results for each of the 23 features can be found in the applicable appendix as specified in Table 3.8. Discussion and application of the validation results from the 23 features is contained in Chapter 5.

Table 4.10 depicts the results of the 2-hr soil drying experiment. Device features with components that are readily accessible to fluid tended to yield better results than those features where flushing was a requirement. For example, features like the ball seal springs and the rough surface passed the acceptance criteria whereas features like the smooth through lumen and threads blind hole yielded results that were substantially higher.

It is also important to note that the acceptance criteria of pass/fail was established based on a water only extraction within the literature. Consequently, the additive extraction value is not used to determine if the feature passed the validation. However, this value is used within the cleaning classification to assess the risk of the feature for non-water-soluble soil retention. If considering the additive protein value, the following features would move from a passing result to a failing result: Mated Surfaces Small Clearance and Leaf Springs.

Sample size (described in section 4.4) provides confidence in the test results, so if the sample size calculation for a feature was higher than the sample size tested, then the passing result was not accepted (e.g., Mated Surfaces). As specified in AAMI ST98, all replicates also have to be lower than the acceptance criteria, so the passing result for the Hinges, Joints, & Pivot Points was adjusted to be a failing result.

The ATP test is intended to indicate the presence of organic residues on surfaces, but due to the nature of the test soil, the cell content was not strictly controlled. ATP showed variable results and did not trend with the results of protein and TOC (see Figure 7.2). As such, the use of this analyte was discontinued and was not reported for all features.

Table 4.11 depicts the results of the 72hr soil dry experiment. Seventeen (17) of the 23 features failed the validation. In addition, all those passing features, Mated Surfaces Small Clearance, Sliding Shaft Short, Snap Rings, Sliding Shaft Long, Buttons – Exposed Springs, and O-rings – External O-ring, all demonstrated passing results for both protein calculations and TOC. The 72hr soil dry experiment further demonstrates that increasing the drying time of soil, will increase the cleaning challenge.

Table 4.15 is reflective of the failed attempt to reverse the chemical changes of the soil drying by adding an additional soaking step. The rejection of the hypothesis that additional soaking can reverse chemical changes was apparent in the first four features tested, so testing on all failed 72hr soil drying experiment features was canceled.

For those features that passed the 2hr soil dry experiment, an additional challenge of removing the no brush/ no flush step in the manual cleaning was performed. Table 4.16 depict results where all features passed with the exception of Mated Surfaces Small Clearance and Mated Surfaces.

For those features that failed the 2hr Soil Dry Experiment, results for their performance in the 1hr Soil Dry Experiment can be found in Table 4.12. Most features with the exceptions of the Sliding Shaft Short, Smooth Through Lumen, Threads Blind Hole and Sliding Shaft Long, passed the 1hr Soil Dry. However, results for the Spring Internal and the O-rings – Internal O-ring could not be accepted due to failed sample size requirements. For those features that failed the 1hr soil dry, a reduced challenge was completed using DBLSO as the soil for a 1hr dry (Table 4.13). There were two features that demonstrated an inability to fully clean unless the soil was not allowed to dry, Sliding Shaft Short and Threads Blind Hole.

The findings presented in Table 4.17 provide evidence backing the semi-automated approach to cleaning, involving sequential soaks and sonication before initiating an automated cleaning cycle in a washer disinfector. Notably, the results indicate that when soil is prevented from drying on the features, the majority of features can be effectively cleaned without the need for manual intervention, except for specific features such as the Ball Seal Springs, Mated Surfaces Small Clearance, Blind Slot, Smooth Through Lumen, Smooth Blind Lumen, and the Sliding Shaft Long. These findings suggest the potential for future advancements towards a fully automated device processing department, facilitated by this innovative cleaning approach.

While the data obtained from experiments involving DBLSO and the semi-automated process were not integrated into the cleaning classification, it nonetheless offers valuable insights for guiding future applications, particularly in the realms of medical device design engineering tools and the automation of validation processes. Conducting such experiments provides essential information that can drive advancements in the processing of reusable medical devices by enhancing our understanding of device feature performance. These insights pave the way for significant progress in the field, facilitating the development of more efficient and effective processing techniques.

Table 4.10: 2hr soil dry experiment results

Device Feature	2 hr Dry						
	Required	Document #	Result	Protein (µg/cm²)	Additive Protein (µg/cm²)	TOC (µg/cm²)	ATP (Fentamoles /cm²)
Ball Detent / Ball Bearing	Yes	TV-VAL-249059	Pass	1.171	3.139	1.436	133.5
Ball Seal Springs	Yes	TV-VAL-251771	Pass	0.3794	4.113	0.08453	35.14
Captured Screw	Yes	TV-VAL-251687	Pass	3.603	3.545	0.0882	8.15
Hinges, Joints, Pivot Points	Yes	TV-VAL-248467	Fail*	5.565	8.53	9.95	27.73
Mated Surfaces Small Clearance	Yes	TV-VAL-249478	Pass	1.825	18.59	0.3362	38.79
Rough Surface	Yes	TV-VAL-250209	Pass	4.676	4.756	0.3118	4.455
Screws Threaded Rod / Threaded Thru Hole	Yes	TV-VAL-249644	Pass	1.338	1.299	1.428	8.95
Sliding Shaft Short	Yes	TV-VAL-250846	Fail	1.135	13.1	0.101	3.93
Snap Rings	Yes	TV-VAL-251521	Pass*	2.481	8.05	0.4888	NA
Through Slot	Yes	TV-VAL-261155	Pass*	4.474	12.42	13.481	7.31
Blind Slot	Yes	TV-VAL-264128	Fail	9.885	24.89	8.09	NA
Smooth Through Lumen	Yes	TV-VAL-256253	Fail	126.9	125.7	290.4	1928
Smooth Blind Lumen	Yes	TV-VAL-250656	Fail	15.5	39.31	4.813	237.5
Threads Blind Hole	Yes	TV-VAL-251192	Fail	11.82	12.26	5.373	12.22
Screws Threaded Rod/ Threaded Blind Hole	Yes	TV-VAL-253128	Pass*	4.496	5.757	0.536	8.59
Sliding Shafts Long	Yes	TV-VAL-261926	Fail	15.07	15.31	2.513	86.6
Button w/ Spring	Yes	TV-VAL-261797	Pass*	2.833	3.462	0.4148	NA
Buttons - Exposed Springs	Yes	TV-VAL-256480	Pass	0.05751	4.881	0.12534	10.92
Spring Internal	Yes	TV-VAL-256426	Fail	17.61	17.41	7.086	27.55
Leaf Springs	Yes	TV-VAL-256469	Pass	1.342	10.57	0.886	88.5
O-rings - External O-ring	Yes	TV-VAL-256517	Pass	2.104	5.498	0.08149	18.52
O-rings - Internal O-ring	Yes	TV-VAL-256442	Fail	7.55	9.52	1.114	393.6
Mated Surfaces	Yes	TV-VAL-255218	Pass**	4.701	4.574	0.12292	44.79

*All results were not below the acceptance criteria.

**Sample size check calculation failed, so passing results cannot be accepted.

Table 4.11: 72hr soil dry experiment results

Device Feature	72 hr Dry						
	Required	Document #	Result	Protein (µg/cm²)	Additive Protein (µg/cm²)	TOC (µg/cm²)	ATP (Fentamoles /cm²)
Ball Detent / Ball Bearing	Yes	TV-VAL-250552	Fail	40.71	33.3	19.45	17.86
Ball Seal Springs	Yes	TV-VAL-251780	Fail	15.01	15.34	20.97	67.5
Captured Screw	Yes	TV-VAL-251690	Pass*	4.867	9.124	8.1	10.31
Hinges, Joints, Pivot Points	Yes	TV-VAL-249432	Fail	112.4	202.7	152.3	275.7
Mated Surfaces Small Clearance	Yes	TV-VAL-249565	Pass	0.058	0.113	1.835	53.74
Rough Surface	Yes	TV-VAL-250210	Pass*	5.382	9.364	1.34	0
Screws Threaded Rod / Threaded Thru Hole	Yes	TV-VAL-249649	Fail	54.49	87.9	1.188	36.45
Sliding Shaft Short	Yes	TV-VAL-250848	Pass	0.05641	0.11	0.08427	0.05641
Snap Rings	yes	TV-VAL-251526	Pass	0.06043	0.755	0.855	NA
Through Slot	yes	TV-VAL-261154	Fail	121.9	151.33	105.5	97.4
Blind Slot	yes	TV-VAL-264118	Fail	89.9	242.9	114.9	376.2
Smooth Through Lumen	yes	TV-VAL-256246	Fail	9.6	35.13	12.88	203.7
Smooth Blind Lumen	yes	TV-VAL-250659	Fail	2.391	24.66	0.3004	9.17
Threads Blind Hole	yes	TV-VAL-251198	Fail	15.9	15.63	6.422	11.63
Screws Threaded Rod/ Threaded Blind Hole	yes	TV-VAL-253130	Pass*	4.496	7.58	3.576	50.22
Sliding Shafts Long	yes	TV-VAL-261925	Pass	0.05866	0.1149	1.159	97.8
Button w/ Spring	yes	TV-VAL-261793	Fail	23.61	20	48.92	NA
Buttons - Exposed Springs	yes	TV-VAL-256479	Pass	0.885	2.935	1.18	45.53
Spring Internal	yes	TV-VAL-256424	Fail	37.59	44.99	58.89	3062
Leaf Springs	yes	TV-VAL-256468	Fail	36.03	65.76	50.12	982
O-rings - External O-ring	yes	TV-VAL-256516	Pass	0.742	1.323	1.385	11.05
O-rings - Internal O-ring	yes	TV-VAL-256441	Fail	2.815	67.7	115.2	665.8
Mated Surfaces	yes	TV-VAL-253221	Fail	25.57	32.920	NA	477.5

*Sample size check calculation failed, so passing results cannot be accepted.

Table 4.12: 1hr soil dry experiment results

Device Feature	1 hr Dry						
	Required	Document #	Result	Protein (µg/cm²)	Additive Protein (µg/cm²)	TOC (µg/cm²)	ATP (Fentamoles /cm²)
Hinges, Joints, Pivot Points	Yes	TV-VAL-256185	Pass	2.946	7.82	2.248	41.11
Rough Surface	yes	TV-VAL-271390	Pass	0.05838	0.1148	2.163	NA
Sliding Shaft Short	Yes	TV-VAL-256187	Fail	6.35	6.56	1.552	142.0
Snap Rings	Yes	TV-VAL-258432	Pass	0.750	5.617	0.08791	4.913
Through Slot	Yes	TV-VAL-270771	Pass	1.991	2.039	4.9885	NA
Blind Slot	yes	TV-VAL-270770	Pass	4.603	9.74	5.572	NA
Smooth Through Lumen	Yes	TV-VAL-266863	Fail	25.73	54.00	1.857	NA
Smooth Blind Lumen	Yes	TV-VAL-261841	Pass*	0.568	8.809	4.212	528.80
Threads Blind Hole	Yes	TV-VAL-261843	Fail	9.10	24.276	11.616	4.341
Screws Threaded Rod/ Threaded Blind Hole	Yes	TV-VAL-271452	Pass	0.05196	0.1026	0.9280	NA
Sliding Shafts Long	Yes	TV-VAL-268804	Fail	9.52	10.32	0.905	NA
Button w/ Spring	Yes	TV-VAL-273134	Pass	1.073	1.924	0.6003	NA
Spring Internal	Yes	TV-VAL-265209	Pass*	3.752	3.578	1.205	20.03
O-rings - Internal O-ring	Yes	TV-VAL-261844	Pass*	4.192	4.981	0.6120	NA
Mated Surfaces	Yes	TV-VAL-273882	Pass	0.885	3.357	0.6920	NA

*Sample size check calculation failed, so passing results cannot be accepted.

Table 4.13: 1hr DBLSO soil dry experiment results

Device Feature	1 hr Dry DBLSO						
	Required	Document #	Result	Protein (µg/cm²)	Additive Protein (µg/cm²)	TOC (µg/cm²)	ATP (Fentamoles /cm²)
Sliding Shaft Short	Yes	TV-VAL-265206	Fail	7.30	7.18	1.642	19.51
Smooth Through Lumen	Yes	TV-VAL-275474	Pass	0.057	12.508	5.727	NA
Threads Blind Hole	Yes	TV-VAL-265207	Fail	10.62	10.46	18.82	50.55
Sliding Shafts Long	Yes	TV-VAL-271386	Pass	0.06136	0.1211	0.2833	NA
Spring Internal	yes	TV-VAL-273754	Pass	2.091	2.801	0.758	NA
O-rings - Internal O-ring	Yes	TV-VAL-273136	Pass*	0.719	0.897	0.918	NA

*Sample size check calculation failed, so passing results cannot be accepted.

Table 4.14: No DBLSO soil dry experiment results

Device Feature	No Dry - DBLSO						
	Required	Document #	Result	Protein (µg/cm²)	Additive Protein (µg/cm²)	TOC (µg/cm²)	ATP (Fentamoles /cm²)
Sliding Shaft Short	Yes	TV-VAL-268019	Pass	0.05641	2.712	0.08427	0.05641
Smooth Through Lumen	Yes	TV-VAL-275474	Pass	1.824	2.208	6.859	NA
Threads Blind Hole	Yes	TV-VAL-267159	Pass	0.06023	22.42	5.772	0.06023

Table 4.15: Extended soak experiment results

Device Feature	Extended Soak						
	Required	Document #	Result	Protein (µg/cm²)	Additive Protein (µg/cm²)	TOC (µg/cm²)	ATP (Fentamoles /cm²)
Ball Detent / Ball Bearing	Yes	TV-VAL-255307	Fail	45.52	104.55	102.5	72.59
Ball Seal Springs	Yes	TV-VAL-258433	Fail	201.7	12246	269.9	178.2
Captured Screw	Yes	TV-VAL-258435	Fail	14.882	13.605	4.752	9.68
Hinges, Joints, Pivot Points	Yes	TV-VAL-252877	Fail	166.4	257.7	279.4	5556

Table 4.16: No Brush / No Flush experiment results

Device Feature	No Brush / Flush						
	Required	Document #	Result	Protein (µg/cm²)	Additive Protein (µg/cm²)	TOC (µg/cm²)	ATP (Fentamoles /cm²)
Ball Detent / Ball Bearing	Yes	TV-VAL-256189	Pass	4.637	24.74	3.8467	15.236
Ball Seal Springs	Yes	TV-VAL-256191	Pass	0.05533	6.516	5.88	301.1
Captured Screw	Yes	TV-VAL-261848	Pass	2.133	4.793	0.982	11.7
Mated Surfaces Small Clearance	yes	TV-VAL-266866	Fail	10.54	10.36	17.29	NA
Rough Surface	Yes	TV-VAL-258441	Pass	0.741	2.536	0.5669	NA
Screws Threaded Rod / Threaded Thru Hole	Yes	TV-VAL-256190	Pass	0.0616	2.221	0.728	NA
Screws Threaded Rod/ Threaded Blind Hole	yes	TV-VAL-268802	Pass	3.922	7.21	3.215	NA
Buttons - Exposed Springs	Yes	TV-VAL-261850	Pass	0.058	4.231	2.959	223.5
Leaf Springs	Yes	TV-VAL-265212	Pass	1.466	7.34	4.773	169.8
O-rings - External O-ring	yes	TV-VAL-261851	Pass	0.05498	6.092	0.32	NA
Mated Surfaces	Yes	TV-VAL-266868	Pass*	5.398	11.787	3.907	NA

*Sample size check calculation failed, so passing results cannot be accepted.

Table 4:17: Semi-Automated experiment results

Device Feature	Semi - Automated					
	Required	Document #	Result	Protein (µg/cm²)	Additive Protein (µg/cm²)	TOC (µg/cm²)
Ball Detent / Ball Bearing	yes	TV-VAL-266872	Pass	0.05816	0.1139	2.037
Ball Seal Springs	yes	TV-VAL-267039	Fail	6.754	6.735	1.951
Captured Screw	yes	TV-VAL-267146	Pass	0.05882	0.1139	0.08882
Hinges, Joints, Pivot Points	yes	TV-VAL-267148	Pass*	4.863	4.994	0.4353
Mated Surfaces Small Clearance	yes	TV-VAL-264604	Fail	8.7	5.56	8.39
Rough Surface	yes	TV-VAL-264309	Pass	3.088	4.528	0.1883
Screws Threaded Rod / Threaded Thru Hole	yes	TV-VAL-264697	Pass	2.034	1.944	0.6185
Sliding Shaft Short	yes	TV-VAL-264602	Pass	2.283	2.495	1.239
Snap Rings	yes	TV-VAL-267044	Pass*	5.496	15.26	0.08791
Through Slot	yes	TV-VAL-264592	Pass	3.475	4.501	4.6152
Blind Slot	yes	TV-VAL-264594	Fail	9.226	10.167	5.502
Smooth Through Lumen	yes	TV-VAL-264596	Fail	4.139	7.111	4.513
Smooth Blind Lumen	yes	TV-VAL-264600	Fail	3.215	24.1	8.339
Threads Blind Hole	yes	TV-VAL-264693	Pass	0.06023	2.483	5.949
Screws Threaded Rod/ Threaded Blind Hole	yes	TV-VAL-264694	Pass	0.05196	0.1026	1.529
Sliding Shafts Long	yes	TV-VAL-266871	Fail	8.811	8.756	0.8582
Button w/ Spring	yes	TV-VAL-267032	Pass	1.763	1.812	0.0861
Buttons - Exposed Springs	yes	TV-VAL-267035	Pass	3.678	3.885	0.5966
Spring Internal	yes	TV-VAL-267037	Pass	4.154	4.074	0.08345
Leaf Springs	yes	TV-VAL-267038	Pass	3.708	3.646	0.08591
O-rings - External O-ring	yes	TV-VAL-267040	Pass	0.05498	2.472	0.08149
O-rings - Internal O-ring	yes	TV-VAL-267042	Pass*	4.987	5.044	1.121
Mated Surfaces	yes	TV-VAL-267153	Pass	0.058	0.111	0.9962

*Sample size check calculation failed, so passing results cannot be accepted.

4.4 Sample Size

The recommended sample size to validate accuracy for an analytical detection method by the US FDA is a minimum or 9 replicates over 3 concentration levels (U.S. Department of Health and Human Services Food and Drug Administration, 2015). A contributing factor to this sample size determination is the demonstration that with well-maintained instruments and a controlled test system, the data will be normally distributed. Normal distribution is demonstrated using the central limit theorem which states that with a large population of independent variables that the mean and variance will be normally distributed. The sample size typically used as a "large population" is 30 and has therefore been used most often as the sample size for validation requirements, but with demonstration of normally distributed data, as in the case of accuracy for an analytical detection method, a lower sample size can be used (Fotis & Bix, 2006).

Sample size must be justified, but in general, as the risk of ineffective cleaning increases, an increased sample size is recommended (Association for the Advancement of Medical Instrumentation, 2022). There are several ways to establish sample number, but it is first important to understand if the data set will be normal. For this evaluation, normality within cleaning validations was established in the Device Feature Approach Validation. The statistical determination of sample size should account for the upper one-sided confidence level and appropriate determination of risk. The proposed sample size is calculated using risk prior to test execution but is verified post testing to demonstrate the statistical power has been achieved.

Protein is the most ubiquitous analyte present in clinical soil, so it is therefore the primary analyte used to evaluate cleaning efficacy of a reusable medical device (Cloutman-Green, et al., 2015). In ISO 15883-5 a lower value of 3µg/cm^2 is established as an alert level where the cleaning efficacy should be investigated and evaluated for risk (International Organization for Standardization, 2021). The alert level provides an additional measure of confidence by reducing the established safety level by 53.13%. By using the alert level, the bias of the protein measurement method of ±15% is mitigated. However, the application of the alert level is typically only used to evaluate the cleaning efficacy of washer-disinfectors as described by ISO 15883-5 and is not widely used when evaluating a cleaning process for effectiveness for reusable medical devices. When using the acceptance criteria of 6.4µg/cm^2, the range of acceptable results of protein residuals for cleaning efficacy falls between 5.44 and 7.36µg/cm^2 when the test method bias is considered. This upper limit of 7.36 µg/cm^2 was also demonstrated for patient safety in the study by Kremer et. al (Kremer, et al., 2019).

Because of the established safety factors imbedded in the acceptance criteria, it is possible to calculate the sample number using the margin of error approach. The margin of error approach has been used in other areas of medical device testing (e.g., packaging) as described by Fotis et.al (Fotis & Bix, 2006), and is also applicable with cleaning efficacy studies since these evaluations stack safety factors to mitigate cleaning variability. When using this approach, the margin of error must first be established. Within cleaning validations, it is not acceptable to have any value exceed the acceptance criteria of 6.4 µg/cm^2.

For each experiment, the minimum sample size of 5 was used. At the conclusion of the testing, a power calculation was performed using Minitab to test the probability of a type I error, i.e., the probability of a result having a value above the established acceptance criteria of 6.4µg/cm^2 for passing experiments. The acceptance criteria of 6.4µg/cm^2 was used as a fixed point in the normal distribution curve, so the curve type selected for the power calculation was two-sided. The confidence interval used was the upper lower bound to account for the risk of a larger portion of the sample population falling near the acceptance criteria.

The α (alpha) value is the significance value or the acceptable level of risk of the accepting a false passing result, i.e., result above the acceptance criteria and considered a passing result or type I error. Conservatively, the confidence interval for this type of study should be 99% so α equates to 0.01 and the power will be 0.99. The margin of error was calculated by subtracting the high value of the 99% confidence interval from the acceptance criteria. The standard deviation was also calculated. Using the sample size for estimation calculation under the power and sample size option in Minitab, the sample size estimation for protein was calculated for each passing experiment.

In addition, an additional check is performed to verify the distribution of the data is acceptable as prescribed by ANSI/AMMI ST98 (Table 4.18). This check was performed by adding the standard deviation to the highest value calculated. A passing result is a value below the acceptance criteria.

Table 4.18: Sample size confirmation calculations – protein

Feature	Experiment	Sample Size	Standard Deviation	Margin of Error	Required Sample Size	Highest Calculated Value µg/cm^2	Check µg/cm^2
Ball Detent / Ball Bearing	2hr Dry	10	0.696	5.229	3	2.023	2.719
	Semi-Automated	5	0.000	6.342	2	0.058	0.058
Ball Seal Springs	2hr Dry	10	0.241	6.021	2	0.818	1.059
	2hr Dry No Brush/No Flush	5	0.000	6.345	2	0.055	0.055
Blind Slot	1hr Dry	5	1.176	1.797	5	4.000	5.176
Button w/ Spring	2hr Dry	10	1.674	3.567	4	5.102	6.802*
	1hr Dry	10	0.646	5.327	3	2.126	2.772
	Semi-Automated	5	0.657	4.637	3	0.056	0.056
Buttons - Exposed Springs	2hr Dry	10	0.000	6.342	2	0.058	0.058
	2hr Dry No Brush/No Flush	5	0.000	6.342	2	0.058	0.058
	72hr Dry	10	0.561	5.515	2	1.871	2.433
	Semi-Automated	5	1.109	2.722	4	3.068	4.177

Feature	Experiment	Sample Size	Standard Deviation	Margin of Error	Required Sample Size	Highest Calculated Value µg/cm²	Check µg/cm²
Captured Screw	2hr Dry	10	1.010	2.797	3	3.432	4.442
	2hr Dry No Brush/No Flush	5	0.414	4.267	2	1.716	2.130
	72hr Dry	10	2.713	1.533	15*	8.386	11.099*
	Semi-Automated	5	0.000	6.341	2	0.059	0.059
Hinges, Joints, Pivot Points	1hr Dry	10	1.661	3.454	4	4.591	6.251
	Semi-Automated	5	1.497	1.537	7*	4.041	5.538
Leaf Springs	2hr Dry	10	0.858	5.058	3	2.331	3.189
	2hr Dry No Brush/No Flush	5	0.562	4.934	3	1.314	1.876
	Semi-Automated	5	1.156	2.692	4	3.4924	4.7754
Mated Surfaces	2hr Dry	10	1.451	1.699	6	5.655	7.106*
	2hr Dry No Brush/No Flush	5	1.365	1.002	10	3.879	5.244
	1hr Dry	10	0.531	5.515	2	1.492	2.024
	Semi-Automated	5	0.000	6.342	2	0.058	0.058
Mated Surfaces Small Clearance	2hr Dry	10	1.191	4.575	3	3.472	4.663
	72hr Dry	5	0.000	6.342	2	0.058	0.058
O-rings - External O-ring	2hr Dry	10	1.379	4.296	3	4.443	5.822
	2hr Dry No Brush/No Flush	5	0.000	6.345	2	0.055	0.055
	72hr Dry	10	0.511	5.658	2	1.671	2.182
	Semi-Automated	5	0.000	6.345	2	0.055	0.055
O-rings - Internal O-ring	1hr Dry	10	2.620	2.208	8	8.185	10.806*
	1hr DBLSO Dry	10	0.493	5.681	2	1.616	2.109
	Semi-Automated	5	1.422	1.413	7	3.303	4.725
Rough Surface	2hr Dry	10	1.821	1.724	5	6.056	7.877*
	2hr Dry No Brush/No Flush	5	0.272	5.659	2	0.668	0.940
	1hr Dry	5	0.000	6.342	2	0.058	0.058

Feature	Experiment	Sample Size	Standard Deviation	Margin of Error	Required Sample Size	Highest Calculated Value µg/cm²	Check µg/cm²
	72hr Dry	5	1.874	1.018	16*	5.607	7.481*
	Semi-Automated	5	0.691	3.312	3	2.287	2.977
Screws Threaded Rod / Threaded Thru Hole	2hr Dry	10	0.880	5.062	3	2.871	3.751
	2hr Dry No Brush/No Flush	5	0.000	6.338	2	0.062	0.062
	Semi-Automated	5	0.787	4.366	3	1.821	2.608
Screws Threaded Rod/ Threaded Blind Hole	2hr Dry	10	1.250	1.904	5	5.867	7.118*
	2hr Dry No Brush/No Flush	5	1.363	2.478	4	3.396	4.759
	1hr Dry	5	0.000	6.348	2	0.052	0.052
	72hr Dry	10	2.484	1.457	14*	5.637	8.121*
	Semi-Automated	5	0.000	6.348	2	0.052	0.052
Sliding Shaft Short	2hr Dry	10	0.681	5.965	3	1.803	2.484
	72hr Dry	15	0.000	6.344	2	0.056	0.056
	1hr Dry	10	3.587	0.050	19,773*	8.827	12.414*
	No Dry DBLSO	5	0.056	6.344	2	0.0843	0.0843
	Semi-Automated	5	0.843	4.117	3	1.3940	1.9797
Sliding Shafts Long	72hr Dry	10	0.000	6.341	2	0.059	0.059
	1hr DBLSO Dry	5	0.000	6.339	2	0.061	0.061
Smooth Blind Lumen	1hr Dry	10	0.379	5.832	3	1.257	1.636
	72hr Dry	10	1.315	4.009	4	3.361	4.676
	Semi-Automated	5	1.144	3.182	5	2.613	3.757
Smooth Through Lumen	1hr DBLSO Dry	5	0.000	6.343	2	0.057	0.057
	DBLSO No Dry	10	1.126	4.576	3	3.349	4.475
	Semi-Automated	5	0.455	2.261	3	3.674	4.129
Snap Rings	2hr Dry	10	1.801	3.919	4	5.757	7.558*
	1hr Dry	10	0.431	5.650	2	1.222	1.653
	72hr Dry	10	0.000	6.340	2	0.060	0.060
	Semi-Automated	5	0.774	0.904	6*	4.716	5.490
Spring Internal	1hr Dry	10	2.011	2.648	5	4.931	6.942*
	1hr DBLSO Dry	10	1.390	4.309	3	4.549	5.939
	Semi-Automated	5	1.634	2.246	5	3.713	5.347

Feature	Experiment	Sample Size	Standard Deviation	Margin of Error	Required Sample Size	Highest Calculated Value µg/cm²	Check µg/cm²
Threads Blind Hole	DBLSO No Dry	5	0.000	6.340	2	0.060	0.060
	Semi-Automated	5	0.000	6.340	2	0.060	0.060
Through Slot	2hr Dry	10	2.163	1.926	8*	6.353	8.516*
	1hr Dry	5	0.677	4.409	3	1.695	2.372
	Semi-Automated	5	0.867	2.925	3	2.467	4.408

* Sample size check calculation failed, so passing results cannot be accepted.

4.5 Conclusion

The results described within this section provided data to inform the development of the risk evaluation described in Chapter 5. The extraction efficiency performance of the 23 features is influenced by the soil's ability to fully dry and the extraction fluid's capacity to access and remove dried soil. This was evident in the comparison of features with internal components (e.g., ball detent/ball bearing, ball seal springs, O-rings – Internal O-ring, etc.) to those with exposed complex features. For instance, both external and internal O-rings were assessed. While the external O-ring met the method validation acceptance criteria for all calculations, the internal O-ring required a less rigorous 2-hour soil dry period to meet the criteria. This disparity underscores the importance of considering the fluid dynamics of the feature when evaluating risk.

Device features with components that are readily accessible to fluid yielded higher extraction efficiency and lower cleaning residual results than those features where flushing was a requirement. For example, features like the ball seal springs and the rough surface passed the acceptance criteria whereas features like the smooth through lumen and threads blind hole yielded results that were substantially higher.

Chapter 5: Cleaning Classification

After the Spaulding Microbial Reduction Classification was introduced and incorporated into industry standards, it provided a needed framework for device manufacturer's, testing laboratories, regulators, and healthcare personal to validate and consistently deliver the appropriate microbiological reduction for reusable medical devices. As discussed above, when only using Spaulding, the entirety of the microbiological quality of the reusable medical device is not considered in detail. Cleaning is a critical aspect of maintaining microbiological quality, is expected as part of the Spaulding Classification but the risk of the device not being cleaned is omitted. The introduction of a complementary cleaning classification system will allow for effective communication between medical device manufacturers and health care facilities on the proper risk mitigation for associated cleaning processes, so appropriate actions can be implemented.

For each device design and associated cleaning process there is a probability of soil retention. This relationship can be quantified to assess patient safety risk. This relationship has been well described in the literature (Southworth, 2014) and evaluated by standards organizations with the intent to inform medical device manufacturers on the cleaning steps that may need to be included in the cleaning IFU based on the device design and associated features. Michels et. al describe an example, based on validations for reusable medical devices, for grouping devices based on their features but did not assess the probability of soil accumulation risk with these features (Michels, et al., 2013). AAMI TIR12:2020 Annex D logically described three categories of devices based on cleaning processes required and designated by device complexity. Category 1 is comprised of simple devices that can be processed using manual or automated cleaning methods. Category 2 devices have features that require a unique human intervention, such as brushing, to remove soil that may be within a difficult to clean location. Category 3 devices included devices that require more detailed cleaning such as sonication to aid in the removal of soil that is not accessible or difficult to remove using brushing and flushing (Association for the Advancement of Medical Instrumentation, 2020). The categorization of these groups was completed by evaluating the cleaning IFUs for marketed devices and applying them to the complexity of device features of the medical device. The assumption of this evaluation is the IFU contained all necessary steps for cleaning the applicable device, but no further guidance is given for how to assess the device for each category.

A cleaning risk-based approach is proposed that considers the probability of risk for residual soil to remain on or in the various design features of a device following cleaning. For effective cleaning to occur, the cleaning chemistry (cleaning agent and water) must have access to the soil with enough exposure (e.g., spray, soak) or force (e.g., brush, flush, sonication) to solubilize and remove the residual soil for surface removal. The device feature is, therefore, the key variable of a reusable medical device that can influence this relationship.

A simple categorization scheme as developed in this study offers the advantage of ease and efficiency in its application. By reducing complexity and streamlining classification criteria, users can quickly and

intuitively assign items or concepts to specific categories. This simplicity not only expedites the categorization process but also enhances clarity and reduces the likelihood of errors. With straightforward guidelines, users are less burdened by the cognitive load associated with intricate classification systems, allowing for a more straightforward decision-making process based on their knowledge of the complexity of the device. The simplicity of the Spaulding Classification is a nice example this.

The proposed cleaning classification follows a similar model where the complex set of cleaning variables can be distilled into three risk categories, maximal, moderate, and minimal (Table 5.1).

Table 5.1: Cleaning classification

Risk Category	Description
Maximal	Complex device features with a high probability of soil retention or accumulation with a medical device.
Moderate	Accessible device features that require specific intervention (e.g., brushing or flow through lumens, mated surfaces requiring disassembly or opening/closing to ensure access).
Minimal	Low risk reusable medical devices where all features are exposed without specific intervention for cleaning.

This classification can provide guidance to manufacturers to improve device designs for cleanability, as well as be included the IFU to effectively communicate the cleaning risk to healthcare personnel. Examples of proposed symbols based on this classification are suggested to be included within the medical device's IFU (Figure5.1).

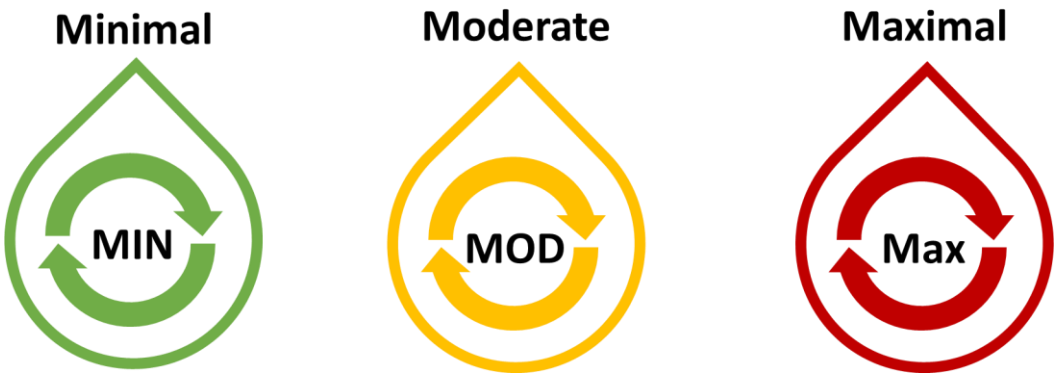


Figure 5.1: Examples of potential cleaning classification symbols

Estimating the number of unique reusable medical devices in any average processing department can be challenging due to variations across healthcare facilities but is typically estimated in the hundreds to thousands (Alfred, et al., 2020). The exact count depends on factors such as the size and specialization of the healthcare facility, the types of medical/surgical services provided, and the complexity of procedures

performed. Effectively funneling large numbers of reusable medical devices into categories that can help streamline the associated cleaning processes requires a systematic and comprehensive approach.

After a thorough evaluation of potential risks associated with various factors, a systematic three-category system can be proposed to classify risks into minimal, moderate, and maximal categories. Clear and objective criteria were established for each category, ensuring a standardized approach to risk assessment. The minimal risk category encompasses scenarios with negligible threats, where the potential for harm is minimal and manageable. The moderate risk category includes situations characterized by a notable degree of uncertainty or potential harm, which can be effectively managed with appropriate measures. The maximal risk category identifies scenarios with the highest potential for severe consequences or significant harm, demanding heightened attention and comprehensive risk mitigation strategies. This three-tiered system allows for a nuanced understanding of risk levels, facilitating efficient decision-making and resource allocation to address potential challenges across various contexts.

By assigning numerical values to the risk factors and finding the sum of the values, the classification of a reusable medical device can be standardized in a quantitative manner. This approach enhances objectivity and reduces ambiguity in categorization processes. The ability to quantify variables enables more accurate comparisons, promotes consistency and reproducibility.

5.1 Evaluation of Medical Device Risk

Ineffective device processing is a major risk for HAIs and other patient complications. The evaluation of risk in accordance with ISO 14971, a standard for the application of risk management to medical devices, involves a systematic and comprehensive approach. This international standard outlines a structured process for identifying, analyzing, and mitigating risks throughout the lifecycle of a medical device. It emphasizes the importance of considering various factors, such as the severity of harm, the probability of occurrence, and the detectability of potential issues. ISO 14971 provides guidance on assigning risk acceptability criteria and establishing risk controls. The standard promotes a proactive mindset, encouraging continuous assessment and adaptation to changing circumstances (International Organization for Standardization, 2019).

The application of this standard is appropriate when establishing the quantitative criteria for a cleaning classification. Within this framework, the “Evaluation of Risk”, defined as a “process of comparing estimated risk against given risk criteria to determine acceptability of the risk”, is used to identify the hazardous situation. As it applies to the processing of reusable medical devices, “hazardous situation” is defined as circumstances that may cause a medical device to not be effectively cleaned, causing harm to a patient.

To establish the cleaning classification, a risk analysis was performed to systematically identify and assess the potential risks associated with the processing of a reusable medical device for a risk evaluation. Harm to a patient is broadly defined as the negative impact on the health or well-being of an individual resulting from exposure to a medical device. Harm to a patient may include any of the following: potential infection,

toxic reaction from process residuals, improper device function or device damage, delayed or cancelled surgery, tissue damage, or surgery complications.

For a reusable medical device, the hazard, defined as the "potential source of harm" (International Organization for Standardization, 2019), includes the device design, material of construction, intended use, and instruction for use. Each of these elements may individually contribute to a hazardous situation for device processing: inability to execute the instructions for use, processing time, IFU comprehension, equipment, and resource requirements, and how to measure "clean". A graphical image of the medical device risk evaluation can be found in figure 5.2.

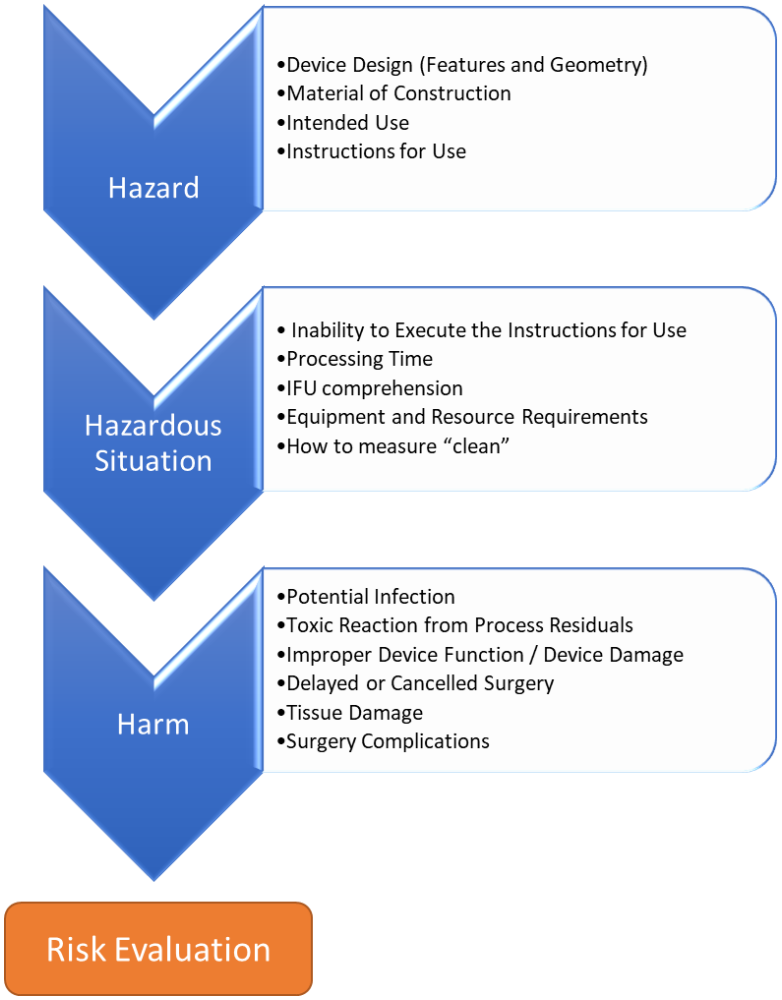


Figure 5.2: Cleaning Classification Risk Analysis

For each hazard and hazardous situation combination, the risk evaluation for harm was assessed and assigned as low, medium, or high risk. For the purposes of this risk evaluation the following definitions were applied (see figure 5.3):

Impact:

- Catastrophic – Results in patient death
- Critical – Results in permanent impairment of life-threatening injury
- Serious – Results in injury or impairment requiring professional medical intervention
- Minor – Results in temporary injury or impairment not requiring professional medical intervention
- Negligible – Inconvenience or temporary discomfort

Likelihood of Occurrence

- Frequent - Higher likelihood of occurrence, indicating that the identified risk is expected to happen regularly or is considered a common occurrence
- Probable – Significant chance of the identified risk materializing but stops short of implying certainty
- Occasional – Not a regular or frequent occurrence but may happen from time to time
- Remote – Indicates that the likelihood of its occurrence is highly unlikely or rare
- Improbable – Indicates that the likelihood of its occurrence is considered highly unlikely, although not ruled out entirely

	Negligible	Minor	Serious	Critical	Catastrophic
Frequent	MEDIUM	MEDIUM	HIGH	HIGH	HIGH
Probable	LOW	MEDIUM	MEDIUM	HIGH	HIGH
Occasional	LOW	LOW	MEDIUM	MEDIUM	HIGH
Remote	LOW	LOW	LOW	MEDIUM	HIGH
Improbable	LOW	LOW	LOW	LOW	MEDIUM

Figure 5.3: Risk Evaluation Rubric

In a low-risk scenario, improper cleaning of a reusable medical device may result in residual contamination that could potentially lead to mild infections or complications for patients, such as localized irritation or minor post-operative complications. While these instances may require additional medical attention and treatment, they are typically manageable and do not pose an immediate threat to patient life.

In a medium-risk scenario, failure to properly clean a reusable medical device could lead to more severe infections or complications for patients, requiring hospitalization and intensive medical intervention. This could include systemic infections or the transmission of antibiotic-resistant strains of bacteria, which may necessitate aggressive antibiotic therapy or even surgical intervention to mitigate the effects. While these situations pose a greater risk to patient health and may result in longer recovery times, they are still generally treatable and do not typically lead to fatal outcomes.

In a high-risk scenario, the failure to adequately clean a reusable medical device could result in the transmission of life-threatening infections or pathogens to patients, ultimately leading to patient death. In such cases, the contamination may be severe and widespread, potentially affecting multiple patients and causing significant harm before the issue is identified and addressed. The literature suggests that a catastrophic event is unlikely, as it would necessitate the failure of all processing steps.

5.2 Device Design

A well-thought-out design for a reusable medical device that considers cleanability from the outset is paramount to patient safety. It not only ensures effective cleaning and sterilization for a critical device but also contributes to the device's longevity and reliability in clinical settings. The manufacturers must evaluate the device for cleaning efficacy and include the design of the device in the risk analysis. Annex C of ISO 17664-1:2021 discusses how the design of a reusable medical device can affect the decontamination processes. The list of design considerations in C.3.2 can be sorted into three groups for discussion on risk, device geometry, material of construction, and device features (International Organization for Standardization, 2021).

For each of these design considerations, there are two elements that are the highest contributors of risk regarding device cleaning efficacy, soil drying and fluid dynamics. Soil drying can be described as the probability of soil drying or not drying on a medical device and present with a spectrum of associated risk. The fluid dynamics can be described as the motion of fluids and their interaction with solid surfaces. Assessing these factors to evaluate risk for the cleaning of reusable medical devices is the foundational principle to establish a quantitative cleaning classification. As the cleaning purpose is to remove residual soil, these factors combined will determine the risk of soil removal.

5.2.1 Geometry

An ideal design for easy and effective cleaning involves smooth and continuous surfaces with rounded edges, minimizing crevices where contaminants can accumulate. The geometry should be free of dead spaces, where fluids may stagnate, ensuring that cleaning agents can reach all areas of the device. Joints and connections should be accessible and designed for easy disassembly, allowing for thorough cleaning between components. Seamless integration of parts reduces the number of seams and joints, simplifying the cleaning process. Additionally, the geometry should facilitate the efficient flushing of fluids, promoting streamlined flow paths that minimize turbulence. Overall, a thoughtfully designed geometry that prioritizes cleanliness considerations enhances the ability to process the device.

Size must be a consideration for cleaning efficacy. Extremely small devices may have intricacies that are challenging to clean manually, while very large devices may have areas that are difficult to access. The small sizes (e.g., microsurgical instruments) of a reusable medical device can significantly impact its cleanability. Miniaturization, while advantageous for certain medical applications, poses challenges in ensuring thorough and effective cleaning. The reduced dimensions may result in intricate geometries with tight spaces, making it challenging to access and clean every nook and cranny. Small-sized components

and intricate features increase the risk of contaminants becoming trapped in confined areas, potentially compromising the device's cleanliness. Additionally, the limited space may restrict the use of standard cleaning tools or impede the effectiveness of cleaning agents. Manufacturers must address these challenges through meticulous design considerations, such as incorporating smooth surfaces, minimizing complex structures, and providing detailed cleaning instructions.

The weight of a reusable medical device can also have implications for its cleaning process. Heavier devices may pose challenges during handling and disassembly for cleaning purposes, potentially increasing the risk of user fatigue or handling errors. Cumbersome components might hinder the ease of thorough inspection and cleaning, especially if intricate or delicate parts require careful disassembly. Moreover, the weight of the device may influence the choice of cleaning methods and equipment, as heavier devices may be more challenging to maneuver and clean manually. Therefore, it is essential for the design of reusable medical devices to strike a balance between functionality and weight, ensuring that the device remains manageable for users during both operation and the critical cleaning process.

Any crevices, recesses, or dead spaces in the geometry of a device can trap contaminants and make it difficult to clean thoroughly. These areas may be challenging to reach with cleaning tools or may not be adequately exposed to cleaning solutions. Examples include small gaps between components or intricate designs with tight spaces. Devices with complex surfaces, especially those with irregular shapes or intricate patterns, can also be harder to clean. The more convoluted the geometry, the more likely it is that contaminants will find shelter in hard-to-reach areas. Simple, smooth surfaces are generally easier to clean as all surfaces are exposed to cleaning chemistries.

The presence of joints, seams, or connections between different parts of a device can create areas where contaminants can accumulate. If these joints are not well-designed and accessible, cleaning may be incomplete, leading to potential infection risks. Seamless or well-designed joints can contribute to easier cleaning. When a device is made of multiple materials or has transitions between different materials, these areas can be susceptible to the accumulation of contaminants. Different materials may have varying porosity or response to cleaning agents, making it challenging to ensure uniform cleanliness.

Sharp corners and angles can be difficult to clean thoroughly because they provide hiding spots for contaminants. Rounded edges are generally easier to clean as they reduce the likelihood of material buildup. Rough or porous surface finishes can trap contaminants, making it harder to clean. Smooth, polished surfaces are generally more conducive to effective cleaning. The geometry should allow for easy access with cleaning tools. If certain areas of the device are difficult to reach, it becomes challenging to clean them adequately. Consideration should be given to how cleaning instruments, such as brushes or wipes, can be effectively used on all surfaces.

5.2.2. Material of Construction

The choice of material for the construction of reusable medical devices is an important consideration when designing a device for cleaning efficacy. The material must withstand rigorous cleaning, disinfection

and/or sterilization processes without degrading or compromising its structural integrity. Optimal materials are those that are resistant to corrosion, do not adsorb fluids or contaminants, and can withstand exposure to harsh cleaning agents and high temperatures. Additionally, the material should be compatible with the intended disinfection / sterilization methods to ensure effective microbial elimination. Smooth and non-porous surfaces are preferred to prevent the accumulation of residues and facilitate easy cleaning. The selection of appropriate materials not only influences the device's intended use and durability but also its ability to be reprocessed for repeated use.

The use of porous or adsorbing materials in reusable medical devices can present a double-edged sword concerning cleaning efficacy. While porous materials may be selected for their specific functional properties or as a means of reducing the overall weight of the device, they also introduce challenges during the cleaning process. The nature of porosity creates additional surface area and intricate structures that can harbor contaminants and be difficult to access with traditional cleaning methods. Porous materials may complicate the thorough removal of biological residues or other substances, potentially compromising the device's cleanliness. To mitigate this, designers must carefully consider the compatibility of porous materials with cleaning agents and sterilization methods. Additionally, incorporating smooth, non-porous surfaces where possible and providing clear guidelines for cleaning and maintenance can help address the challenges associated with porous materials, ensuring that the reusable medical device remains hygienic and safe for clinical use.

The use of shrink tubing and coatings in the construction of reusable medical devices can significantly influence their cleanability. While these materials may serve various purposes such as providing insulation, protection, or enhancing device aesthetics, they can pose challenges during the cleaning process. Shrink tubing, with its tendency to form tight fits around device components, may create potential areas for fluid entrapment and hinder effective cleaning. Similarly, coatings, if not applied uniformly or if they degrade over time, can compromise the device's surface integrity and introduce difficulties in maintaining cleanliness. The choice of these materials should, therefore, prioritize those that are resistant to degradation from cleaning/disinfection agents and sterilization methods. Additionally, designers must consider the ease of cleaning beneath these coverings and coatings to ensure that the device can be thoroughly cleaned.

Materials chosen for reusable medical devices that exhibit limited process chemical compatibility, susceptibility to scratching, or a tendency to corrode can significantly impede cleaning efficacy. Limited chemical compatibility restricts the range of cleaning agents that can be safely employed, potentially compromising the device's ability to eliminate contaminants thoroughly. Materials prone to scratching may develop surface irregularities that harbor debris and make cleaning challenging, leading to potential hygiene issues. Corrosion, on the other hand, not only compromises the structural integrity of the device but can also introduce contamination risks as corroded surfaces are often more difficult to clean effectively. In choosing materials, prioritization should be given to materials that withstand the harsh conditions of sterilization and cleaning processes, maintain a smooth and intact surface, and exhibit

resistance to corrosion. Ensuring optimal material selection is crucial for maintaining the cleanliness and longevity of reusable medical devices, safeguarding both patient well-being and the device's performance in clinical settings.

Heat sensitivity can significantly impact the cleaning efficacy of reusable medical devices. Many standard cleaning, disinfection and sterilization processes involve the application of heat, such as autoclaving or thermal disinfection, to eliminate microbial contaminants. However, if a medical device is heat-sensitive, subjecting it to elevated temperatures may lead to material degradation, warping, or structural changes that compromise its integrity. This limitation hinders the ability to employ heat-based cleaning methods effectively, increasing the risk of inadequate processing and potential cross-contamination. Designers must carefully select materials and components that can withstand the required cleaning temperatures without sacrificing their functionality or structural properties. The consideration of heat sensitivity is thus crucial in ensuring that the cleaning protocols applied to reusable medical devices are both effective and compatible with the materials used, ultimately contributing to the device's reliability and safety in clinical environments.

The materials used in the construction of the device and the cleaning agents employed must be compatible. Some materials may react with certain cleaning solutions, affecting their efficacy. Understanding the chemical interactions between the device and cleaning agents is crucial for optimizing the cleaning process.

Careful evaluation of the material of construction is important when designing a reusable medical device, particularly concerning cleaning efficacy. The chosen materials should be robust enough to withstand the rigors of cleaning, disinfection, and sterilization processes without degradation, corrosion, or compromising structural integrity. The compatibility with a variety of cleaning / disinfecting agents and sterilization methods will aid in thorough contamination removal. Smooth and non-porous surfaces are preferred to prevent the accumulation of residues that might impede cleaning efforts. Any limitations such as heat sensitivity, susceptibility to scratching, or proneness to corrosion must be carefully considered to prevent compromise in the device's cleanliness and longevity.

5.2.3 Device Features

The device feature can be described as a portion of the device that may be considered challenging to clean. The identification of worst-case device (or device set) features has been a well-established validation approach in many areas, such as terminal sterilization, in determining process effectiveness and requirements, including for reusable medical devices. A device feature approach for cleaning validations has many advantages as a more conservative approach to the alternative compendial method of testing the entirety of the device. By focusing on the device feature(s), the most challenging validation variables can be isolated to and studied at the most difficult to clean feature(s).

The concept of specifically targeting validation variables focused on the most challenging portion of the medical device has been used for many years in validating sterilization parameters (International

Organization for Standardization, 2006). The process challenge location (PCL) is defined as a "site chosen within a load as the position at which the least microbiological inactivation is expected to be delivered" and a process challenge device (PCD) is defined as an "item providing a defined resistance to a cleaning, disinfection, or sterilization process and is used to assess performance of the process" (International Organization for Standardization, 2018). The cleaning method for a reusable medical device can be validated using the actual medical device or surrogate devices that are well-designed comparators (International Standard Organization, 2021) (Association for the Advancement of Medical Instrumentation, 2022).

As demonstrated in the study by (Michels, et al., 2013), there is risk of under-reporting the residual soil level on devices if the validation method is not focused on the most difficult to clean area of the device that provides the greatest risk to the patient. By using the entirety of the device to evaluate cleanliness, the surface area of easy-to-clean areas may dilute the most challenging-to-clean features or PCL (Michels, et al., 2013) thus underestimating appropriateness.

ISO 17664-1 describes methods to classify devices for validation by either using a risk-based approach, such as the Spaulding classification, or by the challenge to the process based on the device design (International Organization for Standardization, 2021). Within the list of design considerations in Annex C, there are a number of items listed that can be considered design features. ISO standards are developed through consensus of international experts within the industry, so this list is not exhaustive but contain features that have been shown to be particularly challenging for cleaning. Each feature listed within ISO 17664-1 was challenged within this investigation using a standardized representative device feature (Table 5.2).

Table 5.2: Crosswalk of ISO 17664-1 design considerations and experimental challenge feature

ISO 17664-1 Annex C Design Considerations	Challenged Feature
Crevices	Blind Slot
Shift-shaft arrangement (e.g., Rongeurs)	Hinges, Joints, Pivot Points
Valves	O-rings-Internal O-ring
Fittings with close tolerances	Mated Surfaces Small Clearance
Lumens of flexible design	Smooth Through Lumen
Multiple internal lumens	Smooth Through Lumen
Lumens that are not easily accessible	Smooth Through Lumen
Clamps or joints that do not open fully for cleaning (e.g., pylorus clamps)	Hinges, Joints, Pivot Points
Small internal parts (e.g., springs, magnets)	Spring Internal
Size of mated surfaces and covered gaps	Mated Surfaces
Rough and irregular surfaces	Rough Surface
Connecting parts (e.g., luer locks)	O-rings - Internal O-ring
Junctions between insulating sheaths and activating mechanisms	Mated Surfaces Small Clearance
Dead-ended or blind end chambers	Blind Slot
Tightly coiled metal shafts (e.g., coiled shafts on flexible endoscope forceps)	Spring Internal

Within this list, lumens are mentioned quite often. The lumen has been demonstrated repeatedly in the literature as the most difficult to clean (Ofstead, et al., 2015) (Lopes, et al., 2019)). A dead-end lumen requires a backflow of the eluent flush once it reaches the dead-end to be removed from the feature. This requires competing pressure gradients in the lumen and can limit sheer force of the liquid over the surface resulting in ineffective soil removal (Hariharan, et al., 2019). The longer the lumen and the smaller the diameter the more challenging this feature becomes to clean. As the diameter narrows, the competing flow of the liquid increases. The length of the lumen will require more force for the liquid to reach the dead-end with enough flow velocity for the liquid to exit the lumen. A through lumen has similar challenges in addition to requiring the pressure of the water flow within the lumen to be great enough that the shear rate of the water can remove residual soil in the amount of time the flush occurs. The Smooth Through Lumen was selected as the challenge feature to represent “Lumens of flexible design” due to its more challenging material. Stainless steel, as an adsorbing material, has been demonstrated to be more challenging than repelling materials (Kremer & Ratanski, 2023).

Devices may have a combination of features that contribute to the overall risk. In some cases, the most challenging device feature can be evaluated for cleaning efficacy with confidence that the cleaning procedure will be effective for all features. However, there are also devices where the combination of features present an increased challenge during the assessment. Two of these scenarios are described in the ISO 17664-1 list and will be discussed in greater detail during the categorization assessment.

- Powered instruments with motors and channels which can entrap debris
- Internal moving parts such as multiple control cables within sheaths

5.2.4 Soil Drying

The time before or during the decontamination process that can lead to an increase cleaning challenge (Kremer, et al., 2023), available processing equipment, and effective process monitoring practices. Soil drying on a reusable medical device poses a significant risk for effective cleaning and sterilization. The risk of soil drying is a factor of soil penetration, air flow patterns and time.

Medical devices vary with different degrees and types of contamination. For example, general surgical instruments used for incision surgery in the fields of visceral surgery, urology, gynecology, ear, nose, and throat surgery are described as having an “average degree of contamination” while instruments used in orthopedic and/or trauma surgery, craniotomies, etc. have a heavy load of intraoperative soils, e.g. tissue residues or bone meal (DGKH - German Society for Hospital Hygiene, DGSV - German Society for Sterile Supply, AKI - Working Group Instrument Preparation, 2007). The following methods of soil contamination should be considered.

- Flushing - Soil will be flushed through the device.
- Full Immersion – The device will be fully immersed in the clinical soil.
- Partial Submersion – Only a portion of the device will be submersed in the clinical soil.

- Vacuum – Soil will be vacuumed through the device.
- Handled with Soiled Gloves – Soil will be deposited on the device
- Partially Soiled – Soil may be introduced during use but only in specific area of the device using a method like a paint brush or pipette method.

Full immersion has been demonstrated to be the most challenging of these application methods due to the volume of soil that is typically deposited on the device (Kremer & Ratanski, 2023), but depending on use, other methods of soil application can be as equally challenging.

5.2.4.1 Soil Drying Risk Evaluation

The use of the device can impact the soil penetration or fixation of the soil onto the device. Devices that actuate can move soil into hard to clean locations of the device. An example provided by ISO 17664-1 is a Rongeurs. This is a device that is used to open a window in bone to access tissue underneath. The shift-shaft arrangement of the device can facilitate the migration of soil into features of the device that are harder to clean. Another example is an impact drill type of device. Although only the distal end is immersed into the patient, the vibration of the device may facilitate the movement to bypass the outer housing and enter the interior of the device.

Devices that are used with heat or energy during use can cause cauterization of the soil. This action can affix the soil onto the device due to exposure to higher temperatures and become more difficult to remove (Kremer, et al., 2023). Exposure to saline for some devices can be highly corrosive and lead to pitting of instruments (Association for the Advancement of Medical Instrumentation, 2020). This type of damage to the instrument can lead to an increased cleaning challenge when soil is deposited in these unintended device features.

Wet soil has a higher solubility rate than dried soil (Kremer, et al., 2023) so the ability of air to penetrate the device and evaporate water from the soil must be assessed. Features such as long or narrow lumens may prevent airflow and slow the drying process of devices. Some devices allow for soil to dry more easily than others, depending on the device feature and geometry. For example, the ball detent / ball bearing has a fluid pathway to the internal mechanism of the device that when soil is allowed to dry make it more difficult to remove the soil during cleaning. This is demonstrated by a passing result for the 2hr drying versus a failing result for the 72-hour drying. Among the 23 features assessed, six (Ball Detent/Ball Bearing, Ball Seal Springs, Captured Screw, Rough Surface, Screws Threaded Rod/Threaded Thru Hole, and Leaf Springs) exhibited altered cleaning difficulties when the soil was permitted to dry for 72 hours, as illustrated in tables 35 and 36.

The hazardous situations resulting from soil drying may include an increase in processing time. As soil dries on a device, the solubility rate of the soil changes (Kimble, et al., 2023). If the cleaning instructions do not consider dried soil, the processing time may need to be extended to compensate. A comparison of data for each device feature between 2 hours and 72 hours has revealed differences for most features, as shown in tables 4.10 and 4.11. Furthermore, some features proved unable to be effectively cleaned if the

soil was allowed to dry completely (e.g., sliding shaft short and threads blind hole). In a study by Hoover et al. (Hoover, et al., 2023), a theory was proposed and tested that suggested the chemistry changes induced by dried soil could be reversed by soaking in an alkaline cleaning agent to rehydrate the soil. However, when tested within this experimental framework on features that failed the 72-hour dry time experiment, this theory was not supported. All four features tested failed to yield passing results and exhibited very high levels of residual analytes. Therefore, the duration for which the soil remains dried on the device during the cleaning validation process should be evaluated as part of the risk assessment.

As discussed, soil remaining on a device has multiple risks to patient harm. The presence of residual soil, debris, or organic material on a medical device can contribute to the growth of microorganisms and increase the risk of infection transmission. Damp or wet conditions provide an ideal environment for microbial growth. If residual soil is not effectively removed from a medical device, bacteria and other microorganisms may proliferate, increasing the risk of contamination. Soil that is not removed can also contribute to the formation of biofilms, posing a long-term risk for infection transmission. This can compromise patient safety during subsequent uses of the device. Within the literature, depending on the device, the likelihood of occurrence for residual soil can be all the categories, but can be serious or critical.

Using the cleaning classification categories to describe the risk as minimal, moderate, and maximal the following risk evaluation can be assigned for residual soil dried onto a device.

- Maximal – Frequent / probable occurrence of soil residual risk with serious / critical harm. This category would include devices with a high risk.
- Moderate – Occasional / Remote occurrence of soil residual risk with serious / critical harm. This category would include devices with a medium risk.
- Minimal – Remote / Improbable occurrence of soil residual risk with negligible / minor harm. This category would include devices with a low risk.

To mitigate the risks associated with soil drying on reusable medical devices, devices must be designed with considerations for easy access to all surfaces during cleaning. Narrow crevices and dead spaces in the device's geometry can impede air movement, making it difficult for moisture to evaporate. This can be beneficial if devices are allowed time to dry before processing, but these areas are not fully clean and remain damp, this can lead to potential microbial proliferation and biofilm development. Designing devices with minimized dead spaces and easily accessible surfaces facilitates the cleaning process. Regular maintenance and inspection of devices can also help identify and address any issues related to soil drying and cleaning efficacy.

5.2.5 Fluid Dynamics

Fluid dynamics play an important role in the cleaning of reusable medical devices. Understanding how fluids move and interact with the surfaces of a device must be considered when designing an effective cleaning process. The key aspects of fluid dynamics that will affect the cleaning process are the contact

angle, if the surface is hydrophilic or hydrophobic, flow patterns, eddy currents, accessibility, dead and spaces or crevices.

The contact angle between the cleaning fluid and the surface of the device determines the wetting characteristics. A lower contact angle indicates better wetting, allowing the cleaning fluid to spread more evenly across the surface. This is essential for ensuring that contaminants are effectively reached and removed. Hydrophilic surfaces attract water, promoting better wetting and spreading of the cleaning fluid. In contrast, hydrophobic surfaces repel water, making it challenging for the cleaning solution to cover the surface uniformly. Designing device surfaces to be hydrophilic can enhance cleaning effectiveness. The flow of cleaning fluid can be turbulent or laminar. Turbulent flow, characterized by chaotic movement, enhances the removal of contaminants by promoting better mixing and scrubbing. Laminar flow, characterized by smooth, parallel layers, is less effective at removing debris. Depending on the cleaning requirements, the design of the cleaning system should consider the desired flow pattern. Eddy currents can form in areas with complex geometries or obstacles. These swirling motions can affect the distribution of cleaning fluids and may result in areas of low fluid velocity where cleaning effectiveness is reduced. Designing devices with streamlined geometries minimizes the formation of eddy currents. The geometry of the device influences how easily cleaning fluids can access different surfaces. Intricate designs with complex geometries may create areas that are difficult to reach, leading to incomplete cleaning. Designing devices with accessible surfaces and simplified geometries enhances the accessibility of cleaning fluids. Dead spaces or crevices in the device's design can trap cleaning fluids, hindering their ability to reach all surfaces. Ensuring that the design minimizes dead spaces and provides effective drainage helps prevent the buildup of cleaning fluids and contaminants. If the device is not designed to allow for appropriate fluid dynamics to occur within the cleaning process a hazard will exist.

The hazardous situations resulting from unsuitable fluid dynamics may be the inability to execute the instructions for use effectively, increased processing time, and equipment and resource requirements. Device geometry that does not facilitate effective fluid dynamics may experience sporadic cleaning results. Good fluid dynamics can be described by emphasizing the efficiency and smoothness of fluid flow within a system. This involves characteristics that minimize resistance, turbulence, and the formation of dead zones. Fluid dynamics is considered good when it promotes efficient flow, allowing fluids to move through a system with minimal resistance and energy loss. Turbulence of the fluid can lead to lack of surface contact, especially if the fluid has vorticity, tendency of fluid elements to rotate, causing the formation of eddies and swirls in the flow. Complex geometries also may experience pressure drops due to resistance in fluid flow, thus changing the velocity of the fluid and as an extension the shear rate. The geometry of a device also impacts its ability to drain fluids and remove residues. If the device has areas where fluids can pool and are difficult to drain, cleaning agents may not be effectively removed, leaving behind contaminants. Incorporating features that facilitate drainage, such as proper angulation and well-designed channels, helps improve the overall efficacy of the cleaning process.

5.2.5.1 Fluid Dynamics Risk Evaluation

Effective and reproducible cleaning efficacy depends on fluid dynamics that result in stable and predictable behavior, meaning that the system responds consistently to changes in input conditions without exhibiting erratic or unpredictable flow patterns. Some devices may need an increased processing time or special equipment to stabilize the fluid dynamics and deliver consistently clean devices. The following questions can be used to assess the risk of the hazardous situation:

- Can the device be fully submerged?
- Do all features of the medical device receive exposure to liquid with a constant flow rate?
- Is the device disassembled prior to cleaning so all internal surfaces of the device are exposed?

Devices with a poor geometry that does not facilitate good fluid dynamics also has multiple risks to patient harm. Water is the main solvent responsible for the removal of soil from a reusable medical device. If the cleaning fluid used does not have fully access to the soiled portions of the device, or is inconsistent with contact, the risk increases that the device will not be clean. Therefore, the harm for fluid dynamics is the same as with residual soil. Using the cleaning classification categories to describe the risk as minimal, moderate, and maximal the following risk evaluation can be assigned for poor fluid dynamics.

- Maximal – Frequent / probable occurrence of soil residual risk with serious / critical harm. This category would include devices with a high risk.
- Moderate – Occasional / Remote occurrence of soil residual risk with serious / critical harm. This category would include devices with a medium risk.
- Minimal – Remote / Improbable occurrence of soil residual risk with negligible / minor harm. This category would include devices with a low risk.

The ability to extract soil from a device can be evaluated using and an exhaustive extraction efficiency. This methodology will demonstrate how the geometry of the device will facilitate the movement of liquid through the device. ANSI/AAMI ST98 states that the recovery efficiency of a device must be greater than 70% (Association for the Advancement of Medical Instrumentation, 2022). Although this value is used as part of the cleaning validation as a safety factor when measuring the residual analyte, the value can also be used to understand the fluid dynamics of the device. The extraction efficiency measurement can be used during the design process to promote device geometry the promotes good fluid dynamics. Designing devices with these fluid dynamics principles in mind helps ensure that cleaning solutions can effectively reach and remove contaminants from all areas of the device, promoting thorough cleaning and sterilization in healthcare settings.

5.3 Risk Assessment

US experts as part of AAMI ST-WG12, Instructions for reusable device reprocessing working group, articulated three categories of devices developed from publicly available cleaning IFUs in AAMI TIR12:2020. The intent of this categorization is to provide manufacturers with a system to assess when device features present risk that would need to be mitigated with increased intervention. Section 4.3 of

AAMI TIR12:2020, “Physical design considerations” filters devices into the following categories: easy to clean, complex devices requiring ultrasonic cleaning, and non-critical devices not intended to be sterilized. This is a bit confusing for the reader, as the diagrams in Annexes D and E of AAMI TIR12:2020 depict four categories: simple devices, devices requiring intervention, devices requiring ultrasonic cleaning, and non-critical devices not intended to be sterilized. In Annex D, the “Easy to clean devices” is broken down into two groups: simple devices and devices requiring intervention (Association for the Advancement of Medical Instrumentation, 2020).

Easy to clean devices can appear in any of the Spaulding classification categories. They will have a simple device structure where all surfaces can be exposed for cleaning. Moving parts are limited and are not likely to have soil migrate into areas that are difficult to clean. Devices that are easy to clean can be cleaned in a washer disinfector without manual cleaning interventions like brushing or flushing.

Devices that require intervention may require additional cleaning steps like brushing, flushing or soaking in order for the soil to be completely removed by the cleaning chemistries. These devices may have rough/textured surfaces or surfaces that allow for soil accumulation, such as crevices. Lumens are often required to have intervention requirements to facilitate the soil removal from the inner shaft. Without flushing or bushing, the air trapped in a lumen will prevent exposure from the cleaning fluid to occur and result in residual soil. Devices with multiple mated surfaces, joints and/or hinges that are difficult to access by the cleaning fluid, will need intervention with brushing/flushing/soak to force the interaction of the soil in the feature with the cleaning fluid. Features that are difficult to dry can be challenging in multiple ways. If the feature does not allow for air flow, the soil may not dry, therefore reducing the cleaning challenge, but if the space is restricted that fluid does not have appropriate fluid dynamics, then the feature cannot be cleaned effectively.

Complex devices with very hard to clean features will require additional intervention such as sonication. If surfaces are inaccessible, hidden sliding surfaces, or blind channels, flushing alone may not be sufficient to promote the necessary fluid dynamics for soil contact, and bushing may not be physically possible. The action of cavitation can facilitate fluid movement in the intricate design features that is not possible with other cleaning methods. Design features with flexible components or multiple components that cannot be disassembled during the cleaning process are examples.

Aside from general descriptions of features, there is no guidance for how to assess which device category a reusable medical device should be placed in. It is the responsibility of the device manufacturer to assess the device and validate the instructions for use accordingly.

In the paper by Michels et. al (Michels, et al., 2013), the authors suggest a classification system in which devices are placed using a risk assessment into six categories based on their design. Group 1 consists of instruments without joints, cavities, or lumens. This category seems similar to the “simple devices” from TIR12, but the authors go on to separate out instruments with a joint into group 2. Joints can be defined as “easy to clean” per TIR12 but can also require intervention depending on if the joint is accessible to

facilitate the appropriate fluid dynamics to clean. Group 3 consists of sliding-shaft instruments and group 4 are tubular instruments. Both groups can require intervention for cleaning and may also require the use of ultrasonic cleaning. Microsurgical instruments and complex instruments are separated into groups 5 and 6 respectively. The authors do not give an explanation for the proposed categories but suggest that the validation dataset they evaluated supported this categorization practice.

Both TIR12 and Michels et. al categorizations identify cleaning performance differences depending on the device feature. Both give the responsibility of a risk assessment to complete categorization to the device manufacturer, but with little guidance for how to complete it. The risk assessment used within this this newly proposed categorization system will assign a quantitative number to evaluate and categorize a reusable medical device based on the overall risk stemming from the probability of effectively cleaning a device feature. This strategy employs a scoring system that converts qualitative aspects into numerical values. Each feature is assigned a weighted score reflecting its importance in the overall cleaning performance of the device. This method allows for a more systematic and standardized assessment, facilitating comparison and decision-making during the risk assessment. It not only streamlines the evaluation process but also enhances transparency.

5.3.1 Device Feature Risk Evaluation

As discussed above, the device features selected are representative of the most challenging to clean features on reusable medical devices and were evaluated in a standardized manner so a direct comparison of cleaning performance can be used to evaluate the probability of soil retention. Cleaning validation guidance in AAMI ST98 dictates the cleaning validation should be performed on multiple replicates. The number of replicates required is dependent on the data variability measured against the acceptance criteria (Association for the Advancement of Medical Instrumentation, 2022). Due to the cost of cleaning validations, the number of replicates performed during a cleaning validation should be minimized. The sample size suggested in ST98 was acceptable by industry experts as acceptable as long as all values in the data set demonstrated passing results.

When performing a risk evaluation, additional measures for safety need to be considered for a robust analysis. Prior to this experimental design, it was only assumed that cleaning validation data was normally distributed. Data that follows a normal distribution is characterized by a symmetric bell-shaped curve, making it highly predictable within certain statistical parameters. The normal distribution, often referred to as a bell curve, is governed by the central limit theorem, which states that the sum or average of a large number of independent, identically distributed random variables will be approximately normally distributed. This predictability arises from the fact that the normal distribution is fully defined by its mean and standard deviation. Knowing these two parameters allows for accurate predictions of the likelihood of values falling within specific ranges or intervals. In a normal distribution, approximately 68% of the data falls within one standard deviation of the mean, 95% within two standard deviations, and 99.7% within three standard deviations. This predictability makes normal distribution a powerful tool in statistical

analysis, as it enables researchers and analysts to make informed predictions and draw reliable conclusions about the data based on its distribution characteristics.

The device feature approach validation confirmed that cleaning validation results are indeed normally distributed when enough data points are generated. The data from both coupon types at 20, 30, and 40mm was normally distributed as demonstrated by the probability density of results (Figure 4.2).

Utilizing the upper value of the 99% confidence interval serves as a robust strategy to mitigate the risk of error in statistical analyses and decision-making processes. When estimating parameters, such as means or proportions, the 99% confidence interval provides a range within which the true population parameter is likely to lie with 99% confidence. By focusing on the upper limit of this interval, practitioners adopt a conservative approach, ensuring a margin of safety against potential errors. This upper value serves as a safeguard, acknowledging the inherent uncertainty in statistical estimates and guarding against underestimation of values critical to decision-making. Consequently, relying on the upper bound of the confidence interval helps to promote a more cautious and accurate interpretation of data, reducing the likelihood of making decisions based on overly optimistic or underestimated estimates, and thus enhancing the reliability of statistical inferences.

Within this experimental design, three test markers were evaluated, protein, TOC, and ATP. Protein was evaluated for both the water-soluble proteins and the non-water-soluble proteins. TOC and ATP methodologies are only compatible with the water as the extraction eluent, so total residual soil, especially after extended drying times, can only be evaluated using the protein test marker.

The features were evaluated for both fluid dynamics, using the extraction efficiency, and soil drying/retention, using the cleaning efficacy. By assigning a quantitative value for each of these evaluations, a total performance score for each feature is identified. This score is the foundational measure for risk evaluation and subsequent categorization.

5.3.2 Fluid Dynamics Risk Categorization

The risk associated with the fluid dynamics aspect of the device geometry is measured using the exhaustive extraction efficiency calculation. Soil was deposited on the device using the immersion technique (see section 3.1.5) and then dried for 72 hours (see section 3.1.6) to simulate the most challenging cleaning condition. Each device was extracted using a combination of flushing (when applicable), orbital shaking and sonication.

Flushing was used to ensure that fluid reached areas of the device that might harbor air that prevent fluid from fully penetrating the feature. During the cleaning process, if air was trapped in certain areas of a medical device, this can create barriers that prevent the direct contact of the cleaning fluid with the surfaces they are supposed to clean. Trapped air can form in small crevices, channels, or other intricate parts of the device, hindering the cleaning solution from reaching those areas effectively.

Orbital shaking forces the movement of the fluid within the device feature and facilitates the removal of soil. The purpose of this mechanical agitation is to dislodge and break-up contaminants, such as proteins, or other residues, from the surfaces of the medical device. Orbital shaking facilitates improved fluid dynamics within the extraction eluent. The shaking motion helps distribute the cleaning fluid evenly, ensuring that it reaches all parts of the device, including complex geometries, crevices, and hard-to-reach areas. This enhanced fluid movement helps in maximizing the contact between the cleaning solution and the surfaces of the device, promoting effective cleaning and removal of contaminants.

Sonication involves the use of ultrasonic transducers that generate high-frequency sound waves (typically above the range of human hearing) in the extraction eluent. These ultrasonic waves create microscopic bubbles in the liquid through a process called cavitation. The bubbles rapidly expand and collapse, generating intense shock waves and tiny, localized liquid jets. This process is called cavitation. The collapse of bubbles during cavitation induces powerful microstreaming currents in the cleaning solution. These currents create agitation and turbulence, facilitating the removal of contaminants from the surfaces of the smallest recesses of a medical device.

The purpose of the exhaustive extraction efficiency is to completely remove all soil from the device, but since water is the primary diluent used in cleaning, only water extractions were used for the extraction efficiency calculation for a total of four extractions. Comparing the performance of the extraction efficiency for each feature demonstrates the efficacy of the fluid dynamics at removing residual soil. The recovery efficiency number should be as high as possible, so the low value of the 99% confidence interval was used to rank the features. Utilizing the lower value of the 99% confidence interval is a strategic approach in assessing the probability of risk. The 99% confidence interval represents a range of values within which we can be 99% confident that the true parameter of interest lies. By focusing on the lower bound of this interval, one adopts a conservative perspective, acknowledging the potential for a worst-case scenario. Using the percent exhaustive extraction efficiency, the feature is assigned a value that is then used in the overall risk evaluation (see table 5.3 and 5.4).

Table 5.3: Risk categorization scheme for extraction efficiency

Extraction Efficiency	Risk Value	Extraction Efficiency	Risk Value
≥ 90%	1	49% - 40%	6
89% -80%	2	39% - 30%	7
79% -70%	3	29% - 20%	8
69% - 60%	4	19% -10%	9
59% - 50%	5	< 10%	10

Table 5.4: Device feature fluid dynamic categorization

Device Feature	72hr Dry Water Only Extraction Efficiency 99% CI (low)	Extraction Efficiency Value	Requires Flushes (additive)	Fluid Dynamics Risk Value
Rough Surface	96.133%	1	0	1
Sliding Shaft Short	92.192%	1	0	1
Leaf Springs	92.161%	1	0	1
Sliding Shafts Long	92.100%	1	0	1
Screws Threaded Rod / Threaded Thru Hole	90.634%	1	0	1
O-rings - External O-ring	87.320%	2	0	2
Mated Surfaces Small Clearance	85.259%	2	0	2
Hinges, Joints, Pivot Points	79.900%	3	0	3
Mated Surfaces	79.020%	3	0	3
Snap Rings	73.070%	3	0	3
O-rings - Internal O-ring	68.180%	4	0	4
Button w/ Spring	66.830%	4	0	4
Ball Seal Springs	55.170%	5	0	5
Screws Threaded Rod/ Threaded Blind Hole	97.145%	1	5	6
Threads Blind Hole	93.633%	1	5	6
Smooth Through Lumen	91.980%	1	5	6
Smooth Blind Lumen	90.330%	1	5	6
Captured Screw	49.620%	6	0	6
Buttons - Exposed Springs	45.750%	6	0	6
Blind Slot	70.540%	3	5	8
Ball Detent / Ball Bearing	67.310%	4	5	9
Spring Internal	64.510%	4	5	9
Through Slot	33.120%	7	5	12

The exposed surface area of a reusable medical device plays a pivotal role in augmenting the fluid dynamics of the cleaning process. This is evident in the analysis of the rough surface results. The rough surface is a complex feature that is known to harbor soil (e.g., laryngoscope handle), where the exposure of the surface area increases the effectiveness of the fluid dynamics to remove soil. Although this feature does have moving parts, all complex features can be exposed to fluid without intervention (i.e., flushing).

A larger exposed surface area provides more contact points for the fluid to interact with, promoting enhanced fluid dynamics. As the cleaning fluid comes in contact with the diverse surfaces of the feature, it can effectively reach and dislodge contaminants from intricate features, crevices, and other hard-to-reach areas. This is reflected in the extraction efficiency being greater than 90% for the sliding shaft short,

leaf springs, sliding shaft long, and screws threaded rod / threaded thru hole. Although these are complex features, the increased surface area allows for better penetration of the fluid into all portions of the device, ensuring a more thorough extraction.

The remaining features with extraction efficiency results greater than 90%, screws threaded rod/threaded blind hole, threads blind hole, smooth through lumen, and smooth blind lumen, required repeated flushing (3X) to effectively remove soil during the extraction. The flushing was completed using a syringe so a constant flow rate could be achieved. The shear rate of the fluid from the syringe proved to be effective at moving through the feature and dislodging the soil. For the blind features (i.e., features with a dead end) the fluid was required to move through the feature to the closed or sealed portion of the device where fluid flow is limited or restricted and then exit using the same opening. This type of feature creates challenges for fluid movement without direct intervention. To account for the intervention and normalize the risk across all the device features, devices that required flushes had an increased weight of 5 points added to the total risk categorization score for fluid dynamics. So although the device features, screws threaded rod /threaded blind hole, threads blind hole, smooth through lumen and smooth blind lumen, all achieved a greater than 90% recovery efficiency, the weighted score for the required flushing (i.e., +5) increases their total rank.

For the features that did not require flushing, as the device increased in complexity with non-exposed surface areas, the extraction efficiency decreases (see \Figure 5.4).

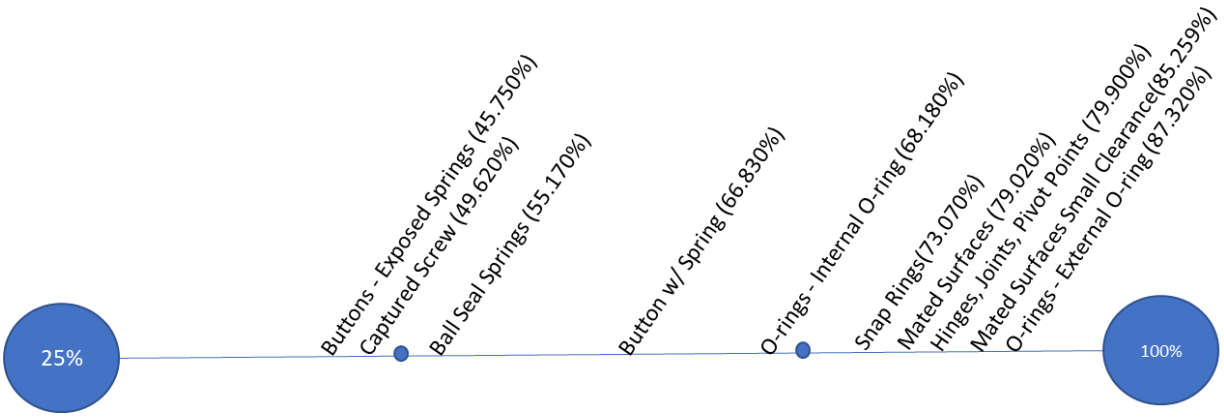


Figure 5.4: Feature Extraction Efficiency < 90% Excluding Flushes

The most challenging features for fluid dynamics proved to be those features that required flushes to reach all surface areas of the feature. The blind slot and the through slot present an increased challenge for the fluid dynamics due to the long narrow space. Fluid must contact all surfaces within the slot with a high enough shear rate to remove adhered soil. The geometry of the device allows for the movement of air and evaporation of water within the soil, therefore, increasing the cleaning challenge compared to other features like lumens. For effective fluid dynamics to aid in soil removal, the flushing must be performed so the fluid is dispensed along the slot. When the slot has a dead end (i.e., blind slot) the flushes

are more effective as the shear force of the liquid is doubled when the fluid exits the same way it enters. Antithetically, the fluid movement in the through slot travels only one way within the feature. This occurrence explains why the extraction efficiency for the blind slot, 70.540%, is significantly higher than the through slot, 33.120% (p-value 0.00).

The two other features that fell below the 70% acceptance criteria (Association for the Advancement of Medical Instrumentation, 2022) where the ball detent / ball bearing, 67.310%, and the spring internal, 64.510%. Both of these features have hidden surfaces that require flushing, but also are restricted with a one-way flow for fluid dynamics. The flushing must be executed in a manner where the fluid comes in contact with the surface at a precise angle to facilitate soil removal. As demonstrated by the lower extraction efficiencies, the effectiveness of the flushes is more variable than with the other features (see Table 31).

The cleaning of reusable medical devices involves inherent fluid dynamic risks that can impact the effectiveness of the cleaning process and, consequently, the safety of patients. The fluid dynamics risk categorization quantitatively (Table 5.4) assigns a score to this risk by assessing the device features. Fluid dynamics play a critical role in determining the flow and distribution of cleaning agents, and the design of the device can either facilitate or hinder this process. Complex geometries, narrow channels, or irregular surfaces can impede the proper flushing of contaminants, leading to incomplete cleaning and a higher risk of microbial residue or other contaminants persisting. Understanding this risk using a quantitative assessment will facilitate the mitigation of this risk by focusing on targeted interventions.

5.3.3 Soil Drying Risk Categorization

The movement of air can also be considered a fluid in the context of fluid dynamics because it exhibits the fundamental characteristics of a fluid. Fluid dynamics is the study of the motion of fluids, which includes liquids and gases. Air, being a gas, conforms to the basic principles of fluid dynamics. Like liquids, gases such as air can flow and take the shape of their containers, displaying the property of fluidity. Additionally, air experiences changes in pressure and velocity as it moves, showcasing behaviors such as turbulence and laminar flow that are central to fluid dynamics.

The interplay between air fluid dynamics and the evaporation rate of water from blood on a reusable medical device is a multifaceted process influenced by several factors. Air fluid dynamics impact the rate of evaporation by governing the movement and exchange of air around the residual soil. The flow patterns and turbulence in the surrounding air can affect the dispersion of water vapor, altering the concentration gradient and, consequently, the rate of evaporation. Temperature and humidity, both influenced by air dynamics, play important roles in this process (Kremer, et al., 2023). The movement of air can enhance or hinder heat exchange, affecting the temperature of the soil and, in turn, influence the rate of evaporation. Furthermore, the design and geometry of the medical device, as well as the airflow patterns in its vicinity, can significantly impact how quickly water evaporates from the residual soil.

Soil drying has a significant impact on the risk for cleaning, so in addition to the fluid dynamic categorization, the device features were sorted by cleaning performance under a variety of experimental conditions designed to test to the point of cleaning failure. To assess the cleaning efficacy for categorization, protein was used as the test marker. Using both the water extraction and the additive extraction technique, the residual soil was assessed for the probability of soil remaining for both water-soluble and non-water-soluble proteins. This total assessment of residual protein assesses the cleaning efficacy of the device feature under standardized cleaning parameters. The number of replicates and robustness of the test design accounted for human factors that may influence the results.

The test design for each feature, as described in Chapter 3, was designed to test the point of failure. Not all of the scenarios tested were applicable for the classification (i.e., semi-automated and DBLSO soiling), but will be included as part of the related discussion on modeling of cleaning and automation. For the classification the following test designs were included as applicable for the device feature.

- 72-hour soil dry with a water only extraction
- 72-hour soil dry with an additive extraction
- 2-hr soil dry with a water only extraction
- 2-hr soil dry with an additive extraction
- 1-hr soil dry with a water only extraction
- 1-hr soil dry with an additive extraction
- 2-hr soil dry, no brush/flush cleaning instructions and a water only extraction
- 2-hr soil dry, no brush/flush cleaning instructions and an additive extraction

The first 6 test designs challenge the device feature to assess the risk of residual soil remaining on the device after cleaning. To assign a quantitative value to the feature performance, the alert and action levels for protein residuals from ISO 15883-5 were used to score the feature. The 99% confidence interval was again used for the assessment, but unlike the recovery efficiency result, the high value of the confidence interval was used to assign the risk value. If the high value of the confidence interval resulted in a value less than the alert level of 3µg/cm^2, then the risk value was 1. If the value fell between the alert level, 3µg/cm^2, and the action level of 6.4µg/cm^2, then the risk value assigned was 2. If the value was greater than the action level of 6.4µg/cm^2, then the risk value assigned was 3 (see table 5.5).

Table 5.5: Risk categorization scheme for cleaning efficacy (except No Brush/Flush)

Protein Residual	Risk Category
<3µg/cm^2	1
3 µg/cm^2 – 6.4 µg/cm^2	2
>6.4 µg/cm^2	3

Those features challenged under the scenario of no brush/flush, were those that had results <6.4 µg/cm^2 for the 2-hr soil dry test design. The additional challenge of removing the manual portion of the cleaning

instruction allows for the further assessment of the fluid dynamics. This additional assessment allows for a deeper understanding of the feature's cleaning efficacy. If acceptable protein residual results, <3µg/cm^2, were achieved without the added intervention of a brushing or flushing step the risk category is decreased by 2. This value is assigned to reflect a lower risk of the feature with residual protein when brushing/flushing are included as part of the cleaning steps. If the result fell between the alert level, 3µg/cm^2, and the action level of 6.4µg/cm^2, then the risk value was decreased by 1. And finally, if the result was greater than the action level of 6.4µg/cm^2, then the risk value not decreased (see Table 5.6).

Table 5.6: Risk categorization scheme for cleaning efficacy for No Brush/Flush

Protein Residual	Risk Category
<3µg/cm^2	-2
3 µg/cm^2 – 6.4 µg/cm^2	-1
>6.4 µg/cm^2	0

Increased time associated with soil drying has an increased risk for the soil challenge (Kremer, et al., 2023). This increased risk is primarily due to changes in solubility of the soil (Kremer, et al., 2023). To assess this risk of soil drying, cleaning efficacy for each feature was challenged at both the 2hr and 72hr time point. Some features (e.g., through slot, hinges, joint, pivot points, ball detent/ball bearing) demonstrated that the manual interventions of brushing/flushing were not sufficient when soil is allowed to dry on the device for extended periods and indicates that additional interventions may be necessary to effectively clean the feature.

Other features (e.g., sliding shaft short, smooth through lumen, threads blind hole, sliding shaft long, O-rings – Internal O-ring) demonstrated that cleaning efficacy is difficult to achieve when soil has been allowed to dry to the point where no moisture content is detectable, >1hr (Kremer, et al., 2023) (see tables 4.12 and 4.13). These features have increased risk to account for the hazardous situation where point of use instructions for use, to not allow soil to dry, are not completed prior to transport for full decontamination.

The semi-automated cleaning instructions demonstrated success with 17 out of the 23 features (Table 4.17), with the procedure involving preventing soil from drying on the device before initiating the cleaning instructions. The positive outcomes underscore the significance of point-of-use treatment in facilitating effective decontamination. Without ensuring this crucial step is performed on every device, the successful automation of device processing will not be feasible.

5.3.4 Device Feature Risk Categorization

By assessing the full risk of the device features, a combined risk category is calculated. The score from the extraction efficiency is combined with the scores from the other six experimental designs to deliver the total risk categorization value (Table 5.7). The risk of the feature can also be assessed for the likelihood of

occurrence based on the numerical value obtained from the experimental designs focused solely on cleaning. Although the extraction efficiency predicts the penetration of the cleaning fluid into the feature, the results from the cleaning only can be used to assess how effective a standardized cleaning process is at removing residual soil. As discussed above, for cleaning classification, the risk for the likelihood of occurrence is condensed into 3 categories to be used to assess the risk. The cleaning only risk value is assessed against the categories using the categorization scheme in Table 5.8.

Table 5.7: Risk categorization scheme for risk for likelihood of occurrence

Risk for Likelihood of Occurrence	Risk Value
Frequent / probable occurrence of soil residual risk	>15
Occasional / Remote occurrence of soil residual risk	10-15
Remote / Improbable occurrence of soil residual risk	<10

Features with a cleaning only risk value of greater than 15 have a higher likelihood of occurrence, so would be considered frequent/probable. Features with this risk level are the smooth through lumen, threads blind hole, spring internal, and the smooth blind lumen. This result is consistent with device features that have been demonstrated in the literature as being very difficult to clean (Ofstead, et al., 2015) (Lopes, et al., 2019).

The next category of occasional / remote includes the following features: hinges, joints, pivot points, button w/ spring, sliding shaft short, O-rings-internal O-ring. This higher risk is likely due to the complexity of the feature and the migration of the soil into difficult to access areas of the feature.

The remaining features fell into the third category of remote/improbable. Although these features also have complicated components, the cleaning process has a higher likelihood of thorough cleaning than the other categories.

Table 5.8: Device feature risk categorization

Device Feature	Total Feature Risk Value	Cleaning Only Risk Value	Risk for Likelihood of Occurrence
Ball Detent / Ball Bearing	17	8	Remote / Improbable
Ball Seal Springs	12	7	Remote / Improbable
Blind Slot	25	17	Frequent
Button w/ Spring	15	11	Occasional / Remote
Buttons - Exposed Springs	8	2	Remote / Improbable
Captured Screw	12	6	Remote / Improbable
Hinges, Joints, Pivot Points	18	15	Occasional / Remote

Device Feature	Total Feature Risk Value	Cleaning Only Risk Value	Risk for Likelihood of Occurrence
Leaf Springs	9	8	Remote / Improbable
Mated Surfaces	15	12	Occasional / Remote
Mated Surfaces Small Clearance	8	6	Remote / Improbable
O-rings - External O-ring	4	2	Remote / Improbable
O-rings - Internal O-ring	18	14	Occasional / Remote
Rough Surface	8	7	Remote / Improbable
Screws Threaded Rod / Threaded Thru Hole	5	4	Remote / Improbable
Screws Threaded Rod/ Threaded Blind Hole	16	10	Occasional / Remote
Sliding Shaft Short	12	11	Occasional / Remote
Sliding Shafts Long	15	14	Occasional / Remote
Smooth Blind Lumen	20	14	Frequent
Smooth Through Lumen	24	18	Frequent
Snap Rings	12	9	Remote / Improbable
Spring Internal	25	16	Frequent
Threads Blind Hole	24	18	Frequent
Through Slot	25	13	Frequent

As described in the device feature approach validation, by selecting the hardest to clean feature on a reusable medical device the entirety of the device risk is assessed. Identifying the most challenging-to-clean feature on a reusable medical device involves a meticulous and systematic process. Initially, a thorough analysis of the device's design and functionality is conducted, with a focus on intricate components, complex geometries, or areas with tight tolerances that may impede effective cleaning.

5.3.5 Compounding Cleaning Risks

The device feature categorization is used as the base value for the risk assessment, but the compound risks must also be assessed to include the device geometry and material of construction. Compounding risk refers to the phenomenon where multiple risk factors, independently manageable on their own, converge or interact to create a heightened and more complex level of risk. In the context of cleaning a reusable medical device, compounding risk manifests when multiple factors converge to create challenges that collectively make the cleaning process more intricate and demanding. Various elements, such as complex device design, intricate components, and hard-to-reach areas, may independently pose difficulties in achieving thorough cleanliness. When these factors interact and compound, their collective

impact can significantly amplify the risk of incomplete cleaning, potentially leading to the persistence of contaminants and compromising patient safety.

Compounding risk in this scenario underscores the necessity for a holistic approach in identifying, assessing, and mitigating the interrelated challenges involved in cleaning. It involves recognizing how different aspects, such as material composition, device geometry, and usage patterns, may synergistically contribute to the overall difficulty of achieving cleaning efficacy.

When assessing a reusable medical device for risk, the most challenging feature that is exposed to soil is identified. Although a device may consist of multiple challenging features, by isolating the feature that is exposed to soil during clinical use, the highest starting risk value for the classification is assigned. Similar to sterilization validations (International Organization for Standardization, 2006), where the most difficult to sterilize location of the device is identified and challenged within the test design, the most difficult to clean feature is used to represent the total risk of a device. As shown in the data set in Figure 4.2, if the cleaning instructions effectively clean the most challenging feature, then all other features will also be cleaned using the instructions.

To assess for these compound risks, in addition to the device feature, additional risks are assessed during the risk evaluation. The base risk value from the most challenging to clean feature that is exposed to soil is increased by an additional value that reflects the total compounded risk. Each compounding risk is discussed with an associated risk rubric.

The material of the feature may impact the probability of risk for cleaning the device. As demonstrated in the research by Kremer & Ratanski, commonly used medical device materials can be classified as either adsorbing or repelling. Materials that repel soil like Silicone, PEEK and Delrin have been demonstrated to allow soil to lift from the surface during application and coagulate. Materials that can adsorb a certain level of soil like Stainless Steel, Nitinol, Aluminum and Titanium have demonstrated a more consistent application pattern for adsorbable metal (Kremer & Ratanski, 2023). Adsorbing materials may have a slightly higher risk for cleaning efficacy due to the nature of the soil attachment to the material (Table 5.9).

Table 5.9: Risk rubric device material

Risk Question	Answer	Risk Value	Risk for Likelihood of Occurrence
Is the material in the most challenging feature adsorbing (e.g., Stainless steel, Nitinol, Aluminum, Titanium) or repelling (e.g., silicone, PEEK, Delrin)?	Adsorbing	1	Remote/Improbable – Serious/Critical
	Repelling	0	Remote/Improbable – Minor/Serious

The amount of soil that a device will be exposed to is directly related to how the device is being used clinically. For example, if a medical device is used during an open-heart surgery, portions of the device

may be fully submerged into the patient. Clinical soil will in this case, have full contact with the medical device and have the opportunity to migrate into difficult to clean features (e.g., hinges, mated surfaces).

The risk therefore needs to consider how the feature will be soiled during contact with the patient. To assess the risk of patient contact, the following categories from ISO 10993-1 are used. As the contact with the patient increases, so does the risk (Table 5.9).

- Non-patient contacting – Medical devices that are used during a patient procedure, but do not come in contact with the patient (e.g., instrument case).
- Intact skin – Medical devices that contact intact skin surfaces only.
- Mucosal membranes – Medical devices that contact intact mucosal membranes (e.g., bronchoscopes)
- Breached or compromised surfaces – Medical devices that contact breached or otherwise compromised body surfaces
- Blood path, indirect – Medical devices or components that do not necessarily directly contact the blood path directly.
- Tissue/bone/dentin – Medical devices that contact tissue, bone, or pulp/dentin systems. Medical devices or components that do not necessarily directly contact tissue or bone but serve as conduits to delivery fluids to the tissue or bone.
- Circulating blood – Medical devices that contact circulating blood.

Table 5.10: Risk rubric patient contacting

Risk Question	Answer	Risk Value	Risk for Likelihood of Occurrence
What contact does the feature have with the patient?	Non-patient Contacting	0	Remote/Improbable – Minor/Serious
	Intact Skin	0	Remote/Improbable – Serious/Critical
	Mucosal Membranes	1	Occasional/Remote – Minor/Serious
	Breached or compromised surfaces	2	Occasional/Remote – Serious/Critical
	Blood path, indirect	3	Probable/Occasional – Minor/Serious
	Tissue/Bone/Dentin	4	Probable/Occasional – Serious/Critical
	Circulating Blood	5	Frequent/Probable – Minor/Serious

The increased risk of prion contamination is a notable concern when medical devices come into contact with high-risk tissues, including the brain, spinal cord, and eye. These tissues are particularly susceptible to prion accumulation, and any medical device that interfaces with them may inadvertently carry infectious prions. Due to the resilience of prions to conventional sterilization methods, there is a heightened risk that these infectious agents may persist on or within the medical devices, but literature has also demonstrated that prion risk can be mitigated using specific decontamination protocols (Fichet, et al., 2004). In cases where surgical instruments or devices are used in procedures involving the central nervous system or ocular tissues, specialized cleaning and sterilization protocols may be prudent to minimize the risk of prion transmission (Table 5.11).

Table 5.11: Risk rubric prions

Risk Question	Answer	Value	Risk for Likelihood of Occurrence
Does the device come in contact with high-risk tissue such as the brain, spinal cord and eye?	Yes	1	Remote/Improbable – Serious/Critical
	No	0	Remote/Improbable – Minor/Serious

The amount of soil a feature is exposed to during clinical use will change the risk level (Table 5.12). ASTM F3293-2018 (ASTM International, 2018) describes soil application methods as the following:

- Handled with Soiled Gloves – Soil will be deposited on the device. The method of contamination is the least exposure to the soil.
- Partially Soiled – Soil may be introduced during use but only in a specific area of the device.
- Vacuum – Soil will be vacuumed through the device. The vacuum implies that the movement of soil is one way, so any residual soil in the fluid pathway would not typically be exposed to a patient. However, there is a potential for backflushing if the vacuum is stopped suddenly.
- Partial Submersion – Only a portion of the device will be submersed in the clinical soil. Note that with submersion the soil has the opportunity to migrate.
- Full Immersion – The device will be fully immersed in the clinical soil. This soil application method deposits the most soil on a device (Kremer & Ratanski, 2023).
- Flushing - Soil will be flushed through the device. Residual soil that is remaining in the fluid pathway will be deposited into the patient during use where soil particles will be implanted in the patient.

Table 5.12: Risk rubric soil exposure

Risk Question	Answer	Value	Risk for Likelihood of Occurrence
How is the soil exposed to the most challenging feature?	Handled with Soiled Gloves	1	Remote/Improbable – Minor/Serious
	Partially Soiled	2	Remote/Improbable – Serious/Critical
	Vacuum	2	Remote/Improbable – Serious/Critical
	Partial Submersion	3	Occasional/Remote – Serious/Critical
	Flushing	4	Probable/Occasional – Minor/Serious
	Full Immersion	5	Frequent/Probable – Minor/Serious

After soiling, how the device may be used will further increase the risk. If the soil is exposed to heat (e.g., cauterization) the proteins can become affixed to the device surface, thus making them more difficult to remove (Kremer, et al., 2023). Chemicals used within the procedure can also have a negative effect on the instrument and affect soil application. For example, saline is corrosive to instrumentation and can lead to pitting of the device material. This change increases the probability of soil absorption and the cleaning challenge (Table 5.13).

Table 5.13: Risk rubric special conditions

Risk Question	Answer	Value	Risk for Likelihood of Occurrence
Will the soil be exposed to heat (e.g., cauterization)?	Yes	1	Remote/Improbable – Serious/Critical
	No	0	Remote/Improbable – Negligible/Minor
Will the soil be exposed to chemicals during use (e.g., saline, chlorine, iodine, simethicone)?	Yes	1	Remote/Improbable – Serious/Critical
	No	0	Remote/Improbable – Negligible/Minor

The orientation, presentation, and size of specific features in a reusable medical device, such as size (e.g., long lumens, microsurgical instruments), or housing enclosures can impact cleaning efficacy risk. For lumens, the orientation and size play a critical role; extended or convoluted lumens may hinder the accessibility of cleaning tools and solutions, making it challenging to reach and effectively remove

contaminants. The presentation of features where they are enclosed within housings or covered but allow for soil migration, can create hidden areas where debris may accumulate, demanding meticulous cleaning approaches to ensure thorough decontamination. The small size of microsurgical instruments, while essential for precision, can pose challenges in handling and cleaning due to the limited space for cleaning tools and potential difficulty in visually inspecting for cleanliness. Thus, the thoughtful consideration of the orientation, presentation, and size of these device features is crucial in designing comprehensive cleaning protocols to maintain the sterility and functionality of reusable medical devices, especially those used in microsurgical procedures.

Lumens have been identified within the literature as being more difficult to clean (Ofstead, et al., 2015) (Lopes, et al., 2019), but are used readily as a feature within reusable medical devices. As the length of lumens in reusable medical devices increases and the diameter decreases, the cleaning process becomes progressively more challenging. Lumens, which are narrow, tubular passages within medical instruments, serve various functions such as irrigation or suction. The longer the lumen, the more difficult it is to access and clean thoroughly, particularly in cases where the diameter is reduced. The reduced diameter poses a challenge as it limits the space for cleaning tools or solutions to penetrate effectively. The length and narrowness create a scenario where debris, bodily fluids, or other contaminants can easily become trapped and adhere to the lumen walls. As a result, the risk of incomplete cleaning or the presence of residual organic matter increases, potentially compromising the sterility and safety of the medical device. Material of construction and designs within the lumen can also play a role in risk. For example, repelling material such as silicone may be easier to clean than stainless steel, but if a junction of material within the lumen creates a mated surface or other complex feature, the cleaning challenge may increase regardless of material used. The meticulous design of cleaning protocols, including specialized brushes, flushing techniques, and suitable cleaning solutions, becomes paramount in addressing these challenges and ensuring the thorough decontamination of extended, narrow lumens in reusable medical devices. The lumen used within this experimental design had a very small diameter of 1.2mm, but a relatively short length of 277.2mm in comparison to lumens that are used within devices like flexible endoscopes. This difference must be accounted for in the total risk assessment (Table 5.14).

Table 5.14: Risk rubric lumen length

Risk Question	Answer	Value	Risk for Likelihood of Occurrence
Does the device have a lumen greater in length than 270mm?	Yes	10	Frequent – Serious/Critical
	No	0	Remote/Improbable – Negligible/Minor

Device geometries that include housing or casings that cover features can be problematic for cleaning. These casings are typically fixed to the devices with captured screws and have mated surfaces. Mated

surfaces and mated surfaces with small clearances allow for soil migration into the housing leaving the opportunity for soil to accumulate around internal features not designed to be cleaned (e.g., a captured screw that is not flush with the surface). Soil that has accumulated over time within a housing or covering is a risk for patient harm if the cleaning process does not sufficiently remove the soil and there is a probability that it can migrate out of the device during use. Housings that allow for soil migration, but do not have accessibility designed for fluid movement have a high risk of cleaning failure. The issue of soil migration is not just during clinical use, but if the device is submerged in fluid, soil can transfer into the enclosed chamber but not be able to exit during the cleaning process. Soil removal may require the use of flushes with enough shear force to remove any soil particles that have migrated in and have been allowed to dry during and after patient use. This risk is mitigated if the housing is completely sealed or can be disassembled prior to cleaning to allow for all features internally to be cleaned (Table 5.15).

Table 5.15: Risk rubric housing

Risk Question	Answer	Value	Risk for Likelihood of Occurrence
Does the device contain a housing that cannot be removed or accessed by fluid without flushing for cleaning where soil can migrate?	Yes	10	Frequent – Serious/Critical
	No	0	Remote/Improbable – Negligible/Minor

The ingress of blood into the housing of powered surgical devices poses a significant concern within medical settings. During surgical procedures, especially those involving vascular or highly vascularized tissues, there is a risk that blood may inadvertently seep into the intricate components of powered surgical instruments. If not thoroughly cleaned, the residual soil within the housing can serve as a reservoir for microbial contamination. Furthermore, when the device is subsequently employed, the mechanical action and heat generated during operation can lead to the aerosolization of soil particles, potentially disseminating infectious agents into the surrounding environment. Aerosolization can also be present with articulating devices that are powered but do not contain housing. This aerosolization poses a risk to both healthcare providers and patients (Table 5.16).

Table 5.16: Risk rubric powered

Risk Question	Answer	Value	Risk for Likelihood of Occurrence
Is the device powered (Powered instruments, which can entrap debris that can later be aerosolized during a surgical procedure)?	Yes	5	Frequent/Probable – Minor/Serious
	No	0	Remote/Improbable – Negligible/Minor

Similar to a powered device, reusable medical devices that have internal movable parts, such as those incorporating multiple cables, sliding shafts, springs, gears, internal moving parts such as multiple control cables within sheaths, etc. pose a serious risk during subsequent use if not fully cleaned prior to disinfection and/or sterilization (Table 5.17). In scenarios where these devices, like robotic instruments or endoscopes, are not thoroughly cleaned, the residual soil can potentially migrate out during operation and come into direct contact with the patient. As supported in the literature, these types of devices have the highest risk (see Chapter 2) for likelihood of occurrence and using the rubric in Figure 5.3 can be assigned as frequent/critical. The complex and intricate nature of the internal components can create challenges in achieving complete cleanliness, making it crucial to employ meticulous cleaning protocols to mitigate the risk.

Table 5.17: Risk rubric internal movable parts

Risk Question	Answer	Value	Risk for Likelihood of Occurrence
Does the device have internal movable parts such as multiple cables (e.g., robotic instruments, elevator shaft, interior lumen) that may have exposure to soil?	Yes	10	Frequent – Serious/Critical
	No	0	Remote/Improbable – Negligible/Minor

Microsurgical devices are small, specialized instruments designed for performing delicate surgical procedures that involve intricate and precise maneuvers at a microscopic level. These devices are used in various medical fields such as ophthalmology, neurosurgery, plastic surgery, and otolaryngology. Microsurgical instruments are typically characterized by their small size, fine tips, and often feature magnification systems to aid surgeons in achieving exceptional precision. Common microsurgical instruments include microscissors, microforceps, microsurgical needles, and microsurgical knives. These devices enable surgeons to work on small and delicate structures with minimal trauma to surrounding tissues, making them essential for procedures like nerve repair, eye surgery, and vascular anastomosis. These designs, although optimal for clinical use, can impose a greater risk for cleaning. The small size of the features can hinder the fluid dynamics. The diminutive size of microsurgical devices poses a unique set of challenges, particularly when it comes to utilizing traditional cleaning methods involving brushes. These miniature instruments, designed for intricate and precise procedures, often feature delicate components and intricate mechanisms that make them susceptible to damage from abrasive cleaning tools. Moreover, the minute dimensions of these devices make it challenging to access and thoroughly clean every nook and cranny, increasing the risk of residual soil. The use of brushes, typically employed for larger surgical instruments, may not be suitable for microsurgical tools, as the force applied during brushing could compromise the structural integrity of the fine components (Table 5.18).

Table 5.18: Risk rubric microsurgical

Risk Question	Answer	Value	Risk for Likelihood of Occurrence
Is the device a microsurgical instrument?	Yes	10	Frequent – Serious/Critical
	No	0	Remote/Improbable – Negligible/Minor

Another element of risk is the exposure to the cleaning solution to ensure it has contact with feature. Within the challenged test system, each feature was fully submerged during the cleaning process. By isolating the feature, it is assumed that the fluid dynamics in the cleaning process allow for complete access to the feature to simulate a condition within the device design where the cleaning fluid will have complete access to each feature of the device with the risk of residual soil. If the device cannot be fully submerged during the cleaning process, the probability of accessibility of the cleaning fluids decreases and depending on the feature, the risk can increase (Table 5.19).

Table 5.19: Risk rubric device submersion

Risk Question	Answer	Value	Risk for Likelihood of Occurrence
Can the device be fully submerged (Facilitation of water exposure to residual soil)?	Yes	0	Remote/Improbable – Negligible/Minor
	No	5	Frequent/Probable – Minor/Serious

As part of the device feature cleaning validation there are some risks that have already been included in the challenge. Each feature was isolated for the test system and exposed to soil, simulating the highest challenge for the feature. Using the immersion/flushing method for the contamination method overchallenged each of the features. Based on the surface area and the average contamination amount established by Cloutman-Green et. al (Cloutman-Green, et al., 2015), the soil volume was calculated. The application method for each feature within this experimental design exceeded the volume for each feature. The soil drying method which included rotating the feature, allowed for consistent air exposure, and even drying throughout the feature to heighten the cleaning challenge. By challenging the feature at both the 72hr and 2hr drying times, the risk of drying was included in the risk value assigned to the feature. However, if the cleaning instructions with the IFU include specific directions for the device to either be rinsed at point of use with the intention to remove as much visible soil as practical and/or the device is placed in a condition (e.g., covered with a wet OR towel, sprayed with enzymatic foam or packaged to retain humidity, etc.) that prevents the soil from drying while awaiting full decontamination, then the risk of soil drying is decreased (Table 5.20).

Table 5.20: Risk rubric soil drying

Risk Question	Answer	Value	Risk for Likelihood of Occurrence
Do the cleaning instructions as part of the IFU contain instruction that prevent soil from drying on the device while awaiting full decontamination processes?	Yes	-5	Remote/Improbable – Negligible/Minor
	No	0	Frequent/Probable – Minor/Serious

The evaluation of risk helps to recognize and assess each risk independently but also in understanding their interconnectedness and how they may amplify each other. The addition of each associated risk value results in a total risk value that can then be used to classify the reusable medical device. This classification is then used to effectively mitigate the compounding risks with a comprehensive cleaning process and proactive mitigation measures to address the interdependencies that can arise, ensuring a more resilient and adaptive response to complex risk scenarios for cleaning efficacy.

5.4 Device Categorization

Assigning a risk classification for the cleaning of a reusable medical device involves a systematic evaluation that incorporates quantitative values to gauge the potential risks associated with the cleaning process. As described, this assessment considers factors such as the complexity of the device, the nature and location of its internal components, and the types of tissues or bodily fluids it may come in contact with during use. This quantitative approach involves assigning numerical values to parameters such as the intricacy of device design, the presence of lumens, and the likelihood of residual contamination using the device feature approach. Considered in the risk value is the likelihood of infection transmission as a result of the device not being effectively cleaned. By systematically analyzing these variables and assigning numerical values, a risk evaluation provides a quantitative framework for prioritizing and tailoring cleaning procedures to ensure the effective decontamination of reusable medical devices and mitigate potential health risks associated with their use.

The quantitative risk evaluation is then used to place the reusable medical device into the applicable classification category (Table 5.21).

Table 5.21: Classification category risk rubric

Classification Category	Category Description	Total Risk Value
Minimal	Low risk reusable medical devices where all features are exposed without specific intervention for cleaning.	<18
Moderate	Accessible device features that require specific intervention (e.g., brushing or flow through lumens, mated surfaces requiring disassembly or opening/closing to ensure access).	18 - 39
Maximal	Complex device features with a high probability of soil accumulation with a medical device.	≥40

To facilitate the remainder of the discussion, an example classification has been performed for each category:

Table 5.22: Minimal classification example

Device:	Scalpel		
#	**Question**	**Answer**	**Risk Value**
1	What is the most challenging to clean feature that is exposed to soil?	Rough Surface	8
2	Is the material in the most challenging feature adsorbing (e.g., Stainless steel, Nitinol, Aluminum, Titanium) or repelling (e.g., silicone, PEEK, Delrin)?	Adsorbing	1
3	What contact does the feature have with the patient?	Circulating Blood	5
4	Does the device come in contact with high-risk tissue such as the brain, spinal cord and eye?	Yes	1
5	How is the soil exposed to the most challenging feature?	Partial Submersion	3
6	Will the soil be exposed to heat (e.g., cauterization)?	No	0
7	Will the soil be exposed to chemicals during use (e.g., saline, chlorine, iodine, simethicone)?	No	0
8	Does the device have a lumen greater in length than 270mm?	No	0
9	Does the device contain a housing that cannot be removed or accessed by fluid without flushing for cleaning where soil can migrate?	No	0
10	Is the device powered (Powered instruments, which can entrap debris that can later be aerosolized during a surgical procedure)?	No	0
11	Does the device have internal movable parts such as multiple cables (e.g., robotic instruments, elevator shaft) that may have exposure to soil?	No	0
12	Is the device a microsurgical instrument?	No	0

13	Can the device be fully submerged? (Facilitation of water exposure to residual soil)	Yes	0
14	Do the cleaning instructions as part of the IFU contain instruction that prevent soil from drying on the device while awaiting full decontamination processes?	Yes	-5
Total Risk Value			**13**

A scalpel typically consists of a slender, handle-like structure with a sharp, pointed blade attached at one end. The blade, usually made of stainless steel, is designed for precise and controlled cutting during surgical procedures. It comes in various shapes and sizes, depending on the intended use and surgical technique. The handle of the scalpel may feature grooves or ridges for enhanced grip and maneuverability, ensuring optimal control for the surgeon. The most difficult to clean feature of a simple, non-blade retracting, scalpel is the rough surface of the handle, which has a relatively low cleaning risk score (Table 5.22).

Table 5.23: Moderate classification example

Device:	**Femoral Reamer**		
#	**Question**	**Answer**	**Risk Value**
1	What is the most challenging to clean feature that is exposed to soil?	Smooth Through Lumen	24
2	Is the material in the most challenging feature adsorbing (e.g., Stainless steel, Nitinol, Aluminum, Titanium) or repelling (e.g., silicone, PEEK, Delrin)?	Adsorbing	1
3	What contact does the feature have with the patient?	Tissue/Bone/Dentin	4
4	Does the device come in contact with high-risk tissue such as the brain, spinal cord and eye?	No	0
5	How is the soil exposed to the most challenging feature?	Full Immersion	4
6	Will the soil be exposed to heat (e.g., cauterization)?	No	0
7	Will the soil be exposed to chemicals during use (e.g., saline, chlorine, iodine, simethicone)?	No	0
8	Does the device have a lumen greater in length than 270mm?	No	0
9	Does the device contain a housing that cannot be removed or accessed by fluid without flushing for cleaning where soil can migrate?	No	0
10	Is the device powered (Powered instruments, which can entrap debris that can later be aerosolized during a surgical procedure)?	No	0
11	Does the device have internal movable parts such as multiple cables (e.g., robotic instruments, elevator shaft) that may have exposure to soil?	No	0
12	Is the device a microsurgical instrument?	No	0

13	Can the device be fully submerged? (Facilitation of water exposure to residual soil)	Yes	0
14	Do the cleaning instructions as part of the IFU contain instruction that prevent soil from drying on the device while awaiting full decontamination processes?	Yes	-5
Total Risk Value			**28**

The smooth-through lumen as a challenging feature to clean is a moderate classification example. The term "lumen" typically refers to a channel or opening within a tubular structure. In the context of a femoral reamer (Table 5.23), the lumen is a hollow space or passage located along the length of the instrument. Specifically, the lumen in a femoral reamer is often situated at the center of the device, running from the proximal (near the handle) to the distal (tip) end. This central channel is designed to accommodate a guide wire or other instruments used during orthopedic procedures such as hip arthroplasty or femoral canal preparation. The lumen allows for precise and controlled placement of the femoral reamer within the femoral canal, contributing to the accuracy and effectiveness of the surgical procedure. The smooth-through lumen of a femoral reamer presents a cleaning challenge that specifically requires intervention. A targeted approach involving manual brushing and flushing is required to effectively dislodge and remove soil adhering to the inner surfaces. Complementing this with high-pressure flushing using cleaning agents is often necessary to ensure a thorough cleaning process.

Table 5.24: Maximal classification example

Device:	**Duodenoscope**		
#	**Question**	**Answer**	**Risk Value**
1	What is the most challenging to clean feature that is exposed to soil?	Smooth Through Lumen	24
2	Is the material in the most challenging feature adsorbing (e.g., Stainless steel, Nitinol, Aluminum, Titanium) or repelling (e.g., silicone, PEEK, Delrin)?	Repelling	0
3	What contact does the feature have with the patient?	Mucosal Membranes	1
4	Does the device come in contact with high-risk tissue such as the brain, spinal cord and eye?	No	0
5	How is the soil exposed to the most challenging feature?	Flushing	5
6	Will the soil be exposed to heat (e.g., cauterization)?	No	0
7	Will the soil be exposed to chemicals during use (e.g., saline, chlorine, iodine, simethicone)?	No	0
8	Does the device have a lumen greater in length than 270mm?	Yes	10
9	Does the device contain a housing that cannot be removed or accessed by fluid without flushing for cleaning where soil can migrate?	No	0
10	Is the device powered (Powered instruments, which can entrap debris that can later be aerosolized during a surgical procedure)?	No	0

11	Does the device have internal movable parts such as multiple cables (e.g., robotic instruments, elevator shaft) that may have exposure to soil?	Yes	10
12	Is the device a microsurgical instrument?	No	0
13	Can the device be fully submerged? (Facilitation of water exposure to residual soil)	Yes	0
14	Do the cleaning instructions as part of the IFU contain instruction that prevent soil from drying on the device while awaiting full decontamination processes?	Yes	-5
Total Risk Value			**45**

Duodenoscopes (Table 5.24) are specialized endoscopic instruments used to visualize and access the duodenum and other parts of the upper gastrointestinal tract. The extended length of the lumen, which is a flexible, tubular channel within the duodenoscope, allows for the passage of medical devices internally and the delivery of therapeutic interventions. Like the femoral reamer, the duodenoscope also has a smooth-through lumen that is the hardest to clean feature. However, the duodenoscope has a lumen length that is greater than the device feature included in the test design, so there is a greater risk of ineffective cleaning. The length of the lumen in a duodenoscope contributes significantly to increased cleaning challenges. The distal and proximal ends of the lumen often form a shelf that interrupts the fluid dynamics and associated shear force during flushes. To overcome these challenges, meticulous cleaning protocols, including manual brushing, high-level disinfection, and specialized cleaning equipment, are often required to ensure the thorough decontamination of the extended lumen in duodenoscopes.

5.4.1 Categorization Application

Although the cleaning classification is similar to the categories of recommended cleaning processes from AAMI TIR12:2020, they do not exactly align. The minimal risk classification does seem to align with the recommendations for the simple device category. The minimal risk devices have features that are relatively easy to clean and have good fluid dynamics that do not need much human intervention for cleaning. The simple devices category reflects this same scenario where it is specified that an automated wash without any manual cleaning may be appropriate based on industry validated IFUs.

The devices requiring intervention (i.e., category 2) tend to be more complex and subsequently require manual cleaning steps like soaking, brushing, flushing before they can be cleaned using an automated method. Devices requiring ultrasonic cleaning (i.e., category 3) have more intricate designs and require the use of ultrasonic cleaning to facilitate full soil removal in addition to the manual brushing and flushing. The moderate risk cleaning classification category includes features are reflecting of the need of both a manual intervention and the physical effects of sonication for cleaning efficacy. Of the 23 features challenged within this experimental design, only two, mated surfaces with small clearance and buttons – exposed springs, successfully passed the 2hr and 72hr soil dry and no brush/flush tests, making them eligible for a no sonication challenge. Both features have a base score of 8 and would therefore result in

a risk value within the minimal risk category which requires no intervention. As reflected by AAMI TIR12, the majority of complex reusable medical devices will be of a moderate risk.

Some devices may seem simple for cleaning, but the score places them in the moderate risk category and demonstrates that manual intervention is necessary to fully clean the device. For example, a slap hammer (Table 5.25), also known as a slide hammer or inertia hammer, is a hand tool designed for extracting objects or components from a surface by utilizing inertia and impact force. It would be an example of a device that is right on the border between minimal and moderate risk. It typically consists of a heavy metal rod or shaft with a weighted head at one end. To use the slap hammer, the user grips the handle and allows the weighted end to slide freely along the shaft. The hammer is positioned over the object to be extracted, and a sudden, forceful "slap" or pulling motion is applied to the handle. The inertia generated by the sliding weight creates a sudden impact on the object, helping to dislodge or extract it from its position. In this classification example, the most challenging feature is a sliding shaft long. The sliding shaft of a slap hammer presents a challenge during the cleaning process due to its elongated design and the potential for contaminants to accumulate within its length. The extended and often tubular structure of the sliding shaft creates internal channels that may be difficult to access and clean thoroughly. Residual debris, lubricants, or contaminants can find their way into these intricate spaces. This risk of residual debris does not negate the need for visual inspection to be conducted as manual intervention may be necessary to fully remove all residual soil as indicated by the failing semi-automated validation results (Table 4.17). Although more difficult to clean than other minimal risk devices such as scalpels (see Appendix 27), the slap hammer presents less risk than devices in the moderate risk category.

Table 5.25: Example of borderline device

Device:	**Slap Hammer**		
#	**Question**	**Answer**	**Risk Value**
1	What is the most challenging to clean feature that is exposed to soil?	Sliding Shafts Long	15
2	Is the material in the most challenging feature adsorbing (e.g., Stainless steel, Nitinol, Aluminum, Titanium) or repelling (e.g., silicone, PEEK, Delrin)?	Adsorbing	1
3	What contact does the feature have with the patient?	Tissue/Bone/Dentin	4
4	Does the device come in contact with high-risk tissue such as the brain, spinal cord and eye?	No	0
5	How is the soil exposed to the most challenging feature?	Partial Submersion	3
6	Will the soil be exposed to heat (e.g., cauterization)?	No	0
7	Will the soil be exposed to chemicals during use (e.g., saline, chlorine, iodine, simethicone)?	No	0
8	Does the device have a lumen greater in length than 270mm?	No	0

9	Does the device contain a housing that cannot be removed or accessed by fluid without flushing for cleaning where soil can migrate?	No	0
10	Is the device powered (Powered instruments, which can entrap debris that can later be aerosolized during a surgical procedure)?	No	0
11	Does the device have internal movable parts such as multiple cables (e.g., robotic instruments, elevator shaft) that may have exposure to soil?	No	0
12	Is the device a microsurgical instrument?	No	0
13	Can the device be fully submerged? (Facilitation of water exposure to residual soil)	Yes	0
14	Do the cleaning instructions as part of the IFU contain instruction that prevent soil from drying on the device while awaiting full decontamination processes?	Yes	-5
Total Risk Value			**18**

The most complex medical devices which require extensive intervention for cleaning are assigned the maximal risk category. This categorization is more reflective of the proposed categorization by Michels et. al (Michels, et al., 2013) where the authors suggest microsurgical instruments and complex instruments are separated into additional groups. The results of this analysis are in agreement with these authors, that complex instruments with compound features and device designs create cleaning challenges that will require additional mitigation activities to address the risk. Additional examples of device categorization can be found in Appendix 27.

Chapter 6: Industry Application

Medical device manufacturers can use this novel cleaning classification in conjunction with the Spaulding definitions to assess the risk for the entire decontamination process for reusable medical devices. This can improve cleaning and disinfection/sterilization validation methods, improve device design, and ensure risks are clearly communicated and mitigated at healthcare facilities. The established Spaulding Classification focusing on sterilization and patient risk provided an easy mechanism to connect manufactures and health care facilities to how devices must be validated and then processed. The simplicity of the Spaulding classification allowed non-microbiologists within the healthcare facility to understand risk in a manner that allowed them to act accordingly. The intent of this cleaning classification is to deepen this knowledge transfer between the medical device manufacturer and those responsible for ensuring patient safety between use. By complementing this with a classification to assess the cleaning risk in more detail, the appropriate processing methods can be defined and optimized, thereby further decreasing the risks to patient safety. This combined approach can help safeguard against and tackle the emergence of increasingly recalcitrant microbial pathogens including drug-resistant microorganisms (Garvey, et al., 2022) (Garvey & Rowan, 2023).

6.1 Medical Device Manufacturer Application

Medical device manufacturers have the challenge of balancing the functionality of a reusable medical device to the specifications of the using physicians while making sure the device can be safely processed throughout the established lifetime. During the device design process, the voice of customer that primarily informs the design comes from the professionals that will be utilizing it on a patient. This feedback focuses on functionality and ease of use. As such, sales efforts are focused on these users.

Sales efforts to promote reusable medical devices to hospitals involve a multifaceted approach focused on addressing the unique needs and concerns of healthcare institutions. Sales representatives engage in thorough research to understand the specific requirements of each healthcare facility, considering factors such as patient demographics, budget constraints, and facility size. By emphasizing the long-term cost-effectiveness and sustainability of reusable devices, sales teams aim to showcase the economic benefits of investing in durable equipment. Comprehensive product demonstrations, highlighting the devices' features, ease of use, and compatibility with existing healthcare infrastructure, play a pivotal role in convincing hospital administrators and procurement teams. Unfortunately, the needs of those responsible for the processing of these devices is rarely considered. This disparity is well understood within sterile processing departments to the extent that the following language was added into documents such as ANSI/AAMI ST79 to promote leadership within these departments having a voice in the decision making of reusable medical devices before they are purchased. Standards and guidelines across the world continue to promote this as best practice, and even requiring processing departments to be part of the purchasing decisions. Purchased devices must include processing instructions that the healthcare facility can follow (Association for the Advancement of Medical Instrumentation, 2020) (International Organization for Standardization, 2021).

The application of a cleaning classification can facilitate medical device manufactures to consider implications for the cleaning process during the design phase and subsequent validations. The recognition of the design risks will inform engineering early in the process so reusable medical devices are designed with cleaning in mind. No longer will cleaning be an afterthought at the end of the process but will instead be recognized as a critical design input to encourage design innovation.

6.1.1 Device Design

The device features challenged were representative of the most common features used today for reusable medical devices. As such, this can provide engineers with valuable information early in the design process. For example, the smooth blind lumen dimensions of 6.0mm depth x 1.0mm in diameter represent a greater challenge than this feature in reusable medical devices. The total risk value of 20 indicates that this feature is more challenging to clean than other features. If provided this information during the design phase, an engineer may consider making adjustments to the design to either eliminate the feature or change the dimensions of the blind hole to be more amendable to a defined cleaning process.

The thought exercise of using the device feature as a design input specifically to cleaning can be completed using the table of features manually but can also be adopted into software for an automated assessment. Imagine a software application where an engineer can engage with the data in way that will provide design recommendations. One can envisage an application where the engineering drawing is uploaded to software and using machine learning the program will identify the hardest to clean feature and assess it against a larger database of validated devices to inform the engineer on the required cleaning process necessary to fully mitigate risk. The data generated to establish this cleaning classification is a foundational data set required to build this type of software. This vision of device design automation would require more cleaning efficacy data on full device designs with an algorithm to connect the results back to the design feature. The process of innovation unfolds gradually, evolving in incremental steps that cumulatively contribute to significant advancements. It is characterized by a series of small, iterative improvements and discoveries rather than sudden, transformational changes. As such, the device design engineering tool using this available data may be relatively simple, but still provide engineers with an understanding of how their decisions will ultimately affect the cleaning process.

A simpler use of this data set for device design may be an engineering tool where the user selects features and associated dimensions used within the current design. The tool will identify the corresponding data set tested (i.e., device feature geometry, cleaning process, and risk score) and assess the proposed device design for a design freeze decision. In the development of a reusable medical device, the design stage gates with design freezes represent checkpoints that structure the product development process. These stage gates serve as systematic evaluation points at various stages of the design lifecycle, ensuring that key criteria are met before progressing to the next phase. A design freeze is a pivotal component within these gates, indicating a point in development where further alterations to the device's design are restricted to minimize potential risks and maintain consistency. Typically, design freezes occur after thorough assessments, incorporating input from multidisciplinary teams, regulatory considerations, and user feedback based on initial design inputs. The duration of the design process for a reusable medical device can vary significantly based on factors such as complexity, regulatory requirements, technological innovations, and the specific requirements of the project. Generally, the design process can take anywhere from several months to a few years. Using a design tool to assess cleaning can give engineers decision criteria for if during this process they should redesign the device, test the device for cleaning efficacy, or freeze the design. This decision making within the design process can significantly speed up the process and shorten the time to market.

The significance of time to market in the realm of reusable medical devices cannot be overstated. Swift entry into the market provides a competitive advantage, allowing manufacturers to establish themselves ahead of rivals, reduce development costs, and potentially capture a larger market share. This expeditious approach is particularly important for ensuring that innovative solutions are promptly available for improved patient care. Timely market entry is also integral to realizing a faster return on investment, a critical consideration given the substantial resources invested in research, development, and regulatory

compliance. The stringent regulatory landscape governing reusable medical devices necessitates efficient navigation through approval processes to avoid delays that could impact the overall product launch timeline. Additionally, rapid technological advances underscore the importance of bringing devices to market quickly, allowing manufacturers to incorporate the latest technologies and remain at the forefront of industry standards. Striking the right balance between a timely market entry and meticulous attention to testing, quality assurance, and regulatory requirements is pivotal to the success of reusable medical devices throughout their lifecycle.

6.1.2 Cleaning Validations

The cleaning validation of a reusable medical device, if completed properly, demonstrates that regardless of hazards, execution of the cleaning instructions will mitigate the risk of residual soil remaining on/in the device. Validation variables in cleaning efficacy studies can significantly impact the outcomes and reliability of the results. These variables encompass a wide range of factors that must be carefully considered and controlled to ensure the validity of the findings.

The selection of validation variables directly affects the accuracy and precision of the measurements or assessments conducted during the study. For example, if a blood soil is selected for the validation but does not reflect the type of soil the reusable medical device is exposed to, the generated IFU may be ineffective during clinical use. Inconsistencies or inadequacies in these variables may lead to biased or unreliable results. The manipulation of validation variables can impact the internal and external validity of the study. Internal validity refers to the degree to which the study accurately reflects the relationship between variables, while external validity pertains to the generalizability of the findings beyond the study's specific conditions. Inappropriately chosen or manipulated validation variables may compromise both internal and external validity, limiting the study's applicability and relevance. Furthermore, the control of validation variables is essential in reducing confounding factors that could distort the true relationship between independent and dependent variables. Failure to control for relevant variables may introduce noise into the study, making it challenging to isolate the effects of the variables of interest and leading to inaccurate conclusions.

The results from this experimental design can be used by medical device manufacturers to inform variable selection. Testing variables such as family grouping decisions, test soil selection, application, and drying, cleaning instructions, extraction methods, and sample size. The standardization of these validation variables will help to ensure the validity, accuracy, and generalizability of validation results.

6.1.2.1 Family Grouping Justification

Cleaning validations can be unnecessarily and time-consuming tests. As such, it is not practical for medical device manufacturers to challenge every one of their devices, especially when assembled into a complex case and tray configuration. Family grouping is allowed as a strategy to challenge the hardest to clean device within the cleaning validation and adopt other devices into the same cleaning instructions.

The US FDA states the following in their 2015 guidance, "It is possible that similarities in design, materials, and other factors may allow for establishing **product families** (e.g., devices with a range of available sizes) for the purpose of minimizing reprocessing validation efforts. That is, it may be possible to establish that validation data for the **most difficult to reprocess devices in a family** (i.e., the worst-case device or "master device") covers devices that present an equivalent or lesser reprocessing challenge. If this method is utilized, all design features of the less difficult to reprocess devices in a family, such as lumen length and diameter, materials, configuration, and texture relevant to reprocessing challenges of the subject device should be evaluated and assured to be less challenging to reprocessing than the master device" (U.S. Department of Health and Human Services Food and Drug Administration, 2015).

This same guidance was adopted into the ANSI/AAMI ST98 cleaning validation standard in 2022 with similar text, "It is possible that similarities in design, materials, and other factors may allow for **establishing product families** (e.g., devices with a range of available sizes) for the purpose of **minimizing reprocessing validation efforts**. That is, it may be possible to establish that validation data for the **most difficult to reprocess devices in a family** (i.e., the worst-case device or "master device") covers devices that present an equivalent or lesser reprocessing challenge. If this method is utilized, all design features of the less difficult to reprocess devices in a family, such as lumen length and diameter, materials, configuration, and texture relevant to reprocessing challenges of the subject device should be evaluated and assured to be less challenging to reprocessing than the master device" (Association for the Advancement of Medical Instrumentation, 2022).

Although this guidance is clear that family grouping is an acceptable validation strategy, instructions for completing the family grouping justification are not included. AAMI/AAMI ST98 points the reader to AAMI TIR12 annex D where the following list of factors for consideration are described: design configuration, number of design components, material of construction, size and density, surface area and porosity, need for disassembly, surface finish or texture, cannulations or lumens, presence of mated surfaces, ability to be cleaned and/or sterilized in a routine cycle, appropriate of test soil and service life. Using the 14 questions to establish a total risk value, the device can be assessed for a quantitative value that will address assess most of these family grouping factors.

Consider the case design example in Figure 6.1. This case can have more than 20 instruments within the configuration. When using the Kremer cleaning classification to establish the family grouping rational, each instrument would be assessed for a total risk value. Assuming all devices in the case have the same predicted service life and all devices are cleaned using the same instructions, the device with the highest

risk value would then be selected for the cleaning validation. Using this quantitative assessment to justify family grouping, removes the subjectivity and potential bias from the family.

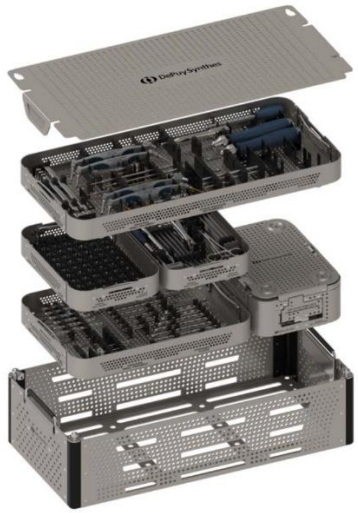


Figure 6.1: Case and Tray Design

6.1.2.2 Test Soil

In a cleaning validation experiment, the importance of test soil selection, application, and drying cannot be overstated, as these factors directly impact the accuracy and reliability of the results. The choice of test soil should closely mimic the types of contaminants or residues that the medical device may encounter during actual use. Careful consideration of the application method ensures uniform and realistic distribution of the test soil on the device surface, reflecting real-world scenarios. The supporting research in this thesis demonstrated that Modified Coagulated Blood was the most challenging test soil as it contains both water soluble and water insoluble proteins. Recognition of this challenge should be reflected in cleaning validations, and unless during the clinical procedure an anti-coagulant is used, should be utilized for a worst-case challenge. The application process of the soil should reflect clinical use, but in an exaggerated way. Immersion should be selected as a worst case, if appropriate to clinical use. As demonstrated in the results, there is a difference between 1hr, 2hr, and 72hr drying times. This validation variable must be considered and reflected in the validation. Cleaning instructions may be drastically different if the soiled device is allowed to dry versus not. This scenario is demonstrated in the results of the Hinges, Joints and Pivot Points feature. With a 72hr dry, the device may not be able to be cleaned, but if point of use treatment includes instructions to not allow the device to dry (e.g., covering with a wet OR towel), the device can be cleaned without any manual intervention (e.g., brushing / flushing). Inconsistent or inadequate soil selection, application techniques and drying conditions may lead to skewed results, either underestimating or overestimating the effectiveness of the cleaning procedure.

6.1.2.3 Cleaning Instructions

The primary goal of reusable medical device manufacturers is to release a product into the market that is effective for its intended use as quickly as possible. As such, there is a delicate balance between meeting the regulatory requirements of a passing cleaning validation and facilitating practical use in healthcare settings. Devices are not challenged to the point of failure during the cleaning validations. Instead, proven cleaning instructions, already validated, are often adopted for new or modified device designs. This creates a challenge for healthcare facilities to optimize cleaning processes and adopt new products into existing processes.

The data generated within this novel evaluation provides visibility for each feature, where the cleaning instructions may be modified for process optimization. For example, if a healthcare facility is attempting to fully automate the cleaning process (i.e., remove all human-device interaction) then medical device manufacturers may support this initiative by designing the device with features that are amenable to an automated process (e.g., rough surface, through slot, screw threaded rod / threaded blind hole) and conduct a reflective cleaning validation. The utilization of the data collected within this novel dissertation will advance the recognition of user-friendly instructions to encourage proper adherence to cleaning protocols by healthcare professionals.

6.1.2.4 Extraction Methods

The choice of extraction methods in a cleaning validation process can significantly influence the accuracy of the results obtained. As demonstrated by the differing results for the 72hr soil dry method validation, the effectiveness of the extraction method can be highly variable. The experimental design for the 23 features included a number of factors that if applied consistently to cleaning validations would substantially increase the robustness of the test method. The physical extraction methodology has been well established in the literature (Kremer, et al., 2021). However, the choice of extraction eluent has been debated for many years, but it is clear from this study that water alone does not sufficiently remove all protein residuals from a device during extraction. To address this gap and prevent the under reporting of residuals, the additive extraction method was developed and proved to be a successful strategy (Kremer, et al., 2023).

The volume of extraction fluid used can also have a serious impact on the test results. The extraction volume is typically determined by balancing the need to capture sufficient residues for analysis while avoiding dilution that may compromise sensitivity. Rigorous testing and optimization within this experimental design has demonstrated the efficacy of an equation to calculate extraction volume.

$$Device\ Maximum\ Extraction\ Volume\ (mL) = \frac{Device\ Surface\ Area\ (cm^2)\ x\ Method\ Acceptance\ Criteria\ (\frac{\mu g}{cm^2})}{Method\ LOQ\ (\frac{\mu g}{mL})}$$

The implementation of a standardized formula for calculating extraction volume in cleaning validation processes offers numerous benefits to the medical device manufacturing industry. This consistent and systematic approach promotes uniformity in testing methodologies across different devices and

scenarios. This consistency enhances comparability between studies and facilitates industry-wide benchmarking, fostering a more cohesive understanding of cleaning efficacy. Moreover, a standardized formula helps streamline regulatory compliance by providing a clear and accepted methodology for determining extraction volumes, ensuring that manufacturers adhere to established guidelines. This not only simplifies the validation process but also enhances transparency and trust among regulatory bodies and healthcare professionals. Ultimately, a standardized formula for calculating extraction volume contributes to the reliability, reproducibility, and efficiency of cleaning validation efforts.

6.1.2.5 Sample Size

ANSI/AAMI ST98 states, “Cleaning validation shall be done using a sample size sufficient to demonstrate reproducibility” and should be determined using the device complexity (Association for the Advancement of Medical Instrumentation, 2022). This requirement is further expanded upon in the informational annex where the document again points to AAMI TIR12 to determine device complexity focused on a cleaning challenge and instructs that device with more complexity should have a higher sample size during validation.

The choice of an appropriate sample size is essential to ensure the statistical robustness and reliability of the validation results. It involves considering factors such as the variability in contamination levels, the desired level of confidence, and the acceptable margin of error. A larger sample size increases the precision of the validation, providing a more accurate representation of the device's cleanliness. Conversely, an overly small sample size may lead to underestimation or overestimation of the cleaning efficacy. Striking the right balance is important to obtain results that are both scientifically sound and practically applicable. Sample size determination, however, can be challenging to determine. Sample and testing costs for cleaning validations can be very high, $30K, so medical device manufacturers are inclined to limit the sample size through a subjective justification.

Use of the Kremer cleaning classification to determine sample size can provide medical device manufacturers the clarity needed to properly challenge their device design. For example, section A.6.6 of ANSI/AAMI ST98 explains that testing is common with a sample size of 3, 6 or 9 devices depending on the cleaning challenge. These values can align with the cleaning risk category. For a device with minimal risk and low complexity, a sample size of 3 may be suitable, 6 for moderate risk, and 9 for maximal risk devices. This sample size assignment brings clarity to the expectations for both medical device manufacturers and regulators.

To ensure the reproducibility of the provided sample size, ANSI/AAMI ST98 incorporates a statistical validation check to assess the acceptability of dataset variability. This involves the addition of the standard deviation of the sample set to the maximum observed value, which is then compared against predefined acceptance criteria. This method of checking the data variation is not described in the literature. The sample size determination included within this experimental design, encompassing the calculation of the high value of the 99% confidence interval and utilizing the resulting margin of error and standard deviation

for sample size determination uses traditional statistical technique. The comparison of this methodology with the ST98 approach reveals that the ST98 approach is more conservative in relation to more intricate statistical techniques that could be employed and has essentially validated ST98 approach. Hence, employing the Kremer cleaning classification for sample size selection and subsequently verifying the dataset variability with the ST98 approach is an approved method for sample size determination.

6.1.3 Instructions for Use

Understanding the impact of the reusable medical device's complexity on behaviors within a healthcare facility should not be underestimated. This cleaning classification serves as a communication tool to alert users to when devices may need special attention (e.g., maximal risk). The IFU acts as a comprehensive guide, providing essential information for the effective processing of the device. Manufacturers, by explicitly addressing device complexity in cleaning processes, can communicate potential challenges and nuances, facilitating the device's integration into existing cleaning practices. This communication may also emphasize features requiring special attention during cleaning or additional training requirements. Clear communication regarding device complexity enhances user awareness, mitigates the risk of inadequate cleaning, and promotes adherence to proper cleaning protocols, thereby contributing to overall patient safety in healthcare settings.

6.2 Healthcare Facilities

Upon the device's arrival at the hospital for utilization, the onus for patient safety transitions from the medical device manufacturer to the healthcare facility. The Instructions for Use (IFU) act as the primary tool for communication to facilitate this shift in responsibility. For established healthcare facilities, comprehensive cleaning protocols must be in place to encompass all devices processed within the department. Evaluation of new devices upon procurement is essential to ascertain their compatibility with existing processes or to design new protocols as necessary. In certain regions, validation of cleaning processes at the healthcare facility and periodic verification, particularly for complex devices like scopes, are standard practices. Given the significant human involvement in the cleaning process, robust training programs are imperative for success. The cleaning classification presents an opportunity to support these endeavors and offer clarity to both medical device manufacturers and healthcare facilities.

6.2.1 New Devices

Documents such as ANSI/AAMI ST79 or similar documents internationally guide sterile processing departments to engage in the procurement of reusable medical devices. Decisions to purchase or take responsibility for loaned instrumentation should be contingent upon the healthcare facility's capacity to adhere to the manufacture’s IFU (Association for the Advancement of Medical Instrumentation, 2020). Recognizing the impracticality of medical device manufacturers independently validating each device, as discussed in Chapter 2, it is equally unrealistic to expect strict adherence to every IFU. Consequently, healthcare facilities have the opportunity to categorize devices into families and institute a comprehensive master cleaning process.

Healthcare facilities receive similar guidelines for establishing device family groups as those provided to medical device manufacturers. For instance, in ST79, factors such as design configuration, number of components, materials of construction, size and/or surface area, need for disassembly, surface finish or texture, presence of cannulations, lumens, or mated surfaces, and manufacturers' reprocessing instructions are considered. These considerations help determine whether a device is integrated into an existing family or identified as a new master product (Association for the Advancement of Medical Instrumentation, 2020). Depending on the volume of devices processed within a department, this evaluation may be necessary for thousands of devices.

The Kremer cleaning classification can play a pivotal role in guiding decision-making processes. If manufacturers furnish healthcare facilities with the comprehensive risk score and device categorization, the facilities can systematically group devices into families and designate a master product, the most challenging to clean, to establish a unified cleaning process for the entire family. This categorization aids healthcare facilities in accurately defining device processing families, enabling the establishment of cleaning practices that suit all devices within a family while maintaining efficiency. The incorporation of the cleaning classification may also be instrumental when devices cannot be grouped into a single cleaning process. In such instances, adherence to the exact cleaning process outlined in the IFU becomes imperative, especially for devices in the maximal risk category with specific cleaning instructions aimed at mitigating the risk of residual contamination from high-risk features. Family grouping may only be deemed acceptable for minimal and moderate risk devices to prevent oversight of critical steps that could pose a risk to patient safety.

It is also possible for the sorting process to become automated. The automation of the family grouping process offers several additional benefits. With the availability of a shared software incorporating both the cleaning risk score and instructions for use, healthcare facilities can streamline the sorting process by inputting their reusable device inventory. The software can then autonomously generate device processing families, proposing an optimal cleaning process that maximizes resource utilization and equipment efficiency. This automated approach not only standardizes the family grouping assessment across hospitals but also significantly reduces the overall burden of this practice. By leveraging automation, healthcare facilities can achieve consistency, efficiency, and a more seamless integration of the family grouping process into their routine operations.

6.2.2 Cleaning Process Validations / Verification

In healthcare facilities where family groupings are employed to streamline cleaning processes, there is an expectation globally that these processes will be validated. However, the specific requirements for this validation activity vary across countries. For instance, in the United States, the requirements are not well-defined, whereas other countries, like Germany, have established stringent and standardized criteria for adherence to the validation process. These validation and verification activities and associated measurements for cleanliness have opportunity for improvement.

When the Spaulding Classification gained widespread adoption, precise measurement techniques or endpoints for determining cleanliness had not yet been established. The prevailing expectation was visual cleanliness, and the Spaulding classification system was founded on the assumption that all devices would be visibly clean before the microbial reduction step of disinfection or sterilization. The assumption was that thorough cleaning would always be conducted, and in many instances, devices (and their features) could be promptly inspected during or after the cleaning process. If the device appeared visually clean, it was presumed that the residual soil level was sufficiently low to ensure the effectiveness of the antimicrobial process, even in the presence of some residual soil.

Over the past 30 years, there has been a growing interest in establishing scientific endpoints for cleaning, driven partly by the identification and characterization of proteinaceous infectious particles (prions). This interest intensified with a specific focus on the risks associated with protein contamination on reusable medical devices following the BSE crisis in the UK and other countries (Kovaleva, et al., 2013). Simultaneously, concerns persisted regarding outbreaks and potential patient risks associated with inadequately cleaned surgical devices, along with the risks of transmitting blood-borne pathogens and antibiotic-resistant bacteria. It is acknowledged that numerous such incidents occurred but remained unpublished, implying that the published literature may have underestimated the true extent of the risks to patients. In response to the potential inadequacy of device cleaning, international efforts have been undertaken to establish cleaning performance requirements during the processing of reusable medical devices. These initiatives aim to prompt medical device manufacturers and healthcare facilities to develop and monitor the effectiveness of their cleaning instructions.

In addition to the traditional requirement for visual cleanliness, the ISO 15883 series now defines acceptance criteria for specific analytes when measuring cleaning efficacy. Quantitative, analytical test methods are justified for use based on a risk assessment with protein detection being highlighted as a recommended analyte. The acceptance criteria for analytes have been defined as both alert and action levels (Table 6.1). Detection levels of analytes below alert levels over multiple test cycles are considered ‘clean’, but those falling between alert and action levels are to be further investigated as they are considered high risk of potential failure overtime. This was designed to minimize the risk of soil accumulation or periodic, insufficient cleaning during normal use of the WD. These levels have been defined, but the standard does note that country-specific requirements may also need to be considered, such as levels of total protein per device (German Society for Hospital Hygiene (DGKH), German Society for Sterile Supply (DGSV), Working Group Instrument Preparation (AKI), 2012) or device side (Department of Health, 2016). Processing residuals are also assessed to evaluate patient impact (Kremer, et al., 2021) or an impact to further processing.

Table 6.1: Analyte acceptance criteria for cleaning efficacy

Analyte	Alert Level	Action Level
Protein	≥3 µg/cm^2	≥6.4 µg/cm^2
Total Organic Carbon	≥6 µg/cm^2	≥12 µg/cm^2
Carbohydrate	≥0.9 µg/cm^2	≥1.8 µg/cm^2
Haemoglobin	≥1 µg/cm^2	≥2.2 µg/cm^2
ATP	≥10 femtomoles ATP/cm^2	≥22 femtomoles ATP/cm^2
Endotoxin	≥2.2 EU/device	≥20 EU/device

The industry's acceptance criteria for cleaning validations have solid support in the literature, with the primary analyte, protein, extensively evaluated for patient safety (Kremer, et al., 2019), and other analytes established as clinically relevant and measurable (Lappalainen, et al., 2009). The establishment of two levels of acceptance criteria ensures a safety margin within the test system, considering variability in analyte detection methods and variables within the test system that may impact detectability, such as sample extraction (Kremer, et al., 2021). A risk assessment aids in identifying the appropriate level to ensure patient safety. For instance, if medical device manufacturers, during cleaning validation, must remain below the action level under the most challenging conditions, it might be suitable for verification testing at a healthcare facility to achieve results below the alert level for an additional safety margin.

There is an opportunity within the standards for healthcare facilities to standardize when cleaning validations need to be conducted. As discussed, current standards for medical device manufacturers are clear on the requirements to validate the IFU, but as healthcare facilities funnel many medical devices into since process flows within the sterile processing department, it is vague as to when additional testing to implement a new cleaning family should be conducted.

In the 2019 Kilmer Conference presentation titled, Cleaning Validations on Robotic Devices – Laboratory to Patient, by Nupur Jain, this concept is explored. Jain discussed the complexity of robotic devices and risk of performing the cleaning incorrectly without healthcare facilities performing a validation of the implementation of the cleaning instructions (Jain, 2019). The complicated nature of the cleaning instructions for robotic instruments poses the same concern as other complicated devices, such as endoscopes (Kremer, et al., 2023).

Scope manufacturers have not advocated for cleaning validations at the healthcare facility but rely on cleaning verification requirements to demonstrate cleaning efficacy. ANSI/AAMI ST91 provides instruction to healthcare facilities to perform cleaning verification to mitigate the concerns of ineffective processing (Association for the Advancement of Medical Instrumentation, 2021). Currently there is no requirement

for medical device manufacturers to validate appropriate verification tests for their devices, so selection is left to the health care facility.

Annex F of ANSI/AAMI ST91 includes a statement regarding cleaning verification tests, emphasizing that the US FDA has not yet reviewed the efficacy of these assays. Instead, manufacturers of cleaning verification assays develop their own methods and criteria to assess the effectiveness of their products (Association for the Advancement of Medical Instrumentation, 2021). This clause serves as a cautionary note for the industry, particularly healthcare facilities employing these test methods. The verification test manufacturer may or may not correlate their tests with cleaning validation criteria. As illustrated by this assessment, verification tests such as ATP may not align with cleaning validation tests like protein and TOC. Variations in test conditions and extraction techniques can also impact result validity, particularly for certain devices. For instance, extensive sonication of robotic devices has been shown to release iron and tungsten particles from cables, potentially interfering with a BCA protein test (Wallace, 2017). The application of Kremer's cleaning classification in this context may offer industry insights into applying validation and verification requirements within a healthcare facility.

Devices categorized as maximal risk using the Kremer cleaning classification have the highest likelihood to experience a disconnect between medical device manufacturers and healthcare users within the IFU. Because these device types have the highest likelihood of causing harm, additional requirements may be appropriate. Below are examples of mitigation actions that should be applied:

- Validation of Verification Tests – During the cleaning validation, medical device manufacturers should include in their cleaning validation verification testing that may be completed at the healthcare facility. Instructions should be included in the manufacturer’s IFU for how to perform the verification testing to include if applicable, extraction methods and warnings on test interference. This type of instruction will prevent testing results that are incorrect or misleading as described by Wallace (Wallace, 2017).
- Healthcare Facility Validation or Verification of Cleaning Processes – Clear requirements should be established for how and when healthcare facilities should perform cleaning validations to confirm their processes and equipment can deliver an effective cleaning process. Within medical device manufacturing, this requirement would be similar to a process validation. This requirement to validate the manufacturing process demonstrates that the manufacturing process will deliver product within specification consistently. As device processing is an extension of the manufacturing process, the same expectation should be established for each cleaning process established for family groups, especially for maximal risk categories.
- Manufacturers should include in their IFU if the device can be adopted into a product family for efficient processing, or if the device instruction is so specialized that it must be its own family.

The Kremer cleaning classification offers a clear framework for device manufacturers and healthcare facilities, guiding the seamless transfer of the cleaning process to ensure patient safety. Establishing

success for the healthcare facility can be accomplished by specifying requirements according to each cleaning classification category and incorporating them into the relevant standards. This approach has demonstrated effectiveness in microbial reduction using Spaulding's classification, making it a viable strategy for enhancing the efficiency of the cleaning process as well.

6.2.3 Training

An important aspect of conveying information from the medical device manufacturer to the healthcare facility involves training, and the extent of training can differ based on device complexity. For devices with straightforward cleaning instructions, a training approach might involve an "in-service" model, where a sales representative conducts a session during lunch, providing informational handouts on the cleaning steps. Conversely, more intricate devices may necessitate a more extensive training program with a representative from the manufacturer providing one-on-one hands-on training. The cleaning of scopes serves as an example where a comprehensive training initiative may be necessary to demonstrate cleaning competence (Ofstead, et al., 2023).

Training and competence development largely adhere to conventional methods despite significant advancements in processes and technology. In specialized training and educational programs, innovative technologies like extended reality, particularly virtual reality (VR), are emerging as potential platforms for delivering learning content in a more ecologically valid manner. At the 2019 Kilmer Conference, a concept was introduced on how augmented reality could enhance training for the processing steps associated with reusable medical devices. The authors illustrated how users could experience the real-world environment with an overlay of information providing instructions (Patel & Flynn, 2019). This instructional enhancement has the potential to substitute direct training from device manufacturers' representatives, offering greater opportunities for enhanced training experiences as described by Kremer et. al in the publication, *Use of real-time immersive digital training and educational technologies to improve patient safety during the processing of reusable medical devices: Quo Vadis?* (Kremer, et al., 2023).

Associating training requirements that corresponds to device complexity can be an effective way to ensure that device manufacturers prioritize training of processing personnel. The Kremer cleaning classification can be a useful mechanism for guiding manufacturers. The cleaning validation demonstrates that with successful completion of the IFU, the reusable medical device can be successfully processed under the worst conditions. The cleaning classification risk score can be used to require specific training activities from the manufactures be completed to mitigate the hazardous situations described in Chapter 5. For example, maximal risk devices may require independent certification to qualify each person individually before they can be certified to process the device.

Linking training requirements to device complexity may prove to be an effective strategy for prioritizing the training of processing personnel by device manufacturers. The Kremer cleaning classification serves as a valuable tool in guiding manufacturers through this process. Successful cleaning validation, aligned

with the Instructions for Use (IFU), demonstrates that reusable medical devices can be effectively processed even under challenging conditions.

6.3 Industry Standards

Since its inception in the 1950s, Spaulding's microbial-focused classification has been extensively employed in device processing standards and global guidance, significantly enhancing industry knowledge in microbial risk mitigation. Similarly, Kremer's cleaning classification holds comparable potential. This quantitative cleaning assessment offers the healthcare industry a structured framework to enhance communication between medical device manufacturers and healthcare facilities.

As part of this initiative, a proposal for a new work item (NWI) was submitted to the ISO/TC 198 committee. The NWI, titled "ISO-NP TS 17664-3, Processing of healthcare products – Information to be provided by the medical device manufacturer for the processing of medical devices – Part 3: Guidance on the designation of a reusable medical device," underwent a global voting process over three months from December 2022 to February 2023. Twenty delegates voted in favor of incorporating the NWI into the program of work, while three opposed it. Consequently, the NWI received approval and was assigned to the working group (WG) 12 subcommittee.

During the November 2023 meeting of the WG12 subcommittee, there was acceptance to incorporate Kremer's cleaning classification into the initial working draft of the document (Appendix 29). The endorsement by this international assembly of experts underscores the practicality of the classification and its relevance to the healthcare industry. The introduction of this ISO document will propagate the adoption of the cleaning classification into various global guidance and standard documents, establishing it as a valuable asset for risk reduction in the healthcare industry.

Chapter 7: Implications of Findings and Further Research

The new cleaning classification developed in these novel studies employs device features as the critical element for risk analysis in the device cleaning process. As mentioned earlier, the new device feature approach yields a more conservative and appropriate estimation of residual analytes on reusable medical devices, enabling the identification of the most probable location for soil accumulation and, consequently, the risk to the cleaning process. This methodology will empower medical device manufacturers to assess risk during the development and validation of the device processing IFU. Device classification categories serve as a means to convey the cleaning risk to healthcare users. The IFU should incorporate suitable risk mitigation actions to address these risks. When considered holistically, the novel findings generated that were disseminated to stakeholders in this thesis will also inform and enable much needed simplification of what has become very complex processes for design and reuse of devices that will positively impact patient risk by way of reduction in unwanted HAIs.

Over the past decade, various standard committees have undertaken efforts to standardize decontamination process flows based on device risk, with some implementations varying by geographical region. In the United States, device manufacturers receive guidance through AAMI TIR 12 Annex D and E, which recommends processing instructions based on device categories and the difficulty of cleaning (Association for the Advancement of Medical Instrumentation, 2020). In Germany, responsibility shifts to healthcare facilities, necessitating a process qualification to validate the cleaning process. This qualification assesses cleaning performance for processing steps, typically using a worst-case device or surrogate device as the process challenge device, and emphasizes complete automated processes, as outlined in ISO 15883-1 (International Organization for Standardization, 2021). However, healthcare facilities still retain discretion in grouping devices and adopting appropriate processing procedures.

Device manufacturers face a similar challenge in validating each device within a product portfolio, often comprising thousands of devices. An efficient approach involves identifying and using representative product families, validating worst-case designs demonstrating commonality in device materials, design features, intended use, and clinical soil exposure.

Understanding the need for devices that can be effectively processed, including design thinking that considers time issues and typical methods of healthcare facilities, is important for manufacturers. The cleaning classification system provides specific guidance to combine with industry guidance, standardizing cleaning requirements for various types of reusable medical devices. This standardization allows healthcare facilities to streamline their processing workflow, offering numerous benefits for overall safety, efficacy, and efficiency:

- Consistency and Reliability: Standardized cleaning instructions ensure uniformity across healthcare facilities, promoting reliability and reducing the likelihood of errors or variations in cleaning procedures.

- Patient Safety: Minimizing the risk of inadequate cleaning contributes to a safer healthcare environment, reducing potential complications for patients and HAIs.
- Compliance with standards and regulations: Standardized cleaning instructions align with regulatory guidelines, facilitating compliance and meeting stringent requirements set by regulatory bodies.
- Efficiency and Time Savings: Clear and standardized instructions streamline training, enabling staff to adhere to consistent and efficient cleaning protocols, saving time and resources.
- Device Longevity: Proper cleaning, as outlined in standardized instructions, contributes to the longevity and durability of reusable medical devices.
- Risk Mitigation: Standardization aids in mitigating risks associated with cleaning.
- Quality Assurance: Standardized cleaning instructions establish a systematic approach to device processing, fostering a culture of accountability and quality control.
- Enhanced Communication: Clear communication is promoted among healthcare staff involved in device reprocessing, reducing misunderstandings or misinterpretations.

In summary, standardizing cleaning instructions for reusable medical devices is informed and advanced by these new novel findings and will be seen as integral to promoting patient safety, regulatory compliance, operational efficiency, and overall quality in healthcare delivery.

7.1 Economics

Enhancing healthcare quality has the potential to drive economic improvements across the entire supply chain, commencing with medical device manufacturers. A substantial investment of time and resources is dedicated to the device design phase. The critical nature of time in this phase often leads medical device manufacturers to prioritize functionality over cleanability. Anticipated applications of the cleaning classification may involve the creation of a device design database. This resource could serve as a guide for medical device engineers during the design process, steering them towards simplifying features that enhance cleanability. Purposeful design for cleaning reduces the testing burden during validation, consequently expediting the time to market. Given the financial impact of delayed market entry, such a database would prove invaluable to medical device manufacturers, as every day a product is not on the market represents potential lost revenue.

As outlined earlier, employing the cleaning classification system enhances the comprehension and predictability of a device's performance during cleaning validation. A sophisticated implementation of this classification offers medical device manufacturers the opportunity to enhance their validation capabilities through equivalency. Validating without extensive testing not only accelerates time to market but also promotes standardized cleaning instructions, resulting in cost savings for the company. This approach necessitates the establishment of an internal database aligning cleaning validations with criteria from the cleaning classification. Stringent usage guidelines for validation within this database would be set through a risk assessment, ensuring a safety factor in line with the cleaning classification category. A thorough

validation of the database itself may be required, incorporating verification testing using data from actual devices. While the automation of validation demands an initial investment, for medical device manufacturers with an extensive product portfolio, this strategy holds considerable benefits.

Improved communication facilitated by the cleaning classification system can yield economic advantages during the transition of reusable medical devices to healthcare facilities. When sales professionals utilize the cleaning classification to guide users in integrating devices into their established cleaning procedures during the transfer process, the risk of non-compliance with the Instructions for Use (IFU) is diminished. The cleaning classification fosters collaborative efforts between healthcare facilities and medical device manufacturers, ensuring adherence to the minimum cleaning requirements outlined in the IFU. Standardizing instrument processing in bustling sterile processing departments can notably enhance efficiency and minimize risks. Achieving higher throughput of instrument processing with maintained quality directly correlates to reduced healthcare costs for patients.

The utilization of the cleaning classification allows for targeted focus on medical devices requiring heightened attention. Initiatives such as training programs for the cleaning process and the implementation of verification testing will be instituted to mitigate associated risks. With continual improvements in device design, the likelihood of residual contamination after cleaning is expected to decrease. Additionally, as healthcare facility processes evolve and enhance the methods for device processing, the risk of Hospital-Acquired Infections (HAIs) stemming from contaminated devices is anticipated to diminish. Given that HAIs represent a substantial financial burden on healthcare facilities, a reduction in occurrences is likely to directly correlate with a lower overall cost of healthcare. This interconnected approach prioritizes safety, efficiency, and cost-effectiveness within healthcare practices.

7.2 Benefits to Environment

In the 6-Sigma philosophy, Muta is defined as the reduction of waste in a process. As explained in Chapter 2 and detailed in Appendix 1, the current processing of reusable devices involves a considerable amount of waste and rework. Rework poses a significant challenge to the process, serving as evidence that it is flawed and incapable of consistently producing high-quality products. When devices are sent back to sterile processing due to contamination, it erodes confidence in the healthcare facility's proficiency in reprocessing surgical devices. This decline in confidence directly impacts trust in the safety of the devices. The implementation of the cleaning classification has the potential to minimize waste in the cleaning process, thereby bolstering confidence in the effectiveness and safety of reusable medical devices.

The capacity to process a medical device offers the industry the opportunity to maximize the utilization of finite resources. With each reuse, the overall cost of the device decreases, presenting a sustainable alternative. In contrast, single-use devices significantly contribute to the waste generated by healthcare facilities. Disposal of these devices often involves methods such as incineration, leading to environmental harm. The single-use supply chain is more detrimental to the environment than the use of reusable medical devices. Despite the consumption of resources during reprocessing, such as water and

detergents, the adoption of water recycling and environmentally friendly cleaning agents is estimated to result in a lower carbon footprint compared to the environmental impact of single-use devices. This highlights the ecological benefits associated with the reusability of medical devices.

The ongoing investment by medical device companies in the production of reusable medical devices, as opposed to their single-use counterparts, is contingent upon customer willingness to purchase them. The dynamics of supply and demand play a pivotal role in influencing medical device manufacturers to sustain investment in this domain. To foster continued investment, healthcare companies must demonstrate a commitment to environmentally friendly options while ensuring an equivalent level of risk to patient safety. The integration of the cleaning classification holds the potential to boost confidence within the healthcare industry regarding medical device processing, thereby encouraging the introduction of more devices into the market. This interplay between consumer demand, environmental considerations, and confidence in safety measures contributes to the sustainability and growth of reusable medical devices in the market.

7.3 Benefits to Society

The cleaning of a reusable medical device should never be considered a competitive advantage for a medical device company. Every patient has the inherent right to receive high-quality care, including the use of meticulously cleaned devices. The moments when a person requires medical attention are often fraught with stress and uncertainty, and medical interventions should not introduce unnecessary risks to the patient. In line with the medical oath to "do no harm," the processing of reusable medical devices should be a steadfast practice that unequivocally prioritizes patient safety, ensuring that the cleaning process itself never poses a threat to the well-being of the patient.

Implementation of the cleaning classification is poised to reduce the risk of HAIs and bolster patient confidence in the utilization of reusable medical devices. The exaggerated narratives of harm associated with reusable devices will no longer dominate discussions; rather, there will be a shift towards language aimed at encouraging their expanded use. Public opinion is anticipated to play a pivotal role in driving increased adoption, fostering a positive perception that, in turn, will drive further innovation in the development and utilization of reusable medical devices. This positive momentum is expected to reshape the discourse surrounding reusable devices, emphasizing their safety and efficacy.

From a personal perspective, I can affirm the significance of this transformation. With a professional background in the healthcare industry and insight into device processing for the past 16 years, I have developed a certain apprehension towards hospitals. I have often guided friends undergoing medical procedures on the best times and days to schedule their treatments for the highest likelihood of encountering uncontaminated reusable medical devices. However, as a mother of two boys, instances of medical intervention have been unavoidable. Being risk-averse, I chose to give birth to both of my children at home with the assistance of a midwife—a decision that, in my case, proved wise. A close friend, who delivered her daughter at the same hospital I considered for my first son's birth, unfortunately, faced the

consequences. Her daughter contracted methicillin-resistant *Staphylococcus aureus*, a bacterium resistant to widely used antibiotics, and continues to grapple with the aftermath 14 years later. As a patient, my anxiety about contracting a Healthcare-Associated Infection (HAI) during a hospital stay diminishes significantly when contemplating the implementation of the cleaning classification. The standardization and regulation in this domain instill confidence in me as a patient, reassuring me that those responsible for the processing of reusable medical devices prioritize my health and safety.

7.4 Automation & Machine Learning

Across various healthcare domains, the exploration of opportunities for automation has become widespread. One notable area undergoing transformation due to robotic automation is device processing. Presently, machines are readily available and being introduced to healthcare facilities, offering the capability to seamlessly transport instruments through each stage of processing. This innovation significantly reduces or eliminates the physical effort involved in lifting instrument trays, transporting them, and loading them into washer-disinfectors and autoclaves. Originating from manufacturing technology, this type of automation has reshaped the landscape of device processing. Additionally, technology has emerged to replace the labor-intensive tasks of sorting, inspecting, and packaging instruments for sterilization. These innovative automation adaptations have been successfully implemented in large-scale sterile processing facilities, serving as effective replacements for corresponding manual activities.

Unsurprisingly, the aspect of the processing steps that has yet to be effectively automated is the manual cleaning requirement outlined in many IFUs. Specifically, the brushing/flushing component within this experimental design's IFU has been proven essential for the thorough removal of residual soil from certain device features. The fluid dynamics of these features create conditions where air flow can dry soil to a point that alters its solubility or prevents cleaning fluids from adequately accessing all areas of the device with the necessary shear force to remove the remaining soil from the device surface. The manual intervention of brushing and flushing compels interaction between the soil and cleaning fluid, facilitating effective removal. However, automating this manual intervention during the cleaning process poses challenges. Visual inspection of the device is required to verify complete soil removal, and automation would require a visual mapping of each device against a master picture for comparison. Establishing this extensive database of imagery is a monumental task given the multitude of unique devices available on the market. Consequently, progress in automating this aspect of processing has been gradual.

Utilizing the cleaning classification has the potential to accelerate innovation in this domain. Certain features, such as ball seal springs, rough surfaces, and threaded rods, have shown the ability to be cleaned without the need for brushing or flushing. However, each of these features, when subject to additive extraction measuring non-water-soluble protein, yielded results surpassing the alert level of 3µg/cm^2 specified in ISO 15883-5 (International Organization for Standardization, 2021). This underscores the significance of drying time in influencing the feasibility of processing these features without manual intervention.

Achieving automation of the manual cleaning process becomes highly feasible if medical device manufacturers incorporate features that facilitate cleaning without manual intervention and healthcare facilities diligently prevent soil from drying on devices during processing delays. Additionally, the semi-automated experimental design explored in this study suggests that cleaning instructions can be crafted to enable the chemistry of the cleaning fluid to carry out the tasks typically performed through manual intervention. Certain features demonstrated effectiveness when the device is kept moist, and the cleaning instructions involve a sequence of soaks, sonication, and utilization of a washer-disinfector, eliminating the need for manual intervention. Leveraging this valuable data, manufacturers and healthcare users can expedite innovation for the comprehensive automation of the entire device processing cycle.

However, it is also essential to note that automation must undergo validation at healthcare sites to showcase its cleaning effectiveness and robustness for all relevant devices. The cleaning classification proves valuable in establishing validation criteria for these automated processes, considering the associated risks. The validation process should encompass theoretical modeling of the cleaning process, focusing on the most challenging feature to clean in the design of cleaning instructions. Subsequently, a comprehensive testing strategy should be implemented, wherein actual devices are subjected to the model to verify effectiveness. Sample size must be sufficient to demonstrate reproducibility and ruggedness of the automated process. Standardization of the validation process should be adopted in the industry before enabling wide scale adoption of automated processes.

Machine learning is a subset of artificial intelligence that involves the development of algorithms and statistical models enabling computers to perform tasks without explicit programming. It revolves around the idea that systems can learn from data, identify patterns, and make decisions or predictions. The process begins with training the machine learning model on a dataset, exposing it to various examples to enable it to recognize patterns and relationships. The dataset established within this experimental design may be used for initial modeling. As the model learns, it adjusts its parameters, enhancing its ability to generalize and make accurate predictions on new, unseen data. Its capacity to adapt and improve over time makes it a powerful tool in solving complex problems and gaining insights from vast datasets.

The adoption of automation in device processing, still in its infancy, requires a robust scientific foundation to advance further. Machine learning plays a pivotal role in this progression, envisioning a scenario where a fully automated system, supported by scientific decision-making, can process a limited range of device types initially. As this system becomes well-established, it consistently and effectively cleans specific types of devices. The process is modeled using machine learning to predictively demonstrate the cleaning efficacy by comprehensively understanding the fluid dynamics of each device. This synergy between automation and machine learning holds the potential to revolutionize device processing, enhancing efficiency and adaptability through continuous learning and improvement.

An example of how to apply machine learning to simultaneously simulate, model and predict utility of new cleaning classification data is potentially as follows:

Emphasis should be placed on the design feature (F1 to F23) based ability to clean effectively. This computationally will then feed into a 'label' that we design as 'patient risk' – this can be a numbered ..1,2, 3 or minimal, moderate, maximal. Input data values can be calculated for a new device from F1 to F23 each feature assigned a value – example, F2 could be 20 (based on ranking), but all features note (for example if only 5 features used then the other 18 are assigned "0"). The model approach 'to learn' will be based on the level of data entry into the system – the more entries that are used – the more the system will learn. The appropriate model to help advance the intuitive learning will be based on coding and its pretty straight forward to apply differ ML algorithms and models based on what we need from needs. This will provide a computational 'learning' model to inform when a device has entered into the various categories of risk, and indeed what predominant feature is contributing to either minimal or say maximal risk – this will allow assignment to a group or family of device features based on ML inputs. This (ML) is an extension of statistical analysis that is based on human calculations of estimates or probabilities – thus, the digital (ML) approach is just automating a human centric activity for efficiencies and to reduce operator error. A real smart approach for us would be also to use connected linkages between device features and heat maps for visualization and verification of devices for stakeholders – this would show red for high-risk feature --- such as using Grad-Cam approach for example – from schematic --- it could even be an elephant (or device) made of component parts, but the contribution of each feature will give a unique heat map that can also be digitized and tracked.

7.5 Further research

Built upon the contributions of industry pioneers, this novel research aims to inspire ongoing exploration in the field of reusable medical device cleaning. Beyond the imperative tasks of integrating automation and machine learning, this study has identified additional gaps in the existing literature that warrant thorough investigation and subsequent publication. The proposed research areas encompass validation variables and verification testing laying the groundwork for continuous advancement in the realm of medical device processing.

7.5.1 Validation Variables

As previously discussed, the validation variables influencing the cleaning efficacy of reusable medical devices can significantly impact testing outcomes. One crucial variable is the effectiveness of the cleaning agent employed. Presently, there is no industry standard in place to ascertain the effectiveness of cleaning agents. While cleaning agent manufacturers may market detergents with added enzymes for enhanced efficacy, there exists no standard defining the required performance. Consequently, a broad spectrum of effectiveness may be observed. In the validation process, medical device manufacturers are advised to incorporate worst-case cleaning agents in their protocols, allowing healthcare facilities flexibility in their selection. The absence of cleaning performance data for medical device manufacturers further contributes to the variability in choosing the cleaning agent for validation.

In the experimental design of this project, a sequence of detergent soaks was incorporated to formulate the semi-automated experiment. During this examination, it became evident that sonication with an enzymatic detergent proved ineffective in solubilizing residual soil, attributed to the inhibitory effect of sonication's cavitation activity on enzyme activity. Consequently, static soaks utilizing an enzymatic detergent were implemented, accompanied by the use of an alkaline cleaning agent during the sonication step. The efficacy of the detergent, leveraging pH variations for soil removal, was demonstrated to be successful when combined with sonication. These findings underscore the impact of cleaning process steps on detergent performance, warranting further investigation. Such exploration is vital to provide medical device manufacturers with pertinent insights for appropriately designing cleaning validations tailored to subsequent steps outlined in the IFU.

In this experimental setup, the sonication bath employed for the cleaning procedure was freshly prepared for each validation. The literature does not provide insights into how the saturation of soil in the sonication bath might impact cavitation action. Further research is necessary to elucidate the saturation point at which the bath would no longer serve as an effective cleaning step. This type of research could also be used to inform healthcare facilities on how often cleaning agents should be replaced in sonication equipment at the healthcare facility.

7.5.2 Verification Testing

ATP testing was incorporated into the experimental design to explore potential correlations between protein, TOC, and ATP. The ATP results exhibited considerable variability and did not reveal a statistically significant relationship (R^2 = 0.00 for most test points) with the other analytes (Figure 7.2). Although ATP is commonly used for cleaning confirmation in healthcare facilities to ensure cleanliness, the outcomes of this experiment suggest a need for additional research to strengthen the association between commercially available verification tests and the quantitative analytes included in cleaning validations.

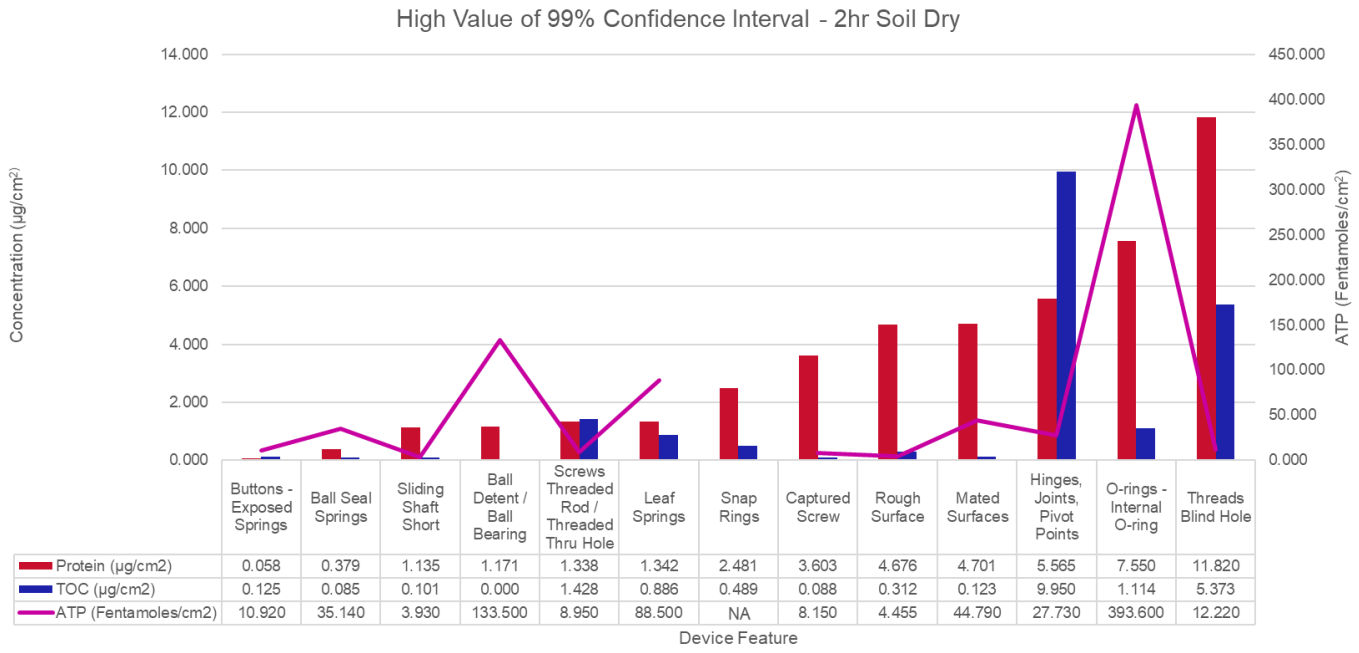


Figure 7.2: Graphical Relationship Between Analytes Demonstrating a Lack of Correlation Between ATP and Other Cleaning Analytes

As proposed in Chapter 6, it is recommended that medical device manufacturers take on the responsibility of evaluating and incorporating these verification tests into their validation strategies for healthcare facility use. Transferring this responsibility to the healthcare facility is not feasible. Instead, the validation of these verification tests should be undertaken by the test manufacturer and subsequently endorsed for application by the medical device manufacturer. These tests should be subject to the same regulations as other cleaning equipment (e.g., washer-disinfectors) and should follow established test requirements and regulatory submission processes.

7.6 Dissemination

Patient safety stands as a collective responsibility shared among key stakeholders. While medical device manufacturers initiate the process through device design, the mantle of responsibility extends through various entities involved in the device's lifecycle. Patient safety can be likened to a robust rope composed of interconnected segments; when all parts work cohesively, the rope attains strength. However, if one segment frays, the entire integrity of the rope is compromised. In this metaphor, the following groups bear responsibility for patient safety (Figure 7.3):

- **Medical Device Manufacturers:** Responsible for designing and validating devices and IFUs.
- **Testing Laboratories:** Conduct validation testing and offer guidance to manufacturers and regulators on validation requirements.
- **Regulators:** Verify that reusable medical devices have undergone validation for cleaning efficacy.

- **Healthcare Facility:** Holds responsibility for patient safety throughout the device's lifecycle.
- **3rd Party Reprocessing Companies:** Entities contracted by healthcare facilities to carry out device processing activities as specified in the device manufacturer's IFU.
- **Academia:** Tasked with collaborating with the industry to advance the scientific understanding of device processing.

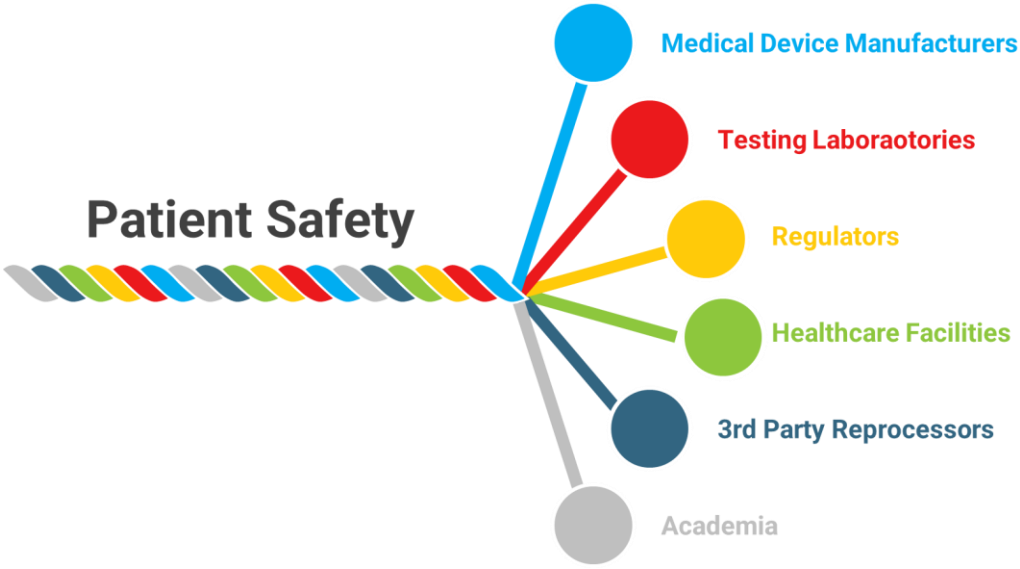


Figure 7.3: Patient Safety Key Stakeholders

The realization of industry applications, as elaborated in Chapter 6, hinges on the widespread distribution of the cleaning classification to key stakeholders across the entire supply chain for reusable medical devices. The duration between the introduction of the Spaulding classification and its incorporation into industry guidance spanned approximately 40 years. Given the direct impact of the dissemination of this cleaning classification on patient safety, a considerably swifter acceptance timeframe is imperative. Consequently, a dissemination plan has been devised for each of the crucial stakeholder groups utilizing industry conferences and focused publications.

Adapting the communication style of a presentation or publication to the target audience is important for enhancing the effectiveness of communication and fostering audience understanding and engagement. Varied audiences possess different levels of familiarity with the subject and diverse knowledge backgrounds. Customizing the communication style enables the adjustment of content complexity, depth, and terminology to align with the audience's comprehension level. This ensures that the message is accessible and pertinent to them, minimizing the risk of confusion or misinterpretation.

A presentation or paper that connects with the audience is more likely to capture their attention and sustain their interest. Aligning content with the audience's interests, concerns, and needs demonstrates a consideration for their perspective, thus increasing the likelihood of audience engagement and active

participation. When the objective is to persuade or influence the audience, tailoring the communication style allows for framing the message in a manner that resonates with their values and priorities. This approach enhances the receptivity of the audience to your ideas and arguments.

The work described in this thesis has been presented 17 times to the various stakeholders described in Figure 7.2. Table 7.1 is a summary of the presentations, but a full description of the presentation content can be found in Appendix 28.

Table 7.1: Summary of dissemination through presentations

Conference	Presentation Title	Audience
2022 Kilmer Conference	Thinking Differently to Unlock and Mitigate Risk in the End-to-End Device Processing Supply Chain	Industry Leaders in the area of Microbiological Quality & Sterility Assurance
2022 Kilmer Conference	Establishing a relationship between an RMM analyte and the CFU	Industry Leaders in the area of Microbiological Quality & Sterility Assurance
Healthcare Sterile Processing Association Annual Conference 2022	Time is Running Out: Importance of Environmental Conditions During Transport and Storage of Soiled Medical Devices	Healthcare Sterile Processing Professionals – US
Infection Control Africa Network: Postgraduate Diploma in Infection Control 2022	Water quality	Post graduate Students in Africa
Infection Control Africa Network: Postgraduate Diploma in Infection Control 2022	Robotic equipment/devices	Post graduate Students in Africa
Infection Control Africa Network: Postgraduate Diploma in Infection Control 2022	Case Studies on Typical Challenges in Decontamination in Outpatient Facilities	Post graduate Students in Africa
Infection Control Africa Network: Postgraduate Diploma in Infection Control 2022	Recycling of single use devices	Post graduate Students in Africa
TUV-SUD Digital Dialogues	Strengthening the Science of Device Processing	Medical Device Manufacturers
OR Manager Conference	Importance of environmental conditions within the healthcare setting during the transport and storage of soiled medical devices	Operating Room Managers and Associated Healthcare Professionals
23rd World Sterilization Congress	The Impact of Time and Environmental Conditions on Contaminated Instrumentation	Global Sterile Processing Professionals
AORN Annual Conference	Mitigating infection risk: What does the evidence really say about POU Instrument Treatment?	Registered Perioperative Nurses

ASTM Cleaning Workshop	A Spaulding Classification System for Establishing Cleaning Limits	ASTM Standard Develop Committee Members
AAMI Cleaning Verification Summit	Utilizing Appropriate Endpoint Analysis into Device Cleaning Evaluations by Incorporating Device Biocompatibility Practices	Industry Leaders in the area of Device Processing
Northeastern University	A Spaulding Classification System for Establishing Cleaning Limits	University Engineering Students
2023 PDA Pharmaceutical Microbiology Conference	Collaborating to Innovate Effective Disinfectant Rotation for Contamination Control	Pharmaceutical Professionals
2024 Nexus	Practical Approaches for Validation of Cleaning Processes	Medical Device and US Regulatory Professionals

Alongside the oral presentations, various aspects of the research have been submitted for publication. Out of the ten submitted papers, nine have successfully undergone the peer-review / acceptance process and are fully published. These publications have been strategically aimed at journals relevant to the intended audience of the periodical, ensuring that the research findings reach and resonate with the target readership.

- Biomedical Instrumentation & Technology (BI&T) – The audience of this journal are US healthcare facilities and medical device manufacturers. This journal is supported by AAMI as a dedicated space for disseminating research related to medical devices and healthcare practices.
- Science of the Total Environment - The audience of this journal comprises interdisciplinary researchers, environmental scientists, and professionals who are interested in gaining comprehensive insights into the various aspects of the global environment. The readership includes experts in environmental chemistry, ecology, biology, and related disciplines, as well as policymakers and stakeholders seeking a holistic understanding of environmental issues and solutions.
- Journal of Hospital Infection – This journal primarily targets healthcare professionals, including clinicians, researchers, and infection control practitioners, who specialize in hospital-acquired infections and related fields. The audience comprises individuals actively involved in infectious disease prevention, surveillance, and management within healthcare settings, aiming to disseminate and exchange the latest research findings, clinical practices, and strategies for infection control and patient safety.

Table 7.2 is a summary of the foundational publications written to support the selection of test variables, but a full description of the publication content can be found in Appendix 28.

Table 7.2: Summary of dissemination through publications

Publication Title	Journal	Audience	Reference
A Standardized Method for Evaluating Test Soils Used to Demonstrate Cleaning Efficacy	Journal of Hospital Infection	Medical Device Manufacturers, Testing Laboratories, Regulators	(Kremer, et al., 2022)
Test Soil and Device Material Affinity for Reusable Device Cleaning Validations	Biomedical Instrumentation & Technology	Medical Device Manufacturers, Testing Laboratories, Regulators	(Kremer & Ratanski, 2023)
Effects of Time, Temperature, and Humidity on Soil Drying on Medical Devices	Biomedical Instrumentation & Technology	Medical Device Manufacturers, Testing Laboratories, Regulators	(Kremer, et al., 2023)
Chemical Changes Over Time Associated with Protein Drying	Biomedical Instrumentation & Technology	Medical Device Manufacturers, Testing Laboratories, Regulators	(Kimble, et al., 2023)
Cleaning Challenges: Can Extended Soil Dry Times be Reversed?	Biomedical Instrumentation & Technology	Medical Device Manufacturers, Testing Laboratories, Regulators	(Hoover, et al., 2023)
Improving Protein Assay Methods to More Accurately Assess Medical Device Cleanliness	Biomedical Instrumentation & Technology	Medical Device Manufacturers, Testing Laboratories, Regulators	(Kremer, et al., 2023)

The additional four peer-reviewed articles were published to disseminate the research described in the thesis. By making research findings publicly accessible, the thesis enables other scholars, practitioners, and interested individuals to benefit from and engage with the research, fostering a culture of shared knowledge and continuous learning.

Paper Title	A review of Spaulding's classification system for effective cleaning, disinfection and sterilization of reusable medical devices: Viewed through a modern-day lens that will inform and enable future sustainability
Publication/Submission Date	22 March 2023
Audience	Medical Device Manufacturers, Testing Laboratories, Regulators, Healthcare Facilities, 3rd Party Reprocessing Companies, and Academia
Journal	Science of the Total Environment
Reference	(Rowan, et al., 2023)

Abstract

Despite advances in medicine and innovations in many underpinning fields including disease prevention and control, the Spaulding classification system, originally proposed in 1957, remains widely used for defining the disinfection and sterilization of contaminated re-usable medical devices and surgical instruments. Screening PubMed and Scopus databases using a PRISMA guiding framework generated 272 relevant publications that were used in this review. Findings revealed that there is a need to evolve how medical devices are designed, and processed by cleaning, disinfection (and/or sterilization) to mitigate patient risks, including acquiring an infection. This Spaulding Classification remains in use as it is logical, easily applied and understood by users (microbiologists, epidemiologists, manufacturers, industry) and by regulators. However, substantial changes have occurred over the past 65 years that challenge interpretation and application of this system that includes inter alia emergence of new pathogens (viruses, mycobacteria, protozoa, fungi), a greater understanding of innate and adaptive microbial tolerance to disinfection, toxicity risks, increased number of vulnerable patients and associated patient procedures, and greater complexity in design and use of medical devices.

Common cited examples include endoscopes that enable non- or minimal invasive procedures but are highly sophisticated with various types of materials (polymers, electronic components etc.), long narrow channels, right angle and heat-sensitive components and various accessories (e.g., values) that can be contaminated with high levels of microbial bioburden and patient tissues after use. Contaminated flexible duodenoscopes have been a source of several significant infection outbreaks, where at least 9 reported cases were caused by multidrug resistant organisms [MDROs] with no obvious breach in processing detected. Despite this, there is evidence of the lack of attention to cleaning and maintenance of these devices and associated equipment. Over the last few decades there is increasing genomic evidence of innate and adaptive resistance to chemical disinfectant methods along with adaptive tolerance to environmental stresses. To reduce these risks, it has been proposed to elevate classification of higher-risk flexible endoscopes (such as duodenoscopes) from semi-critical [contact with mucous membrane and intact skin] to critical use [contact with sterile tissue and blood] that entails a transition to using low-temperature sterilization modalities instead of routinely using high-level disinfection; thus, increasing the margin of safety for endoscope processing. This timely review addresses important issues surrounding use of the Spaulding classification system to meet modern-day needs. It specifically addresses the need for automated, robust cleaning and drying methods combined with using real-time monitoring of device processing. There is a need to understand entire end-to-end processing of devices instead of adopting silo approaches that in the future will be informed by artificial intelligence and deep-learning/machine learning. For example, combinational solutions that address the formation of complex biofilms that harbors pathogenic and opportunistic microorganisms on the surfaces of processed devices. Emerging trends are addressed including future sustainability for the medical devices sector that can be enabled via a new Quintuple Helix Hub approach that combines academia, industry, healthcare, regulators, and society to unlock real world solutions.

Paper Title	Use of real-time immersive digital training and educational technologies to improve patient safety during the processing of reusable medical devices: Quo Vadis?
Publication/Submission Date	03 March 2023
Audience	Medical Device Manufacturers, Testing Laboratories, Regulators, Healthcare Facilities, 3rd Party Reprocessing Companies, and Academia
Journal	Science of the Total Environment
Reference	(Kremer, et al., 2023)

Abstract

Hospital acquired infections stemming from contaminated reusable medical devices are of increasing concern. This issue is exaggerated with the introduction of complex medical devices like endoscopes and robotic instrumentation. Although medical device manufacturers validate their cleaning instructions for use, evidence in the literature demonstrates that effective device processing is not being performed consistently within sterile processing departments in clinical settings. The result is increased risks to patient safety. As a solution to this problem, focused one-on-one training increases compliance to the medical device manufacturer's processing instruction. However, often this is not a practical solution for the volume of healthcare staff responsible for device processing activities. This constitutes the first paper to address the blended use of educational and digital technologies to address these challenges and as a result inform safety and sustainability for the medical device sector. Cognitive learning theory is an evidence-based framework for learning. It supports the use of immersive educational experiences using emerging extended reality technologies (e.g., virtual or augmented reality) to increase learning comprehension. The delivery of educational content via these technologies provides an innovative option for repeatable leaning and training outcomes. The motivation is to decrease patient risk of contaminated reusable medical devices. The proposed approach while primary motivated by safety can also enhance sustainability and efficiency enabled by artificial intelligence and robotic instrumentation.

Paper Title	Validation of the Device Feature Approach for Reusable Medical Device Cleaning Evaluations
Publication/Submission Date	2023
Audience	Medical Device Manufacturers, Testing Laboratories, Regulators
Journal	Biomedical Instrumentation & Technology
Reference	(Kremer, et al., 2023)

Abstract

The identification of worst-case device (or device set) features has been a well-established validation approach in many areas (e.g., terminal sterilization) for determining process effectiveness and requirements, including for reusable medical devices. A device feature approach for cleaning validations has many advantages, representing a more conservative approach compared with the alternative compendial method of testing the entirety of the device. By focusing on the device feature(s), the most challenging validation variables can be isolated to and studied at the most difficult-to-clean feature(s). The device feature approach can be used to develop a design feature database that can be used to design and validate device cleanliness. It can also be used to commensurately develop a quantitative cleaning classification system that will augment and innovate the effectiveness of the Spaulding classification for microbial risk reduction. The current study investigated this validation approach to verify the efficacy of device cleaning procedures and mitigate patient risk. This feature categorization approach will help to close the existing patient safety gap at the important interface between device manufacturers and healthcare facilities for the effective and reliable processing of reusable medical devices. A total of 56,000 flushes

of the device features were conducted, highlighting the rigor associated with the validation. Generating information from design features as a critical control point for cleaning and microbiological quality will inform future digital transformation of the medical device industry and healthcare delivery, including automation.

Paper Title	A proposed cleaning classification system for reusable medical devices to complement the Spaulding classification
Publication/Submission Date	14 December 2023
Audience	Medical Device Manufacturers, Testing Laboratories, Regulators, Healthcare Facilities, 3rd Party Reprocessing Companies, and Academia
Journal	Journal of Hospital Infection
Reference	(Kremer, et al., 2023)
Abstract	
A central tenet in infection prevention is application of the Spaulding classification system for the safe use of medical devices. Initially defined in the 1950s, this system defines devices and surfaces as being critical, semi-critical or non-critical depending on how they will be used on a patient. Different levels of antimicrobial treatment, defined as various levels of disinfection or sterilization, are deemed appropriate to reduce patient risk of infection. However, a focus on microbial inactivation is insufficient to address this concern, which has been particularly highlighted in routine healthcare facility practices, emphasizing the underappreciated importance of cleaning and achieving acceptable levels of cleanliness. A deeper understanding of microbiology has evolved since the 1950s, which has led to re-evaluation of the Spaulding classification along with a commensurate emphasis on achieving appropriate cleaning. Albeit underappreciated, cleaning has always been important as the presence of residual materials on surfaces can interfere with the efficacy of the antimicrobial process to inactivate micro-organisms, as well as other risks to patients including device damage, malfunction and biocompatibility concerns. Unfortunately, this continues to be relevant, as attested by reports in the literature on the occurrence of device-related infections and outbreaks due to failures in processing expectations. This reflects, in part, increasing sophistication in device features and reuse, along with commensurate manufacturer's instructions for use. Consequently, this constitutes the first description and recommendation of a new cleaning classification system to complement use of the traditional Spaulding definitions to help address these modern day technical and patient risk challenges. This quantitative risk-based classification system highlights the challenge of efficient cleaning based on the complexity of device features present, as an isolated variable impacting cleaning. This cleaning classification can be used in combination with the Spaulding classification to improve communication of cleaning risk of a reusable medical device between manufacturers and healthcare facilities and improve established cleaning practices. This new cleaning classification system will also inform future creation, design thinking and commensurate innovations for the sustainable safe reuse of important medical devices.	

While presentations offer a platform for real-time interaction and feedback, publications endure as enduring contributions to academic literature, enabling a broader audience to access and reference the research over time, thus contributing to the collective body of knowledge. Both presenting and publishing play integral roles in the academic discourse, each serving distinct purposes in the scholarly communication process.

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