Laboratory methods with graphic support as a UDL strategy for science students in laboratory sessions

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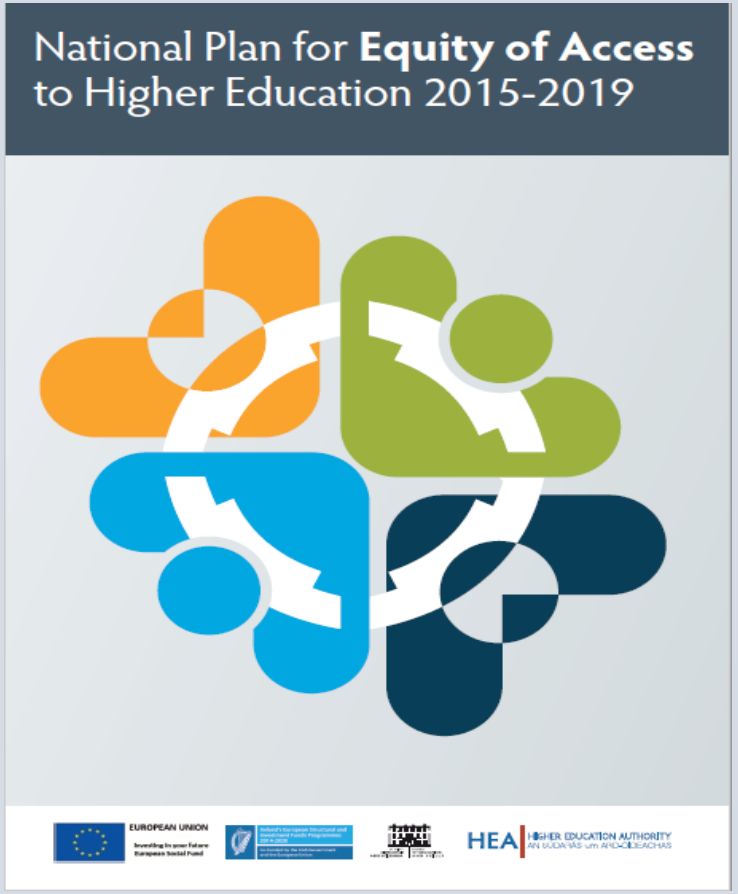
Presentation	• Background Information • Study Design
Audience Participation	• Activate Knowledge
Video	• Putting Theory into Practice!
Presentation Summary	• Key Findings • Future Work
Q & A	• Critical thinking • Peer-sharing

Introduction: Equity of Access

- The National Access Plan (2015-2019) provides a framework for broadening participation in higher education:

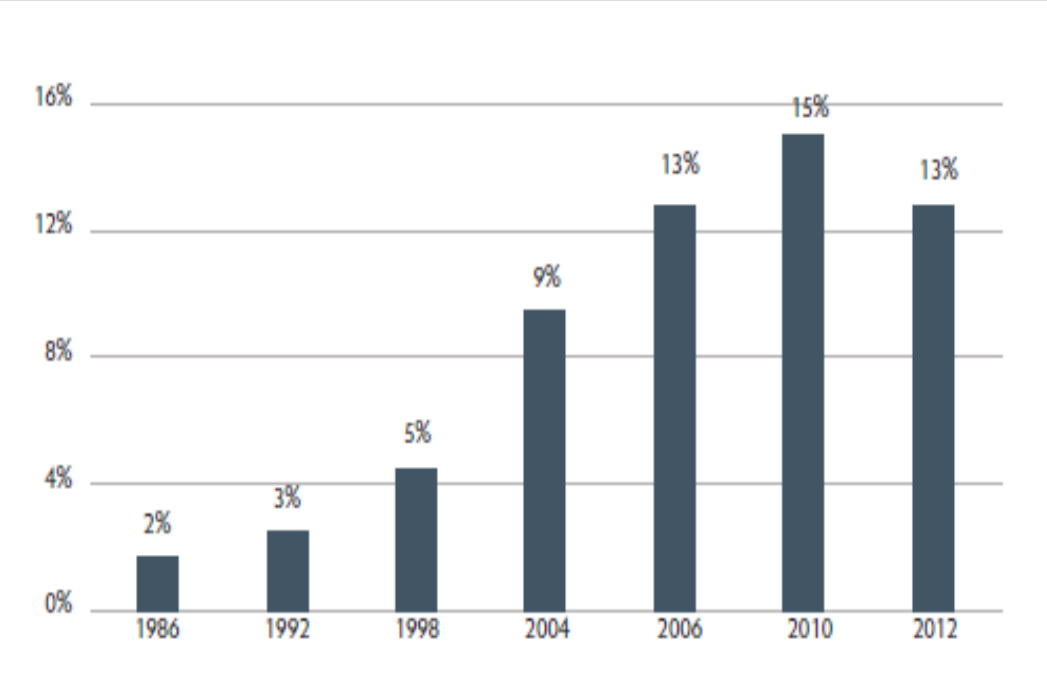


- ❑ People with disabilities
- ❑ Mature students
- ❑ Socio-economic disadvantage

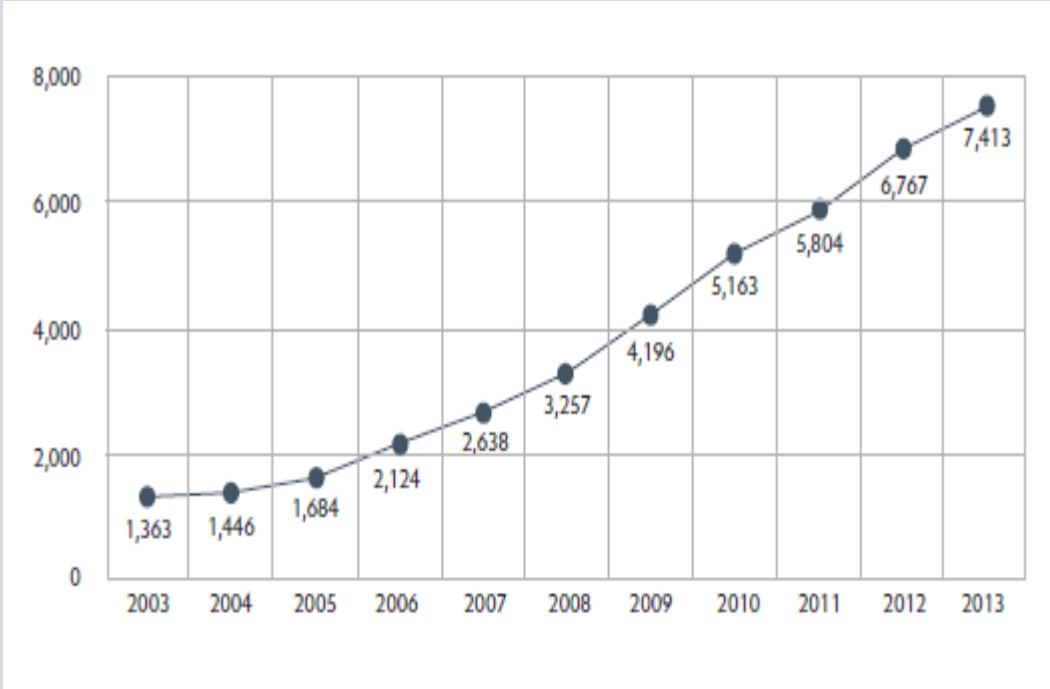
- Reflecting diversity makes sense:
- ❑ Socially
- ❑ Economically

Introduction: Equity of Access

- Target group participation trending upwards ?
- Access ✓?



Mature Entrants (23 yrs+)



Students with Disabilities

National Access Plan (2015-2019)

Introduction: Access Targets (2015-2019)

1: Socio-economic; 2: Mature Students; 3: Disabilities; 4: Holders of further Ed
5: Flex/Part-time; 6: Irish Travellers

National Access Plan (2015-2019)

Introduction: Target Group Subject Areas

	Number of students				
Category of disability	**2003**	**2006**	**2009**	**2012**	**2013**
Specific learning difficulties	831	1,482	2,729	3,814	3,929
Significant ongoing illness	93	114	303	588	681
Multiple disability	48	85	144	548	703
Physical/mobility	175	190	235	398	416
Deaf/hard of hearing	94	126	173	212	201
Blind/vision impairment	76	65	116	140	160
Other disabilities (including ADD/ADHD, ASD (Autistic Spectrum Disorder), Mental Health Conditions)	46	62	496	1,067	1,323
Total	**1,363**	**2,124**	**4,196**	**6,767**	**7,413**

Field of study	Socio-economic target groups	Mature students	Students with disabilities	General student population
Agriculture and Veterinary	1%	1%	3%	2%
Education	3%	2%	2%	3%
Engineering, Manufacturing and Construction	11%	9%	11%	11%
General Programmes*	1%	0%	0%	0%
Health and Welfare	14%	25%	13%	14%
Humanities and Arts	19%	20%	24%	19%
Science, Mathematics and Computing	18%	17%	19%	18%
Services	10%	7%	7%	7%
Social Sciences, Business and Law	25%	19%	21%	24%
	100%	100%	100%	100%

* General Programmes are courses in literacy, numeracy and personal development.

- Specific learning disabilities and ADD/ADHD/ASD the largest and second largest by sub-category (HEA SRS, 2012-2014)

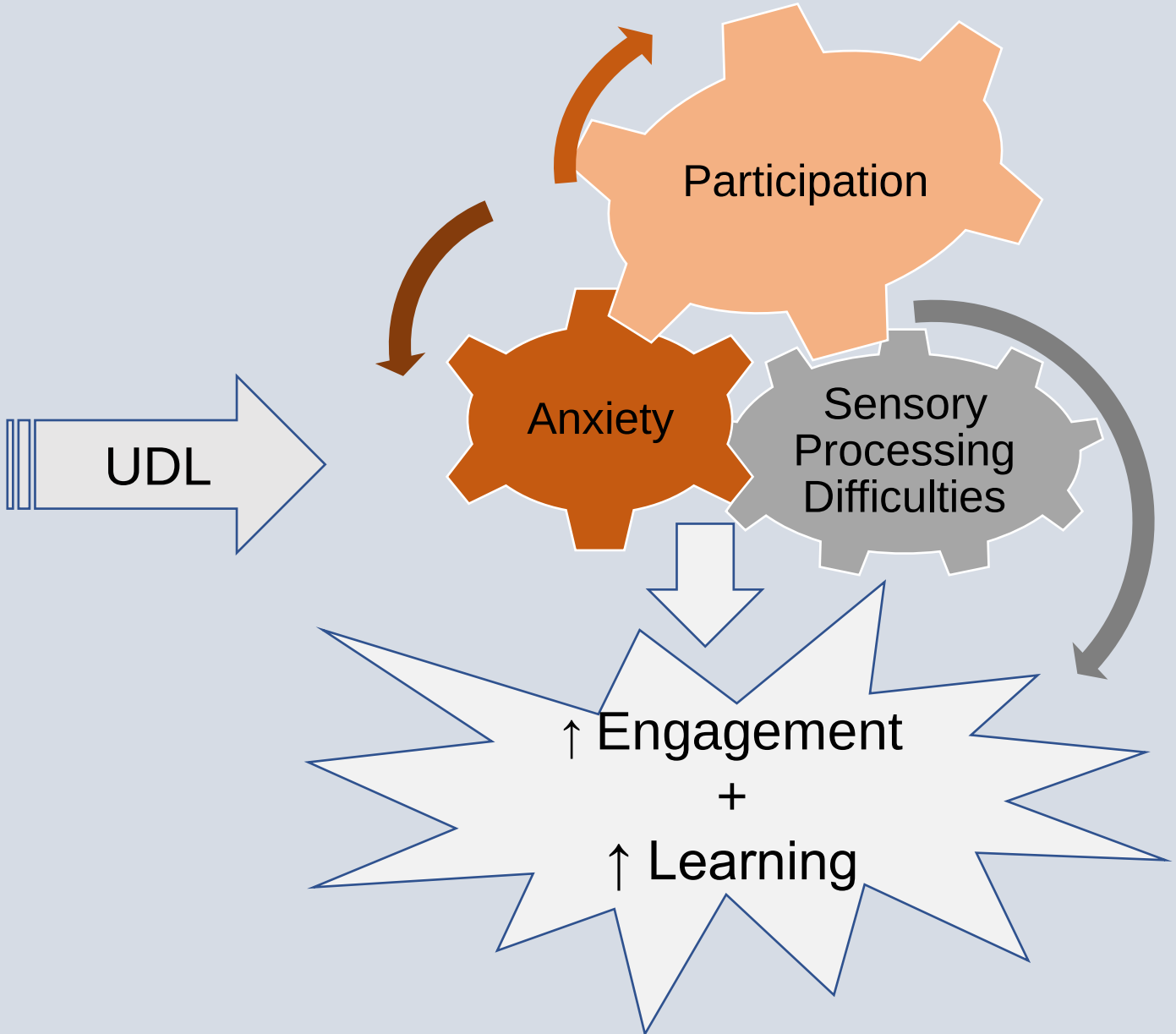
Science, Mathematics and Computing…. (HEA SRS, 2012-2014)

Introduction: Priority Goals & UDL

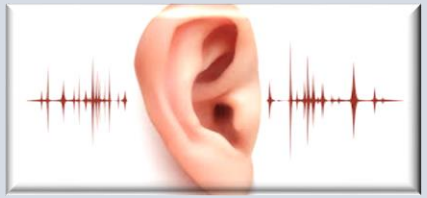
- To mainstream the delivery of equity of access in HEIs, Nat. Access Plan, (2015-2019)

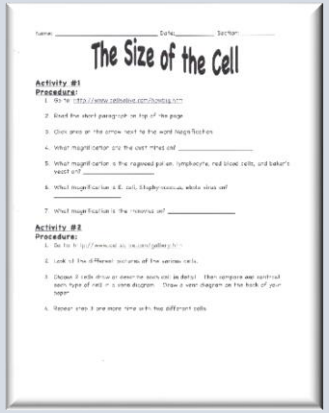
- Proactive strategies to increase accessibility for diverse learners in line with the UDL

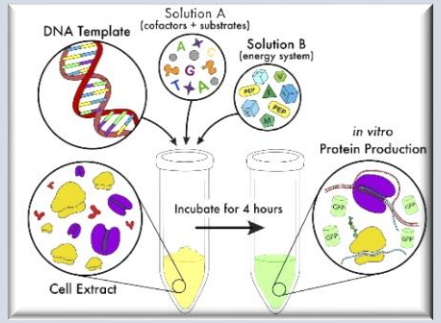
- increased access ✓
- participation and engagement….
- achievement …..



Science & UDL:







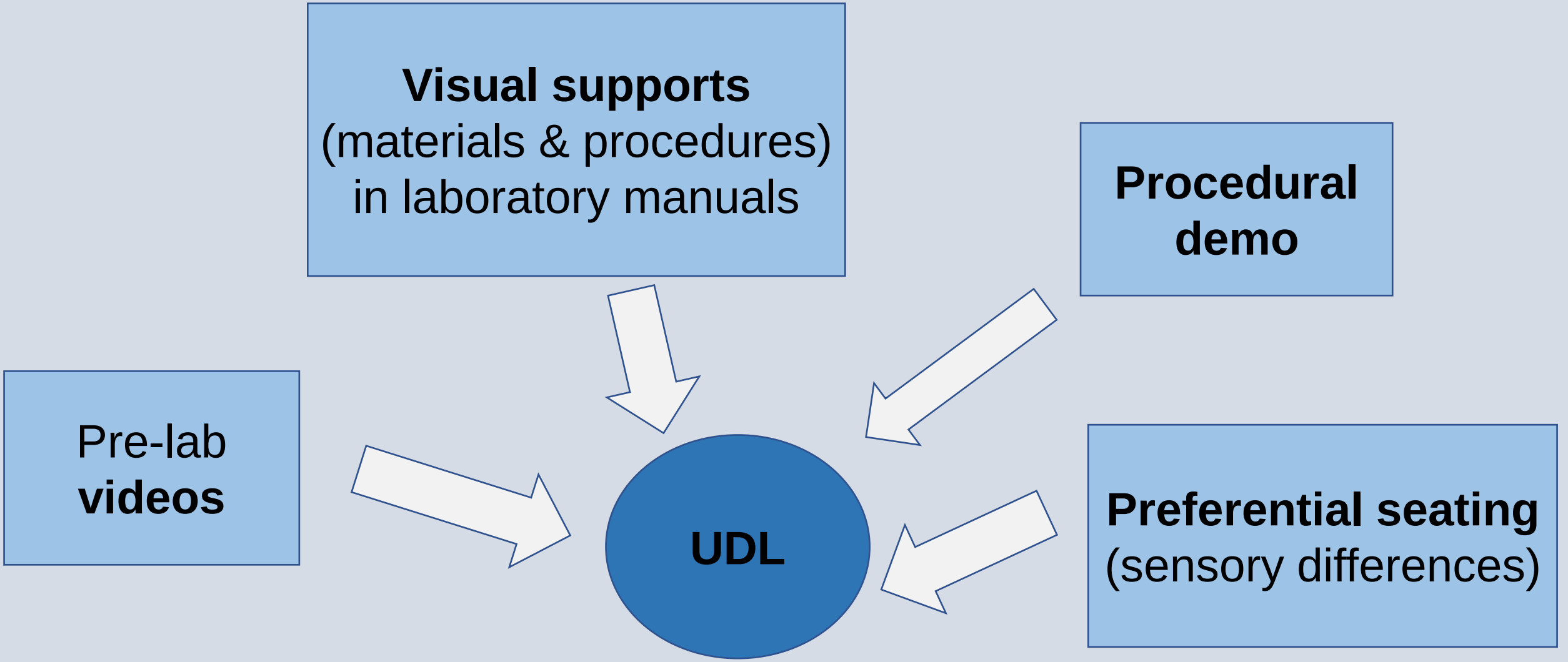
- Preferential seating
- Pre-lab videos (captions and audio)
- Demonstrations
- Multi modal instructions with scaffolding
- Equipment labels/graphics of materials and equipment
- Breaks/indicators of time allocation
- Electronic lab notebooks (ELNs)
- Address safety procedures for students with sensory and mobility abilities

Aims & Objectives:

- The implementation of a UDL approach to the delivery of science laboratory practical classes

- Increase participation and engagement
- Increase knowledge and understanding
- Increase success…….module, programme, employment, personal

Study Design: Multi-modal



General Lab Safety Rules

Read your manual in advance of the practical so you are familiar with the work you are about to do

PPE to be Worn

- **Do not wear lab coats in the canteen area.** Lab Coats must be fastened, all "hoodies, scarfs etc." to be tucked inside of lab coat. "Howie" lab coat are the recommended type, as they provide additional protection. No Graffiti permitted on coats.
- **Eye protection** (Safety glasses/Goggles) an absolute must, except for microscope use.
- **Adequate footwear** - the entire foot must be covered. Do not wear open-toed mesh or canvas shoes these will absorb chemicals, infectious fluid, and offer no protection against broken glass. Wear leather/synthetic, fluid impermeable.
- **Hand protection** – always wear gloves when handling hazardous substances. Always wash your hands after removing gloves and before leaving the lab.

Tie long hair back. No hair gel, **No** hats, **No** hairspray
No eating, **No** drinking, **No** chewing gum, **No** use of electronic cigarette devices
Turn off all mobile phones (or as instructed). No music players permitted
Do not leave personal belongings (use lockers provided) or **congregate in corridors.**
Follow all written and verbal instructions carefully. Ask if in doubt, never take a chance, many accidents can be prevented by getting advice.
Conduct yourself in a responsible manner at all times. Be alert and proceed with caution
NEVER attend a laboratory session under the influence of alcohol or other drugs.
Inform Lecturer (in confidence) of any relevant illness, disability or a pregnancy that might affect the conduct of your work.
Never work alone in the laboratory. **Never** carry out unauthorized experiments.
Never taste or smell chemicals. Only use spatula for its intended use, never place it in the mouth. Use a fume hood when working with volatile chemicals or as instructed.
Know the location of the emergency exits, first aid kits, eye wash stations, chemical shower, 'sharps bin', fire extinguishers – report anything that is missing.
Report all accidents/incidents/near misses, no matter how small they are.
Label all containers/glassware with a marker. Examine all glassware prior to use, broken glass is a hazard. Carefully dispose of broken glass into designated containers.

Department of Life & Physical Sciences AIT

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Table 3. **Positive control chemicals used in the Ames Assay**

Strain	Without "S9-mix"	With "S9-mix"
TA 98	(NQNO); 2-Nitrofluorene; 4-Nitro-o-phenylenediamne	2-Aminoanthracene
TA 100	Nitroquinoline-N-oxide; Sodium azide	2-Aminoanthracene
TA 97	9-Aminoacridine	2-Aminoanthracene
TA 102	Mitomycin-c; Cumene hydroperoxide	2-Aminoanthracene
E.coli WP2uvrA	Methylmethane sulphonate	2-Aminoanthracene

Practical 2: Ames (Bacterial Reverse Mutation) Assay
Dr. S. Devery

Background:

The Ames assay is a reverse point mutation assay, used to assess the mutagenic potential of chemicals. Chemicals that damage bacterial DNA, and induce mutations, are also likely to cause mutations in mammalian cells. Generally any chemical which can interact with cellular DNA and cause a mutation in the genome may also have carcinogenic potential. About 70% of chemicals screened in the Ames assay are found to be rodent carcinogens. However some non-mutagenic compounds have also been found to be rodent carcinogens and are classified as epigenetic carcinogens.
The principle of the bacterial reverse mutation assay is that it detects chemicals that induce mutations which revert mutations present in the tester strains and restore the functional capability of the bacteria to synthesise an essential amino acid. The revertant bacteria are detected by their ability to grow in the absence of the amino acid required by the parent tester strain (i.e. histidine auxotrophy→ histidine prototrophy). Prototrophic strains of *S. typhimurium* are able to synthesise the essential amino acid histidine, but the TA strains used in this test contain mutations in the histidine operon which impose a requirement for histidine in the growth medium. The most commonly used strains in mutagenicity testing, under toxicity testing legislation, are TA 97a, 98, 100 and 102. All four strains are used for screening to ensure detection of mutations induced by base substitution or frameshift (Table 1).

Practical 2 Assessment of the mutagenic response of *S. typhimurium* strains to diagnostic mutagens.

You are provided with:

S. typhimurium strain TA 98 at ~ 10^9 cells/ml
NQNO (1mg/ml in DMSO)
Glucose
VB salts (4 ml)
Histidine/Biotin 0.5 mM
100% DMSO
Top agar (6ml/tube) x 5 tubes .....per group
Molten agar @55°C (200 ml).....per group
Sterile water (10 ml)
Pipettes (p1000, p200), tips
37°C H_2O Bath
45°C Heating Blocks

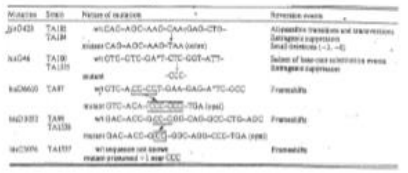
Procedure 1: Preparation of Bottom Agar Minimal Glucose Plates

1. You are provided with 200 ml molten agar maintained at 55°C, to which you add glucose and inorganic salts before pouring.
2. To 10 ml of sterile H_2O add 4g glucose and dissolve. (Solution can be placed at 37°C to facilitate dissolution). Next add to molten agar, continuously shaking to prevent localised precipitation.
3. You are provided with 4 ml VB salts, add to molten agar.
4. Pour the agar to approximately 15 ml volumes, ensuring to obtain a minimum of 15 plates per 200ml agar.

Procedure 2: Preparation of Test Chemical (work within fume hood)

1. Dilute the stock solution of test chemical provided (NQNO) to 4 dose ranges (100µg/ml, 10µg/ml, 1µg/ml, 0.1µg/ml) using DMSO.

Table 1. **Base modification of *S.typhimurium* strains.**



In addition these strains carry other mutations that make them more sensitive to mutagens (Table 2). These include:

1. **The rfa mutation:** causes partial loss of the LPS wall of the bacterium and increases permeability to large molecules.
2. **The UvrB mutation:** this is a deletion of the operon for UV excision repair. This ensures that no repair is induced when a chemical mutagen reverse mutates DNA. Because this deletion has also included the inadvertent deletion of the Biotin operon, Biotin must also be added to the growth medium.
3. **PKM101 Plasmid:** this plasmid was inserted carrying a gene for ampicillin resistance. Its retention by the bacterial strain also indicates genetic stability in the strain.
4. **PAQ1 Plasmid:** this plasmid inserted carries a gene for tetracycline resistance.

Table 2. **Phenotype characterisation of *S.typhimurium* strains**

Strain	Histidine requirement	Rfa mutation	UVrB	PKM101	PAQ1
TA 97	+	+	+	+	-
TA 98	+	+	+	+	-
TA 100	+	+	+	+	-
TA 102	+	+	+	+	+

In the standard protocol a pour-plate incorporation assay is established by pouring a mixture of bacterium in minimal broth, containing histidine, biotin and test chemical. Sufficient histidine is added to encourage bacterial growth for the first 24 h to increase the sensitivity of rapidly dividing DNA to mutagens. Once revertant bacteria are established they are allowed to grow on minimal medium for a further 24-48 h to facilitate colony counting.

The more potent the mutagen the greater the number of revertant colonies. A traditional colony count is determined to establish a dose-response curve for the tested chemical. Since many chemicals are non-mutagenic in their parent form, and require metabolic activation in the liver before their mutagenic and/or carcinogenic potential is expressed, extracts of rat liver enzymes ("S9 mix") are included in the test system as bacteria do not have the same metabolic capabilities as mammals. (***Note***: more recent approaches have also involved genetic engineering of *S. typhimurium* strains to include mammalian CYP450 genes).

To validate an assay each strain must be screened against a diagnostic mutagen or chemical for which there is a well established induced reversion response (i.e. a positive control). For each strain there is a specific diagnostic mutagen with/without "S9 mix" (Table 3).

Procedure 3: Preparation of Top Agar

1. Top agars are provided in 6 ml volumes to be evenly poured over triplicate plates. Place in 45°C water bath to liquefy.
2. To each top agar add: 0.3 ml His/Bio 0.5Mm
   0.3 ml test chemical / solvent (control)
   0.3 ml Test strain
3. Vortex for 3 secs and pour evenly over 3 minimal glucose plates.
4. When agar has set invert plates and incubate for 48-60 h.

Interpretation of Results:

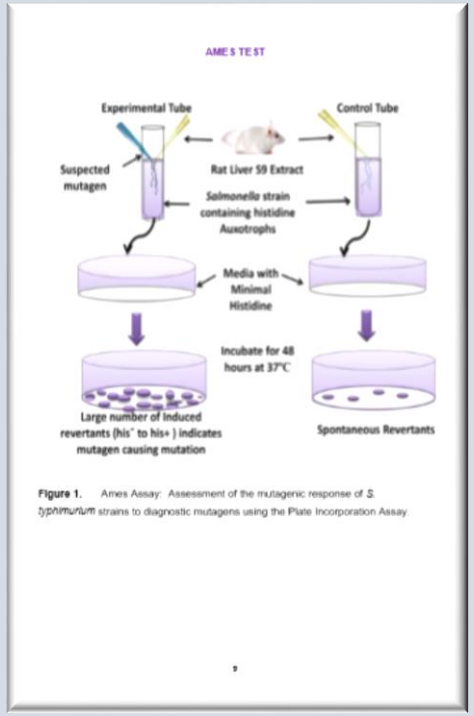
A bacterial lawn of very small, closely spaced, colonies will be present on the negative control plates (i.e. DMSO). These colonies arise from the auxotrophic growth of the tester strains added to the top agar layer (contains histidine). When the histidine is used up the auxotrophic tester strains stop growing.

1. Count the number of spontaneous revertant colonies present on the zero-dose plates (larger colonies varying in size). Get the average value for 3 plates.
2. Count the number of revertant colonies present on treated plates and get the average for each NQNO conc used. Calculate the concentration of NQNO used per plate in the Ames Test.
3. Data should be presented as the number of revertant colonies per plate
4. Also subtract the number of spontaneous revertants from the number of chemically-induced revertants
5. Record the corrected average number of revertant colonies +/- Std. Dev.
6. Establish a dose-response curve by plotting the corrected colony count for each NQNO conc V NQNO conc. Use a point to point graph. Insert SEM error bars. Determine where the [NQNO] gives optimum mutagenicity and the concentration that causes cytotoxicity.

Assignments:

1. Distinguish between mutagens and carcinogens.
2. Define the following terms: mutagen, carcinogen, auxotroph, prototroph, revertant.
3. Name three characteristics of the *S. typhimurium* tester strains that make them useful for mutagenicity testing.
4. What is S9 and why is it used with some test chemicals?
5. What are the advantages of the Ames assay when compared to *in vivo* animal-based carcinogenicity studies.
6. What histidine mutation is present in the TA 98 *S. typhimurium* strain? What is the DNA sequence change?

Inclusion of Visuals: Procedure & Data Analysis


AMES TEST

Experimental Tube

Control Tube

Suspected mutagen

Rat Liver S9 Extract

Salmonella strain containing histidine Auxotrophs

Media with Minimal Histidine

Incubate for 48 hours at 37°C

Large number of induced revertants (his⁻ to his+) indicates mutagen causing mutation

Spontaneous Revertants

Figure 1. Ames Assay: Assessment of the mutagenic response of *S. typhimurium* strains to diagnostic mutagens using the Plate Incorporation Assay.

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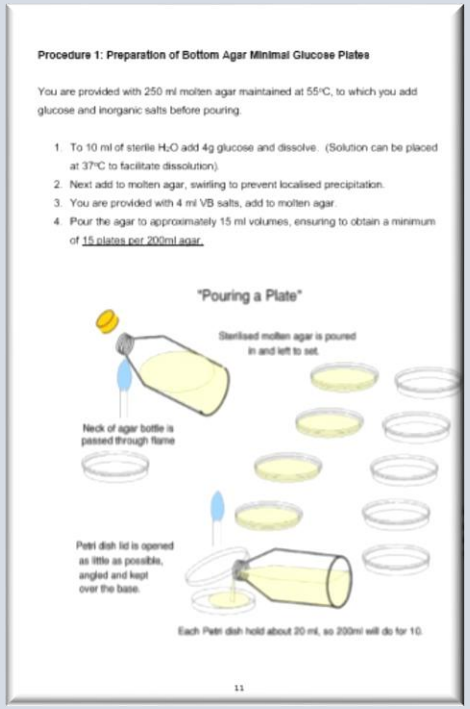
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You are provided with 250 ml molten agar maintained at 55°C, to which you add glucose and inorganic salts before pouring.

1. To 10 ml of sterile H_2O add 4g glucose and dissolve. (Solution can be placed at 37°C to facilitate dissolution).
2. Next add to molten agar, swirling to prevent localised precipitation.
3. You are provided with 4 ml VB salts, add to molten agar.
4. Pour the agar to approximately 15 ml volumes, ensuring to obtain a minimum of 15 plates per 200ml agar.

"Pouring a Plate"

Sterilised molten agar is poured in and left to set.

Neck of agar bottle is passed through flame

Petri dish lid is opened as little as possible, angled and kept over the base.

Each Petri dish hold about 20 ml, so 200ml will do for 10.

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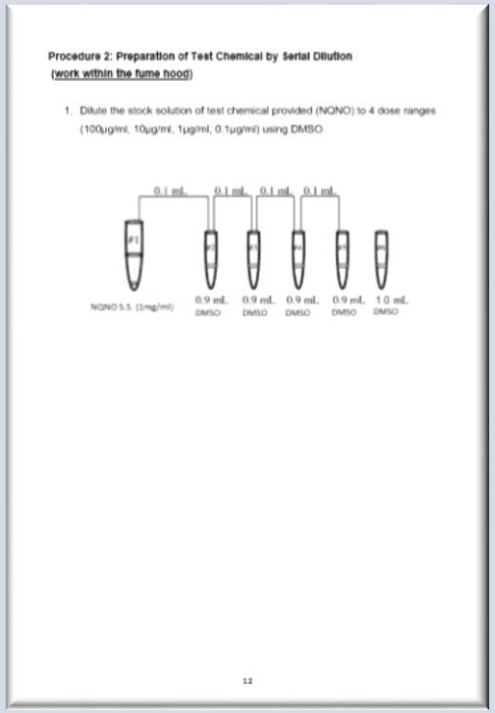
Procedure 2: Preparation of Test Chemical by Serial Dilution
(work within the fume hood)

1. Dilute the stock solution of test chemical provided (NQNO) to 4 dose ranges (100µg/ml, 10µg/ml, 1µg/ml, 0.1µg/ml) using DMSO.

0.1 mL 0.1 mL 0.1 mL 0.1 mL

NQNO 5.5. (1mg/ml)

0.9 mL DMSO | 0.9 mL DMSO | 0.9 mL DMSO | 0.9 mL DMSO | 1.0 mL DMSO

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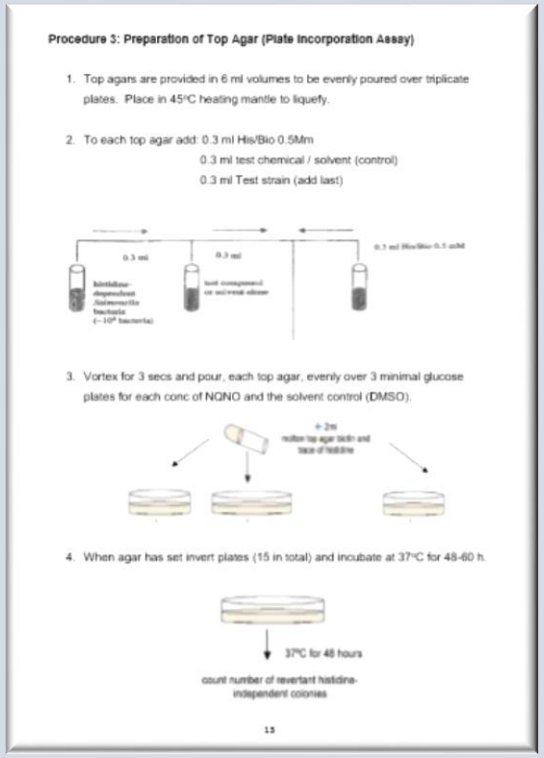
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2. To each top agar add: 0.3 ml His/Bio 0.5Mm
   0.3 ml test chemical / solvent (control)
   0.3 ml Test strain (add last)

0.3 ml

0.3 ml

3. Vortex for 3 secs and pour, each top agar, evenly over 3 minimal glucose plates for each conc of NQNO and the solvent control (DMSO).

+ 2ml molten top agar biotin and trace of histidine

4. When agar has set invert plates (15 in total) and incubate at 37°C for 48-60 h.

37°C for 48 hours

count number of revertant histidine-independent colonies

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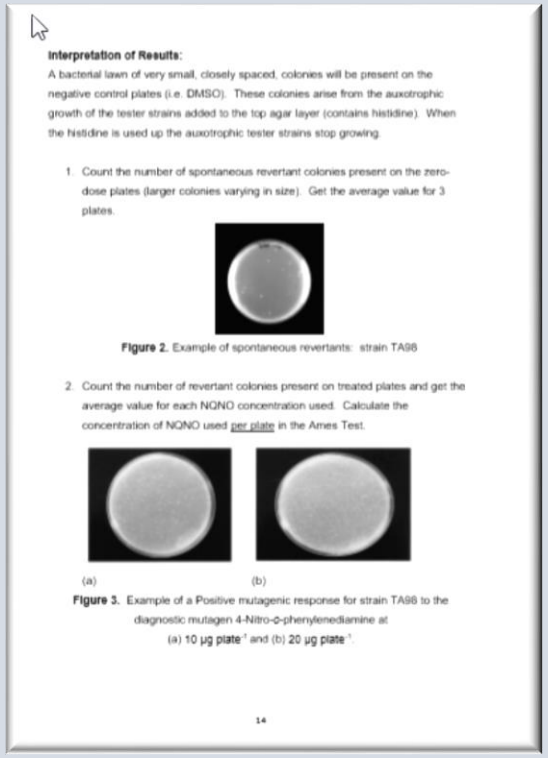
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1. Count the number of spontaneous revertant colonies present on the zero-dose plates (larger colonies varying in size). Get the average value for 3 plates.

Figure 2. Example of spontaneous revertants: strain TA98

2. Count the number of revertant colonies present on treated plates and get the average value for each NQNO concentration used. Calculate the concentration of NQNO used per plate in the Ames Test.

(a) (b)

Figure 3. Example of a Positive mutagenic response for strain TA98 to the diagnostic mutagen 4-Nitro-o-phenylenediamine at (a) 10 µg plate^{-1} and (b) 20 µg plate^{-1}.

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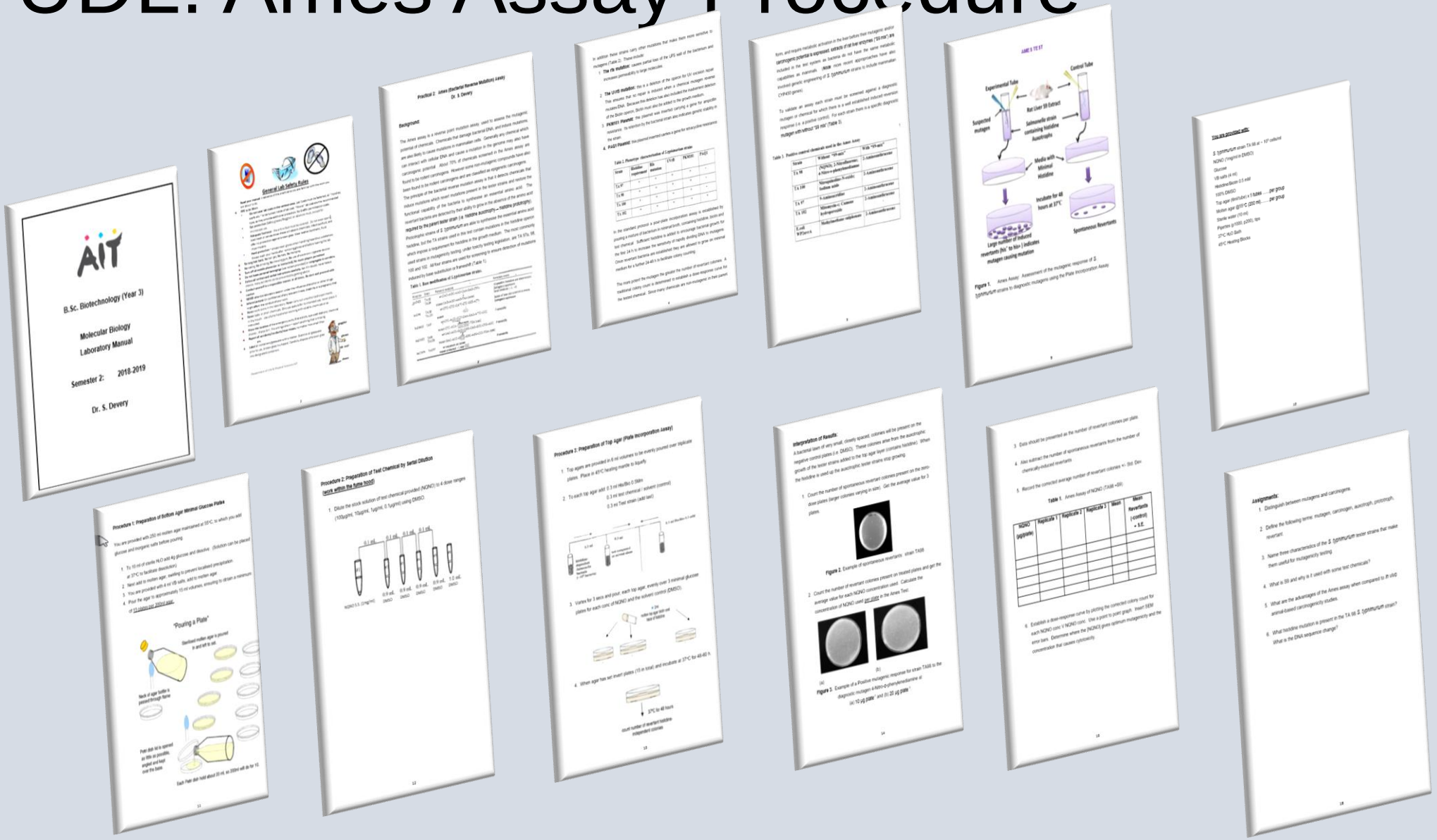
UDL: Ames Assay Procedure



General Lab Safety Rules

- **PPE to be Worn**
  - **Do not wear lab coats in the canteen area.** Lab Coats must be fastened, all "hoodies, scarfs etc." to be tucked inside of lab coat. "Howie" lab coat are the recommended type, as they provide additional protection. No Graffiti permitted on coats.
  - **Eye protection** (Safety glasses/Goggles) an absolute must, except for microscope use.
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- **Turn off all mobile phones (or as instructed), No music players permitted**
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- **Never** work alone in the laboratory. **Never** carry out unauthorized experiments.
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- **Know the location** of the emergency exits, first aid kits, eye wash stations, fire extinguishers – report anything that is missing.
- **Report all accidents/incidents/near misses,** no matter how small they are.
- **Label** all containers/glassware with a marker. Examine all glassware prior to use, broken glass is a hazard. Carefully dispose of broken glass into designated containers.
- **Carefully dispose** of biohazard waste in the provided biohazard bags and **contaminated** needles, glass slides, cover slips, scalpels, capillary tubes etc. into designated puncture proof containers.
- **If a spillage occurs,** inform lecturer /technician immediately, as a quick clean-up is essential. Clean balances after use. Ensure all containers are sealed immediately after use.
- **Leave the lab as you find it.**

logy Year 3

Environmental Biotechnology Laboratory Manual Semester 2

2017

impossible to enumerate all microorganisms by viable plating. This same disadvantage, however, becomes advantageous when one is interested in only a specific microbial population. For example, we can design selective procedures for the enumeration of coliforms and other physiologically defined microbial groups. The viable count is an estimate of the number of cells. Because some organisms exist as pairs or groups and because mixing and shaking of the sample does not always separate all the cells, we actually get a count of the "colony forming units". One cell or group of cells will produce one colony, therefore when we record results for a viable count, it is customary to record the results as colony forming units per ml (cfu/ml) or per gram (cfu/g) of test material.

Another drawback to accurate microbial quantitation using viable plate counts, even with normally culturable species, is that a proportion of these organisms may exist at any one time within a sample in a viable but non-culturable state (VNBC). It was discovered in 1982 that *Escherichia coli* and *Vibrio cholerae* cells could enter this distinct state (Xuetal., 1982) and since then many bacterial species have been found to persist in this state for periods of time. Unlike normal cells that are culturable on suitable media and develop into colonies, VBNC cells are living cells that have lost the ability to grow on routine media on which they normally grow (Oliver,2000). VNBC cells are impossible to enumerate using viable count methods but may still pose a risk to human health, for example in contaminated drinking water.

Since the 1970's, epifluorescence microscopy has become the major technique for direct and accurate enumeration of total numbers of microbes in water and soil samples. In principle, a known amount of water or homogenized soil suspension is placed on a known area of a microscope slide, the microscopes are stained with a fluorescent dye and numbers tallied with a microscope (Bloem & Vos, 2004). For total microial counts in water and soil samples, nucleic acid binding dyes such as acridine orange (AO) and 4',6-diamino-2-phenylindole (DAPI) have commonly been used to stain cells. Other fluorescent dyes have gaining popularity in recent years including SYBR Gold. This nucleic acid gel stain is one of the best cyanine dyes, characterized by high affinity to nucleic acids and large fluorescence enhancement upon binding and providing high effectiveness of DNA and RNA visualization (Kirsanov et al., 2010). SYBR Gold nucleic acid gel stain, has two fluorescence excitation maxima when bound to DNA, one centered at 300 nm and one at 495 nm (Tuna *et al.*, 1999). While essentially nonfluorescent in its unbound state the fluorescence emission maximum of

Viable Plate Count Calculations:

$$N = \frac{a \times 10}{b}$$

N is the number of CFUs from the dilution
a is the total number of colonies counted (30-300)
b is the number of plates counted

For CFUs present in the original sample (per ml or g of sample) don't forget to account for your dilution factors.

C. Staining the water and soil serial dilutions with a nucleic acid stain and filtering for enumeration using epifluorescent miscroscopy.

(**NOTE:** UV light is damaging, do not look directly at the beam from the microscope.)

For the water and soil samples:

1) Add 200 ul of Sybr Gold (10X stock solution in TE buffer pH 8) to all of the serial dilutions.
2) Shake to mix and leave to incubate for 5 minutes.
3) Set up a support filter (GF/C) on the filtration stand.
4) Using a forceps place a black Isopore® membrane filter directly on top of the support filter, shiny side up.
5) Attach the filter column directly over the Isopore® membrane filter and secure in place with a clamp.
6) Add the full volume of stained diluent to be filtered and turn on the vacuum to filter. For the water sample filter the 10^{-1} and 10^{-2} dilutions; for the soil sample filter the 10^{-4} and 10^{-5} dilutions). (**NOTE**: depending on the microbial load within the samples it may be necessary to make up, stain and filter other dilutions).
7) Add 5 ml of water and immediately filter this volume through.
8) Add a drop of oil to the middle of a slide, carefully remove the black Isopore® membrane filter and place gently on top of the oil drop, shiny side up.
9) Add a drop of oil on top of the Isopore® membrane filter and gently place a coverslip on top, avoiding trapping air bubbles.
10) Finally add a drop of oil on top of the coverslip and view under the 100X objective lens (filter 2).
11) Count the number of bacteria within a single field and move at random to another field to repeat the process.

12) Continue until at least 300 cells have been counted

Total Microbial Enumeration Calculations:

$$N = \frac{B \times E}{A \times C \times D}$$

N is the number of bacteria in the dilution.
A is the total volume of sample filtered (ml).
B is the total number of bacteria counted.
C is the number of fields counted.
D is the area of a field (mm^2), measured with a stage micrometer. 0.186 for this microscope.
E is the area of the filter (mm^2) used for filtration. 176 mm^2.

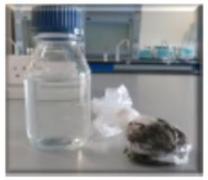
For a count of microbes present in the original sample (per ml or g of sample) don't forget to account for your dilution factors.

D. Recording and comparing results from both methods

Set up a lab archives notebook from the link on the Environmental Biotechnology Moodle page and fill in your results.

Inclusion ... als ... als a... uctions

Required Materials


Water and Soil sample


Filtration set up

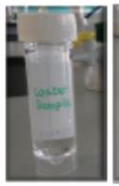
Whatman


Support filter quarters


Isopore Membrane Filters


Filters (s


Vortex


Saline dilutions


Agar plates


Pipette, tips, Sybr safe DNA stain


Glass Slides, Coverslips


Forceps an

6

C. Staining the water and soil serial dilutions with a nucleic acid stain and filtering for enumeration using epifluorescent microscopy.


Filtration set up

Add 200 µl Sybr Gold per sample


Invert sample and leave 5 mins.

Wet supporting filter


Place wet supporting filter on column. Remove isopore filter with shiny side up.


Place isopore filter on centre of supporting filter. Gently place glass column on top.

13


Gently secure column with clamp and secure vacuum. Add stained sample.


Turn on suction and allow sample to completely filter through. Gently remove column.


Add drop oil to centre of glass slide. Remove filter carefully to slide.


Drop of oil on filter. Add coverslip avoiding bubbles. Drop oil on coverslip. Slide is now ready to visualize under UV light (epifluorescent microscope)

See page 15 for further details of this procedure.

14

B. Performing viable plate counts from the water and soil serial dilutions.


Wearing PPE, ensure work space is clear and clean. Carefully light bunsen.


Set pipette for 100 µl.

Mix dilution and remove 100 µl.


Pipette on nutrient agar plate.

Spread sample using aseptic technique.

Label plates and incubate upside-down at room temperature for 4 days.

Spread 3 plates per dilution. For water samples 10^{-1} to 10^{-3} dilutions; for soil samples 10^{-2} to 10^{-4} dilutions. See page 12 for further details of the procedure.

11

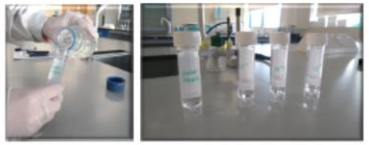
Procedures

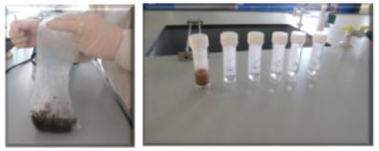
A. Generating serial dilutions from samples of water and soil (10^{-1}-10^{-6})

Generate serial dilutions of the water sample (10^{-1} to 10^{-3}) and the soil sample (10^{-1} to 10^{-6})


Wear PPE and ensure work area is clear and clean.

Summary water sample dilutions:


Summary soil sample dilutions:


7

Generating dilutions: Soil Sample Procedure

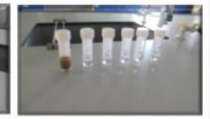

Zero balance with weigh boat.

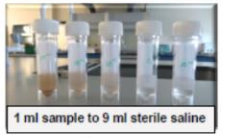

Start to weigh out soil.


Weigh 1g of soil.


Add soil to 9 ml sterile saline.


Vortex soil vigorously


Prepare serial dilutions 10^{-1} to 10^{-6}



Generate 5 serial dilutions (10^{-1} to 10^{-6}) of the original soil sample.

See page 10 for further details of this procedure.

9

Generating dilutions: Water Sample Procedure

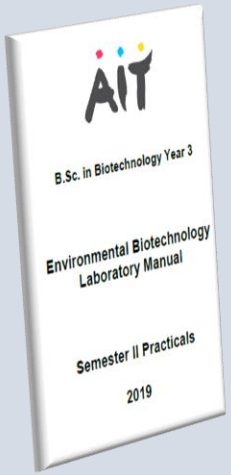
Generate 3 serial dilutions (10^{-1} to 10^{-3}) of the original water sample.

See Page 10 for further details of this procedure.

8

UDL: Great Plat Cou And y cedure

A


AIT

B.Sc. in Biotechnology Year 3

Environmental Biotechnology Laboratory Manual

Semester II Practicals

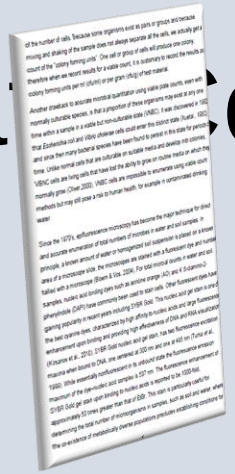
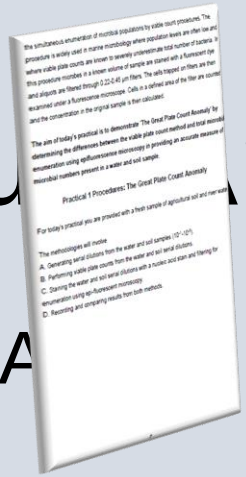
2019


General Lab Safety Rules


Practical 1: Thursday 14.02.19

Dr Gethin Kavanagh

The Great Plate Count Anomaly

Required Materials



Procedure

A. Generating serial dilutions from samples of water and soil (10-1-10-6)

Generate serial dilutions of the water sample (10-1 to 10-6) and the soil sample (10-1 to 10-6

Wear PPE and ensure work area is clear and clean.

Summary water sample dilutions:

Summary soil sample dilutions:

Generating dilutions: Water Sample Procedure

Water sample

Remove 1 ml

Take 9 ml sterile saline

Add 1 ml water sample to 9 ml sterile saline and mix (10-1 dilution)

Generate 3 serial dilutions (10-2 to 10-4) of the original water sample.

See Page 10 for further details of this procedure.


Generating dilutions: Soil Sample Procedure

Zero balance with weigh boat

Start to weigh out soil.

Weigh 1g of soil.

Add soil to 9 ml sterile saline.

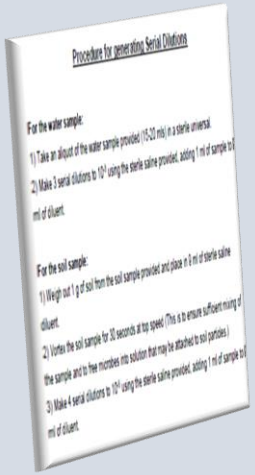
Vortex soil vigorously

Prepare serial dilutions 10-2 to 10-4

1 ml sample to 9 ml sterile saline

Generate 3 serial dilutions (10-2 to 10-4) of the original soil sample.

See page 10 for further details of this procedure.


Procedure for generating Serial Dilutions

For the water sample:

1) Take an aliquot of the water sample provided (10-20 ml) in a sterile universal.

2) Make 3 serial dilutions to 10-3 using the sterile saline provided, adding 1 ml of sample to 9 ml of diluent.

For the soil sample:

1) Weigh out 1 g of soil from the soil sample provided and place in 9 ml of sterile saline diluent.

2) Vortex the soil sample for 30 seconds at top speed (This is to ensure sufficient mixing of the sample and to free microbes into solution that may be attached to soil particles.)

3) Make 4 serial dilutions to 10-4 using the sterile saline provided, adding 1 ml of sample to 9 ml of diluent.


B. Performing viable plate counts from the water and soil serial dilutions

Wearing PPE, ensure work space is clear and clean. Carefully light flambe.

Set pipette for 100 µl.

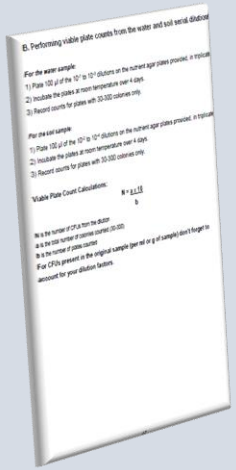
Mix dilution and remove 100 µl.

Pipette on nutrient agar plate.

Spread sample using aseptic technique.

Label plates and incubate upside-down at room temperature for 4 days.

Spread 3 plates per dilution. For water samples, 10-2 to 10-4 dilutions, for soil samples 10-3 to 10-5 dilutions. See page 12 for further details of the procedure.


B. Performing viable plate counts from the water and soil serial dilutions

For the water sample:

1) Plate 100 µl of the 10-3 to 10-5 dilutions on the nutrient agar plates provided, in triplicate.

2) Incubate the plates at room temperature over 4 days.

3) Record counts for plates with 30-300 colonies only.

For the soil sample:

1) Plate 100 µl of the 10-4 to 10-6 dilutions on the nutrient agar plates provided, in triplicate.

2) Incubate the plates at room temperature over 4 days.

3) Record counts for plates with 30-300 colonies only.

Viable Plate Count Calculations:

$$N = \frac{n \times 10}{b}$$

N is the number of CFUs from the dilutor

n is the total number of colonies counted (30-300)

b is the number of plates counted

For CFUs present in the original sample (per ml or g of sample) don't forget to account for your dilution factors.


C. Staining the water and soil serial dilutions with a reactive acid stain and filtering for enumeration using epifluorescent microscopy.

Filtration set up

Add 200 µl of Sybr Gold per sample

Invert sample and leave 5 mins.

Wet supporting filter

Place wet supporting filter on column. Remove isotone filter with shiny side up

Place tongue filter on centre of supporting filter. Gently place glass column on top


Gently secure column with clamp and secure vacuum. Add stained sample.

Turn on suction and allow sample to completely filter through. Gently remove column

Add drop oil to centre of glass slide. Remove filter carefully to slide.

Drop of oil on filter. Add coverslip avoiding bubbles. Drop oil on coverslip.

Slide is now ready to visualise under UV light (epifluorescent microscope).

See page 13 for further details of this procedure.


C. Staining the water and soil serial dilutions with a reactive acid stain and filtering for enumeration using epifluorescent microscopy.

NOTE: UV light is damaging, do not look directly at the beam from the microscope.

For the water and soil samples:

1) Add 200 µl of Sybr Gold (10X stock solution in TE buffer pH 8) to all of the serial dilutions.

2) Shake to mix and leave to incubate for 5 minutes.

3) Set up a support filter (SFC) on the filtration stand.

4) Using a forceps place a black isopore® membrane filter directly on top of the support filter, shiny side up.

5) Attach the filter column directly over the isopore® membrane filter and secure in place with a clamp.

6) Add the full volume of stained diluent to the filtered and turn on the vacuum to filter. For the water sample filter the 10-3 and 10-4 dilutions. For the soil sample filter the 10-4 and 10-5 dilutions. (NOTE: depending on the microbial load within the samples it may be necessary to make up, stain and filter other dilutions).

7) Add 5 ml of water and immediately filter this volume through.

8) Add a drop of oil to the middle of a slide, carefully remove the black isopore® membrane filter and place gently on top of the isopore® membrane filter and gently place a coverslip on top, avoiding trapping air bubbles.

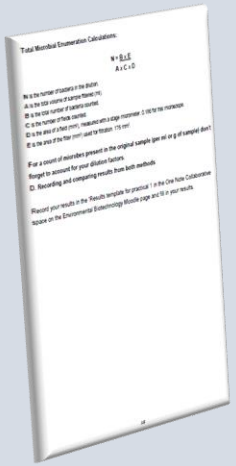
9) Add a drop of oil on top of the coverslip and view under the 100X objective lens.

10) Finally add a drop of oil on top of the coverslip and view under the 100X objective lens.

(Filter 2)

11) Count the number of bacteria within a single field and move all random to another field and repeat the process.

12) Continue until at least 300 cells have been counted


Total Microbial Enumeration Calculations:

$$N = \frac{B \times E}{A \times C \times D}$$

N is the number of bacteria in the dilutor

A is the total volume of sample filtered (ml)

B is the total number of bacteria counted

C is the number of fields counted

D is the area of a field (mm2), measured with a stage micrometer (1 mm for me microscope

E is the area of the filter (mm2) used for filtration. (15 mm)

For a count of microbes present in the original sample (per ml or g of sample) don't forget to account for your dilution factors.

D. Recording and comparing results from both methods

Record your results in the Results template for practical 1 in the One Note Collaborative space on the Environmental Biotechnology Moodle page and fill in your results.

Audience Participation:

Custom made Pre-prac Video:

- Demonstration video on the preparation of a gel filtration column

❑Captions ✓

❑Audio description ✓

- https://www.youtube.com/watch?v=9lSd0v0LI9Y#action=share

Findings: UDL Student Feedback

"Listening to the student voice"

Q1. The laboratory procedures with graphic support were clear and of high quality?

- I strongly disagree
- I disagree
- I agree
- I strongly agree

Q2. The images made it easier to understand and follow the laboratory procedure?

- I strongly disagree
- I disagree
- I agree
- I strongly agree

Q3. Do you consider including graphic images of the materials and reagents used was helpful to you?

- Yes
- No

Q4. What did you think about the number of images in the protocols?

- There were too many images, I found it confusing.
- The number of images used was fine.
- There were too few images, it was difficult to follow the whole method.

Q5. Do you find it easier to carry out a protocol by reading written instructions, listening to an instruction or by having access to a graphic or visual image?

- I like to have written instructions only.
- I find that listening to instructions is better for me.
- Having access to a graphic or visual image is better for me.
- I like having access to text, visuals and oral instruction.

Q6. Would you recommend laboratory procedures with graphic support are used in general across all labs?

- Yes
- No
- Maybe for some labs but not all.

Q7. Did you watch the accompanying video(s) and if you did were they beneficial?

- I didn't watch the videos.
- I watched the videos but didn't find them helpful.
- I watched the videos and found them helpful.

Q8. Did the graphic supports make it easier for you to understand the important concepts under investigation and help with analysis and interpretation of your data?

- Yes.
- No

Q9. Do you have any other suggestions on how we could improve lab manuals/protocols in the future?

Include a short note on what each step is actu
doing i.e when ad
certain reagent
brief note on
reagent

Perhaps the inclusion of model results in the lab manual would be useful. It could aid with the explanation of results by knowing what to look out for

emonstrations at
ning of the lab
a long pre-
k, also more
the board.

Conclusions:

- This **multi-modal instructional delivery** approach:
  - ❑facilitates the implementation of a **proactive UDL** style **into existing laboratory classes.**
  - ❑supports **access and participation for all** students in science laboratories (Q2 – 92% agreed/strongly agreed)
- The majority of **students' prefer a mixed-format delivery** (Q5 – 58% strongly agree)

Conclusions:

- **Graphic supports** also help **increase students' understanding** of concepts investigated and data obtained (Q8 – 83% strongly agreed)

  - ❑Thereby contributing to increased knowledge and attainment.

- The **majority** of students would **recommend laboratory procedures with graphic support** to be used in general across all labs (Q6 – 83%)

Future Work:

- Quantitative evaluation of effectiveness.
- Adapt graphic supports (materials, procedures, scaffolding, time allocation and breaks) to more laboratory procedures across modules and programmes.
  - ❑Inclusion of ‘typical’ example data to aid understanding ✓
- Create more in-house pre-lab videos……marks allocated for engagement.
  - ❑More procedural demonstrations at the beginning of labs

Future Work:

- Expand accommodations for sensory differences.
- Apply to practical-based assessments (with cross-modular implementation)…….ELNs ✓
- Encourage the use of a multi-modal, UDL, approach to the delivery of practical classes across faculties and as per Institute Strategic Plan.

“Clearing the route for diverse learners clears the route for all”

References:

1. National Plan for Equity of Access to Higher Education 2015-2019, available: http://hea.ie/policy/national-access-plan/ [Last accessed 15/1/19].
2. HEA, Student Record System (SRS), 2012-2014.
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5. Middletown Centre for Autism Best Practice Resource, Strengths and Skills in Students with Autism, available: http://best-practice.middletownautism.com/ [Last accessed 28/2/19].
6. Making Science Labs Accessible to Students with Disabilities, Burgstahler, (2012) available: https://www.washington.edu/doit/sites/default/files/atoms/files/Making-Science-Labs-Accessible-Students-Disabilities.pdf [Last accessed 20/1/19].

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7. Creating an accessible science laboratory environment for students with disabilities, available: http://www.accessiblecampus.ca/wp-content/uploads/2017/01/Creating-an-Accessible-Science-Laboratory-Environment.pdf, [Last accessed 28/2/19].
8. Universal Design in Higher Education: From Principles to Practice (2008), Burgstahler, S., Cory, R.C. (Eds), Cambridge, M.A.: Harvard Education Press.
9. Universal Design for Learning Applied to Science Curriculum, available: https://www.montgomeryschoolsmd.org/departments/hiat/udl/Science_UDL_Intro.pdf, [Last accessed 20/2/19].

Thank You!