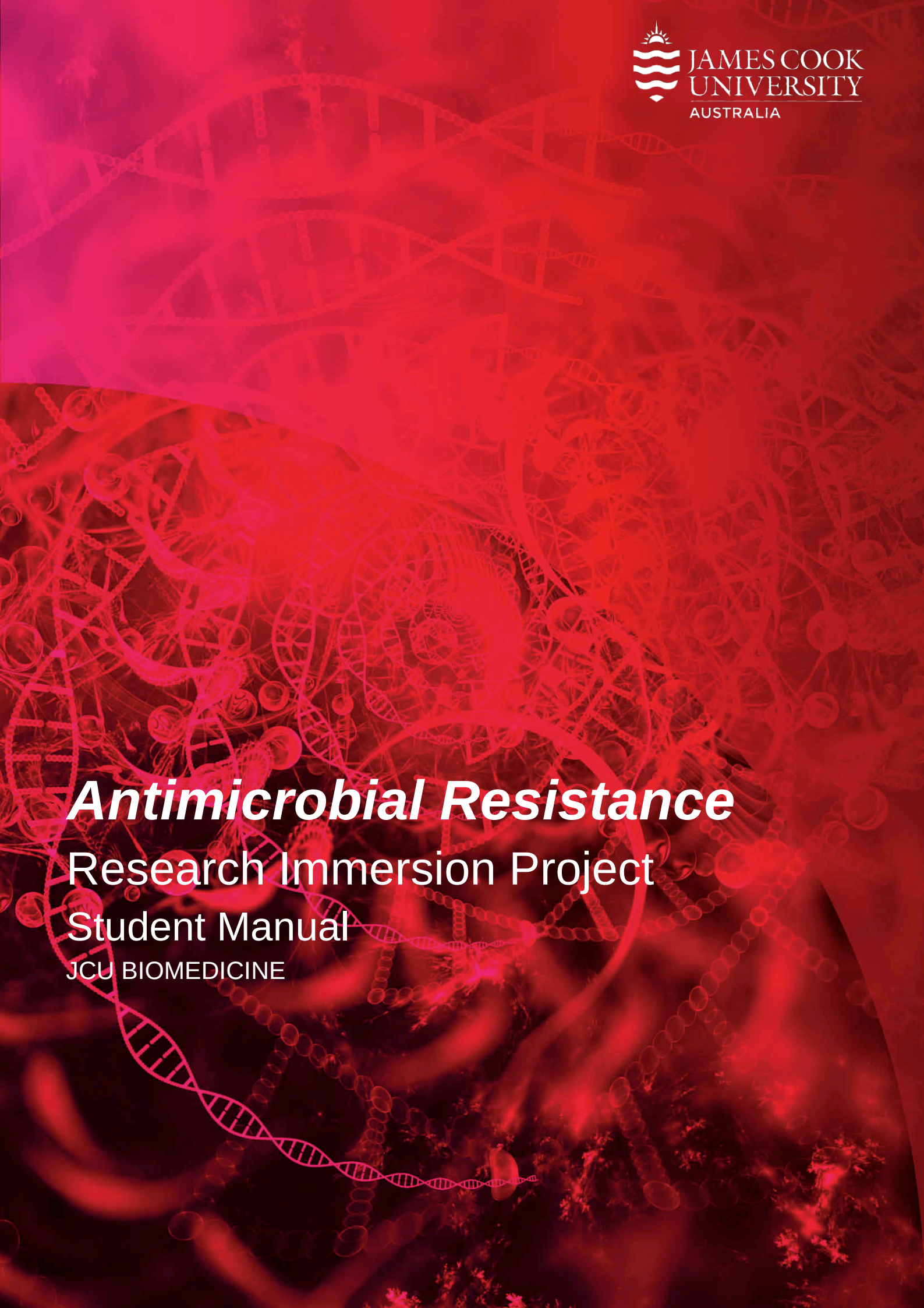

JAMES COOK UNIVERSITY
AUSTRALIA

Antimicrobial Resistance

Research Immersion Project

Student Manual

JCU BIOMEDICINE

Student name:

School:

Supervisor:

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Introduction

Timetable

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<tr><td>9:00AM</td><td>Welcome</td><td>Tutorial: Scientific Literature</td><td>Project Work</td><td>Project Work</td><td rowspan="2">Presentations</td></tr>
<tr><td>10:30AM</td><td colspan="4">Morning Tea</td></tr>
<tr><td rowspan="2">11:00AM</td><td>Precinct Tour</td><td rowspan="2">Project Work</td><td rowspan="2">Project Work</td><td>Project Work</td><td>Awards</td></tr>
<tr><td>Tutorial: Experimental Design</td><td>Preparation for Presentation</td><td rowspan="3">BBQ</td></tr>
<tr><td>1:00PM</td><td colspan="4">Lunch</td></tr>
<tr><td rowspan="2">2:00PM</td><td rowspan="2">Question Time with Academic & Supervisors</td><td rowspan="2">Project Work</td><td rowspan="2">Tutorial: Science Communication</td><td>Preparation for Presentation</td></tr>
<tr><td>Research Symposium</td><td rowspan="2">Finished</td></tr>
<tr><td>4:00PM</td><td colspan="4">Finish Time</td></tr>
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Expectations

Have a go!
Read the manual
Complete the lab work
Clean up after yourself
Do extra online research into your topic
Create a group PowerPoint presentation about your project & experience.

The Project

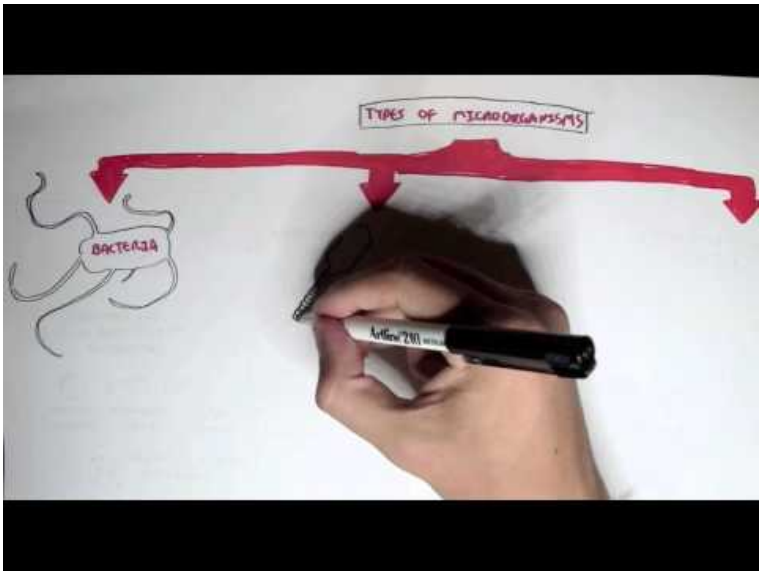
What is Biomedicine?

BIOMEDICINE = MEDICAL SCIENCE+ BIOLOGICAL SCIENCE

The discipline of Biomedicine is a multidisciplinary hub within the College of Public Health, Medical & Veterinary Sciences, in the Division of Tropical Health and Medicine at James Cook University. Biomedicine is a branch of life sciences which closes the gap between natural sciences and industry by applying principles from biology and other science disciplines. Biomedicine graduates contribute to the diagnosis and treatment of disease, by working in academic research, or the medical industry. The aim of JCU Biomedicine is to bring together the North Queensland community, researchers in biology and medicine, clinicians and health workers from across the northern region. The result being collaborate research has implications for the prevention, detection or treatment of diseases relevant to Indigenous health and our tropical communities.

What is Microbiology?

Microbiology is a core branch of Biomedicine at James Cook University. It is the study of microorganisms emcompassing bacteria, viruses, fungi, algae, and other eukaryotic organisms. Microorganisms play a major role in our daily lives, sometimes for good, aiding digestion and sometimes for bad, causing **infectious diseases**. Our understanding of these organisms are directly linked to the control and prevention of infectious diseases. **Immunology** plays a key role in understanding how humans and animals respond to the challenge of these disease-causing organisms. During your time here you will participate in a process that will broaden your understanding for alternative treatments for infectious diseases. Feel free to watch the below YouTube video “Microbiology – overview” to gain an in depth introduction to Microbiology and have a look at Samantha’s Biomedicine at JCU experience.



Research Immersion Project

The threat of bacterial resistance to antibiotics requires new approaches in antimicrobial development. The use of metal and metalloid complexes to treat bacterial infection were extensively used before the rise of antibiotics in the 1940s. Although not a novel approach, there have been promising advances in this field.

Metal complexes that were once considered as anticancer compounds are currently being investigated for their potential as antimicrobials. Past studies under the supervision of Associate Professor Jeff Warner into metal based agents, particularly compounds incorporating ruthenium complexes show promise with low inhibitory and bactericidal concentrations, while maintaining low toxicity in eukaryotic cell lines. However, these complexes in *in vivo* studies show poor tolerability in murine models, indicating the need for future research.

Recent studies into synthetic ligands with lanthanide show potential as future antimicrobials with **toxicity** concentrations significantly higher than the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)**.** Certian ruthenium and lanthanide compounds feature preferential activity against Gram negative bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa*, which may suggest inhibitory mechanisms that are unaffected by membrane bound active efflux pumps; a key antimicrobial resistance feature of P. aeruginosa. The objective of this proposed study is to investigate the lanthanide compounds as potential antimicrobials in regards to their *in vitro* efficacy against pathogens and *in vivo* tolerability and kinetics in a murine model.

The Aim of your project is to:

- Determine the *in vitro* activity of newly synthesised metal based compounds against a range of bacteria including Gram negative, Gram positive and Mycobacteria in a micro broth minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assay.

Antimicrobial Resistance

What is antimicrobial resistance?

Antimicrobial resistance (AMR) is the ability of a microorganism (bacteria, viruses and parasites etc.) to stop an antimicrobial (such as antibiotics) from working against it. As a result, standard medical treatments become ineffective and infections persist. Health care professionals are left with limited or in some instances, no available treatment options.

While AMR is a natural phenomenon, certain human actions have accelerated this process of increased resistance. The single most powerful contributor to resistance is the global unrestrained use of antibiotics. The misuse in both human and animal health and in agriculture; overused, incorrectly prescribed, cutting short duration of treatment time etc.

Why is AMR a problem?

AMR is an urgent global health priority, with the World Health Organization (WHO) describing it as a looming crisis in which common and treatable infections are becoming life threatening. Resistance is increasing at a pace that exceeds the pharmaceutical industry's capacity to develop new antimicrobial drugs.

In human health, AMR infections can necessitate additional investigations, more complex and expensive treatments, longer hospital stays and lead to greater mortality. Estimated costs of AMR have largely focussed on the increased financial costs of longer hospital stays. However, to accurately predict the potential cost of AMR, estimates also need to take into account other factors, such as the impact that an absence of effective antimicrobials poses for modern medicine. Surgery, intensive care, organ transplants and cancer treatment are only possible with effective antimicrobials.

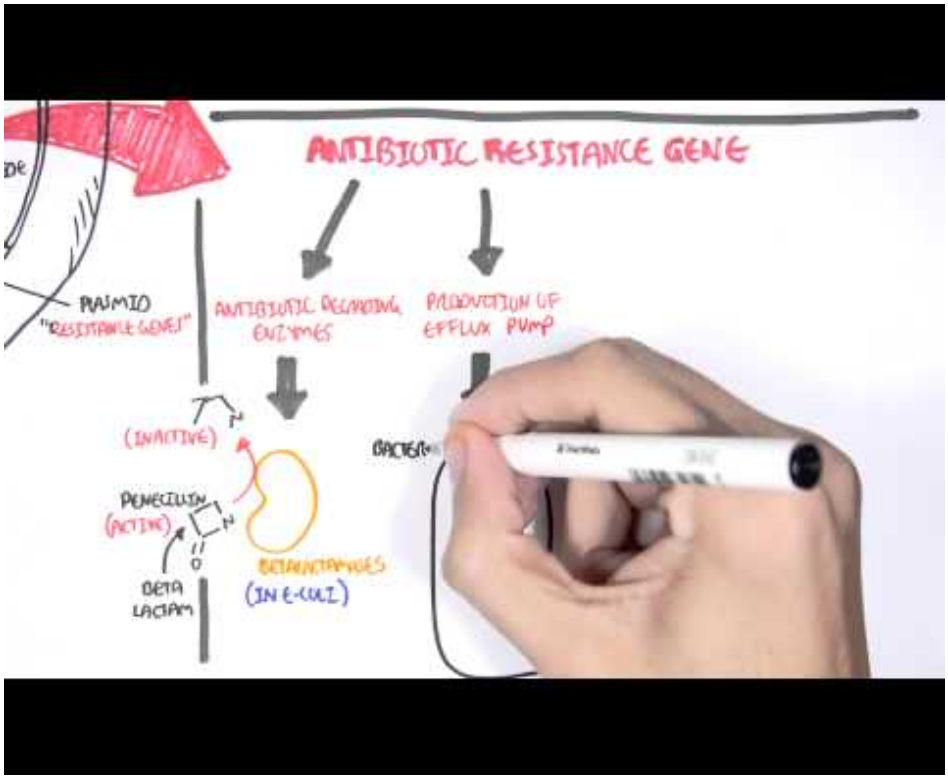
In animals, AMR infections result in reduced animal health, welfare, biosecurity and production outcomes. A major concern of AMR in organisms affecting animals is that these AMR organisms can be transferred to humans through contact, handling or consumption. The risk of AMR is also increased if animal products have been sold before the antibiotic withholding period; antibiotics introduced through food products.

Recommended reading: Chan, M. Combat drug resistance: no action today means no cure tomorrow, World Health Day 2011, 6 April 2011. World Health Organisation.
http://www.who.int/mediacentre/news/statements/2011/whd_20110407/en/

Watch the following videos to gain a better understanding of antimicrobial resistance.

TED TALK - Rise of the Super Bug

Microbiology – Bateria Antibiotic Resistance



What are you going to be doing?

Please see the subsequent *Common Techniques in Microbiology* for more in depth detail on how to conduct each step:

1. Synthesize metal compounds to use as antimicrobial treatment (will be provided by Supervisor).
2. Prepare culture and diagnostic media for bacteria.
3. Conduct MIC and MBC assays to quantitatively establish compound **efficacy** against selected bacteria.
4. Examine the result and make conclusions about the compounds potentials as antimicrobial treatment.
5. Give a PowerPoint presentation on your findings & experience in the program (refer to Appendix B for Presentation Outline).

***Activity**: Information about the Bacteria*

Complete the table below to gain an understanding of the bacteria you will be working with:

Bacteria	**Abbreviation**	**Description**
Escherichia coli		
Pseudomonas aeruginosa		
Staphylococcus aureus		
Methicillin Resistant *S. aureus*		

Common Techniques in Microbiology

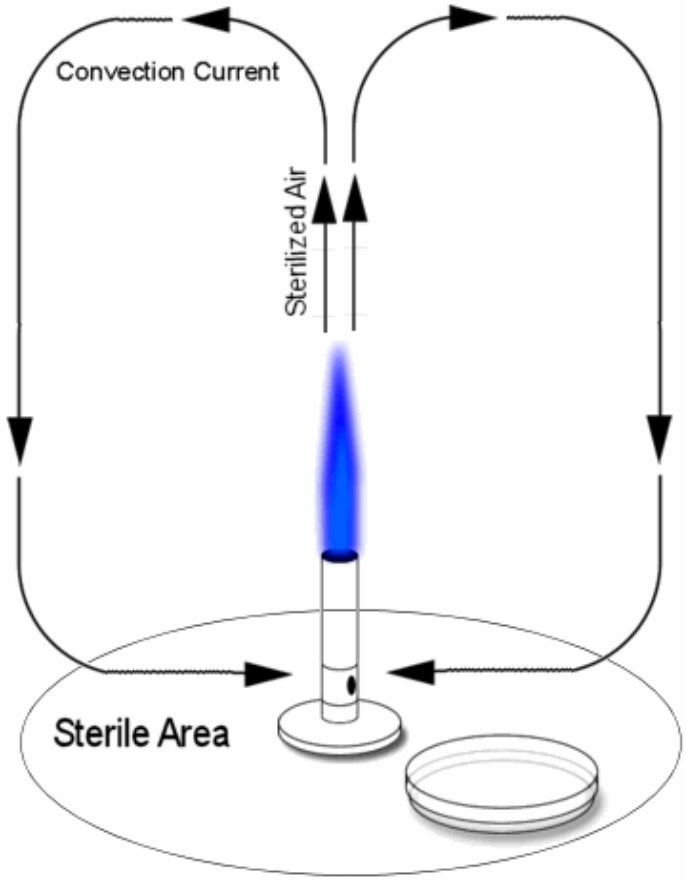
Aseptic Technique

Microbiologists use aseptic technique for a variety of procedures such as transferring cultures, inoculating media, isolation of pure cultures, and for performing microbiological tests (MIC or MBC). Proper aseptic technique prevents contamination of cultures from foreign bacteria common in the environment, and prevents infection of the user.

There are three environments you can conduct aseptic technique:

1. P1 Biosafety Cabinet - Laminar Flow Hood: protects media inside the hood from contamination but not the user (Please refer to Appendix D for SOP for Laminar Flow).
2. P2 Biosafety Cabinet: protects media from outside contamination and the user from any infection material inside the cabinet (Please refer to Appendix D for SOP for P2 Biosafety Cabinet).
3. Open bench using a Bunsen burner & the **zone of sterility** (Please refer to Appendix D for SOP for Bunsen burner).

The most commonly used option for students is a Bunsen burner and the zone of sterility (sterile area pictured below). A zone of sterile air is created via convection currents within 15cm radius of the Bunsen burner while the blue flame is in use. Keeping culture, media and any other vessel close as possible to the Bunsen. Working quickly and accurately is essential to maintaining all items sterile.



While working in the zone of sterility it is essential that you use sterile glassware and instruments. In the discipline of Biomedicine the glassware is **Autoclaved** sterilised and instruments such as **inoculation loops** or dissection needle can be sterilised using heat treatment. Heat sterilization is used both with the Bunsen burner and autoclave is because it kills microorganisms resistant to boiling water and detergents.

For the sterilisation of instruments the air hole control should be open to give a hot blue flame. With the metal handle held at 45degree the loop should be placed in the hottest part of the flame and then draw the loop upwards as when it turns cherry red. Do not leave the loop in the flame for an extend period of time. The loop will now be sterile. To maintain sterility keep instrument within the zone of sterility, do not place flat on the bench, place in an instrument holder or angled off the bench so the loop does not come in contact with contaminants, such as the bench.

To sterilise glassware your supervisor will show you the general physical process for Autoclaving. In theory an autoclave is a pressure cooker. It heats the items: glassware, equipment, instruments, or media, to 121°C under pressure for a certain period of time until sterile.

Culturing Micro-Organisms

Before you can grow a microorganism you need to prepare the necessary mixture of nutrients in a suitable media.

Complex media are rich in nutrients, they contain water soluble extracts of plant or animal tissue (e.g., enzymatically digested animal proteins such as peptone and tryptone). Usually a sugar, often glucose is added to serve as the main carbon and energy source. The combination of extracts and sugar creates a medium which is rich in minerals and organic nutrients, but since the exact composition is unknown, the medium is called complex.

Defined media are media composed of pure ingredients in carefully measured concentrations dissolved in double distilled water i.e., the exact chemical composition of the medium is known. Typically, they contain a simple sugar as the carbon and energy source, an inorganic nitrogen source, various mineral salts and if necessary growth factors (purified amino acids, vitamins, purines and pyrimidines).

Selective/differential media are media based on either of the two categories above supplemented with growth-promoting or growth-inhibiting additives. The additives may be species- or organism-selective (e.g., a specific substrate, or an inhibitor such as cyclohexamide (artidione) which inhibits all eukaryotic growth and is typically used to prevent fungal growth in mixed cultures).

It is a simple task:

1. Add ingredients at desired concentration together.
2. Add specified water to your media.
3. pH the media if required.

4. Boil to dissolve ingredients with lid firmly shut.
5. Then autoclave to be sterilised. Always make sure your media has autoclave indicator tape labeled with your initials, date and type of media (broth or agar). Make sure the lid is loose, close it all the way than half a turn backwards. This is to insure the steam can get into the bottle and the bottle doesn't explode under pressure.
6. Dependent on the end state of the media – liquid or solid, and the culturing technique will determine what glassware or vessel you will place the media into to culture.
   a. Liquid or ***Broth***: you can place it in conical flasks, small bottles or any container that is sterilised prior to use.
   b. Solid in petri dish – ***Plate***: for media that solidifies when cooled can be poured into petri dishes using aseptic technique inside a P1 biosafety cabinet or laminar flow (Please refer to Appendix D for SOP of Laminar Flow).
   c. Solid in small bottles – ***Slopes****:* for media that solidifies when cooled can be poured into small bottles on an angle using aseptic technique inside a P1 biosafety cabinet or laminar flow (Please refer to Appendix D for SOP of Laminar Flow). The angle allows the media surface to solidify on an angle.

Sub-Culturing

Streak Plate Method:

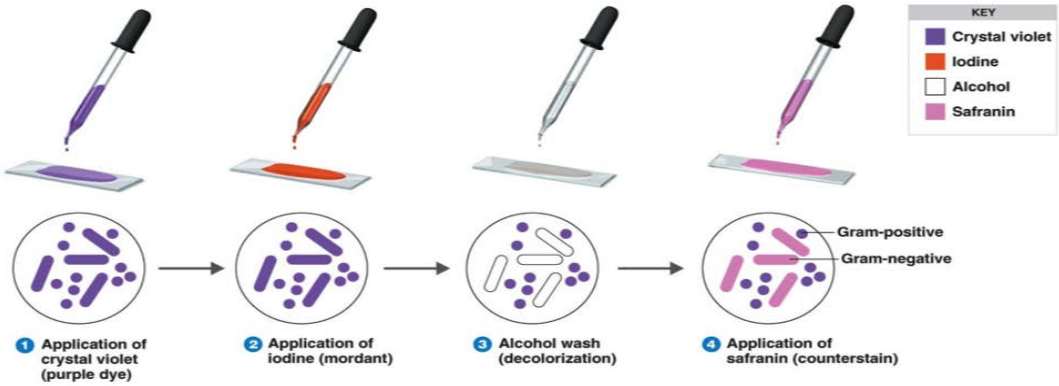
1. Have both plates (overnight culture and fresh agar plate) agar side up on the bench inside the zone of sterility.
2. Pick up overnight culture plate so lid stays on bench, use a sterile loop to pick up a single colony. Replace plate on lid.
3. Pick up new plate so lid stays on bench, gently rub the loop back and forth over 1/5 of the plate. This is your source streak.
4. Sterilise loop, allow to cool, then pull 4 lines (one direction only) away from the source streak.
5. Sterilise loop, repeat the process twice more, pulling lines away from previous streak area.
6. Last steak is a zig zag through previous streak area, be careful not to touch inoculated areas.

Inoculating Broth

1. Label the liquid medium with your name, the date and the organism that you are sub-culturing.
2. Place the previously cultured agar plate upside down on the bench so that the lid is on the bench.
3. Hold the agar plate within the zone of sterility leaving the lid on the bench.
4. Using a sterile loop pick a single colony of bacteria from the plate.
5. Replace the agar plate lid.
6. Remove the lid of the liquid medium bottle and retain in the hand avoiding contamination.
7. Sterilise the top of the bottle by waving it through the hottest part of the flame.
8. Tilt bottle on its side and rub inoculation loop on the side of the bottle low enough the media will cover it.
9. Twist the inoculated loop back and forth within the liquid medium, fairly vigorously to remove all remaining bacterial cells from the wire loop.
10. Sterilise the top of the bottle by waving it through the hottest part of the flame.
11. Replace the lid.
12. Re-sterilise the loop.

Gram Staining

Gram staining is a common technique used to differentiate two large groups of bacteria based on their different cell wall constituents. The Gram stain procedure distinguishes between Gram positive and Gram negative groups by colouring these cells red or violet. In microbiology, gram staining is an important test used because it can determine the presence of bacteria in a sample.



1. Add a loop of saline to a slide.
2. Then add a small amount of your bacteria of interest using a sterile loop.
3. Mix with the saline until you have a small film of bacteria on the slide.
4. Fix bacteria to the slide by running it through the flame 3 times (smear side facing up) or dip in methanol.
5. Place you prepared side in the staining area.
6. Add Crystal violet (60sec).
7. Tip off excess then add iodine (60 secs).
8. Tip off excess, then rinse the slide.
9. Add decolouriser (10 secs), then rinse the slide.
10. Add counter stain Safranin (60secs), then rinse the slide.

Antimicrobial Susceptibility Testing

Minimum Inhibitor Concentration (MIC)

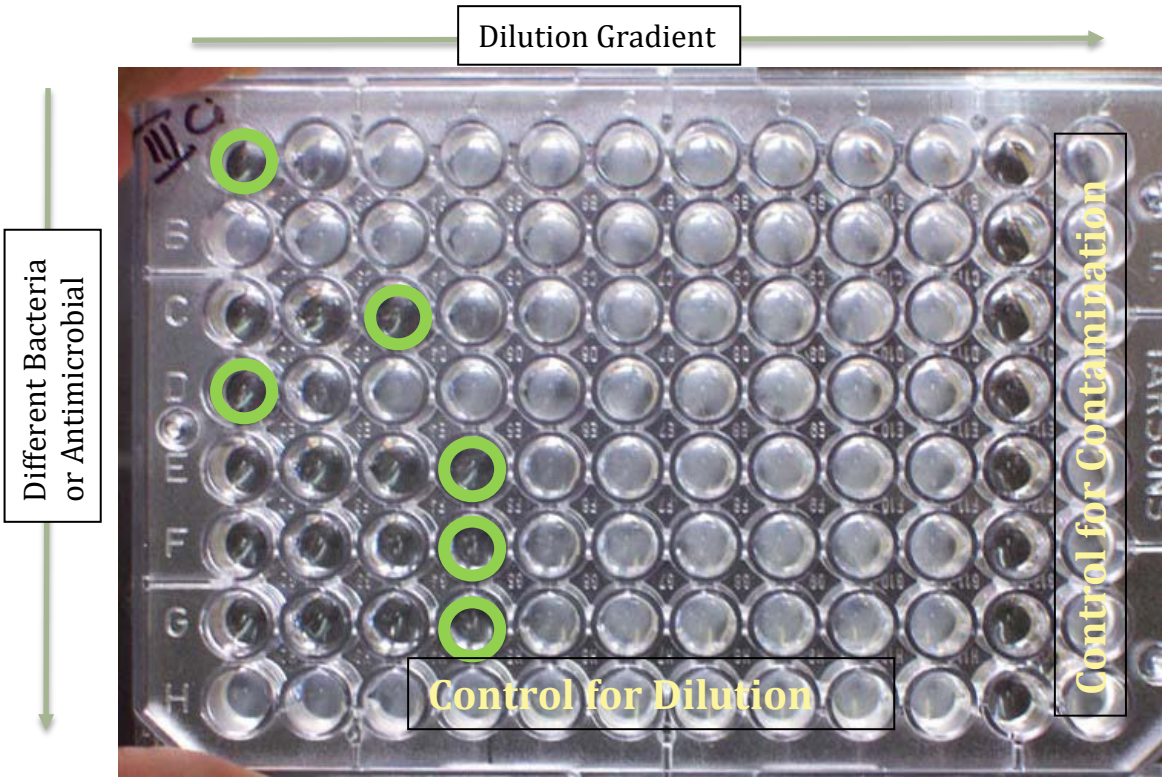
In microbiology, minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial (like an antifungal, antibiotic or bacteriostatic) drug that will inhibit the visible growth of a microorganism after overnight incubation. MICs can be determined on plates of solid growth medium (Kirby-Bauer Disk Susceptibility Test) or broth dilution methods after a pure culture is isolated. For example, to identify the MIC via broth dilution, overnight culture of bacteria is added to wells of liquid media containing progressively lower concentrations of the drug. The minimum inhibitory concentration of the antibiotic is between the concentrations of the last well in which no bacteria grew and the next lower dose, which allowed bacterial growth.

1. In a 96 well-plate add 180ul of Mueller Hinton (MH) broth to column 1. Number of rows filled in column 2 depends on number of bacterial isolate being tested or antimicrobial per bacteria.
2. Add 20ul of antimicrobial (metal compound solution) to corresponding wells used in in column 1.

   Total volume in well = 200ul
3. Add 100ul of MH broth to all other wells required across the plate.
4. Perform two fold dilutions across the plate:
   a. Remove 100µl from well A1 and place into A2.
   b. Remove 100µl from A2 and place into A3.
   c. Continue this until you reach well A7 or the number of dilutions you wish to make.
   d. Discard the last 100µl from the last well.
5. Repeat the dilution process (step 5) for every row of bacteria being testing and control wells.
6. Make a suspension of the isolate in a bijoux of sterile saline to 0.5 McFarland (just visibly turbid). Your supervisor will demonstrate this.
7. Use the remaining 0.5 McFarland suspensions (created in instruction 1 of Section 1) to complete the following instructions.
   a. Place 100ul of the 0.5 McFarland for the microorganism you are testing into 1000ul of MH broth.
   b. Repeat for each microorganism.
   c. Place 100ul of bacterial suspension into each well on the corresponding row in the 96 well plate.

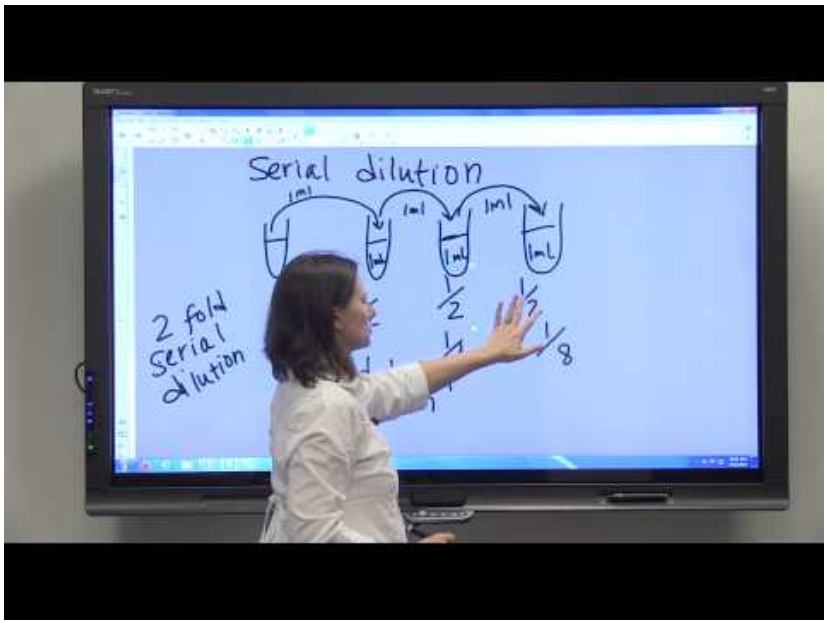
Total per well = 300ul

8. Incubate for 24-48hrs at 37°C.
9. Following incubation, inspect each well for turbidity, the lowest concentration that completely inhibits visible growth of the organism is recorded as the MIC figure (green rings below).

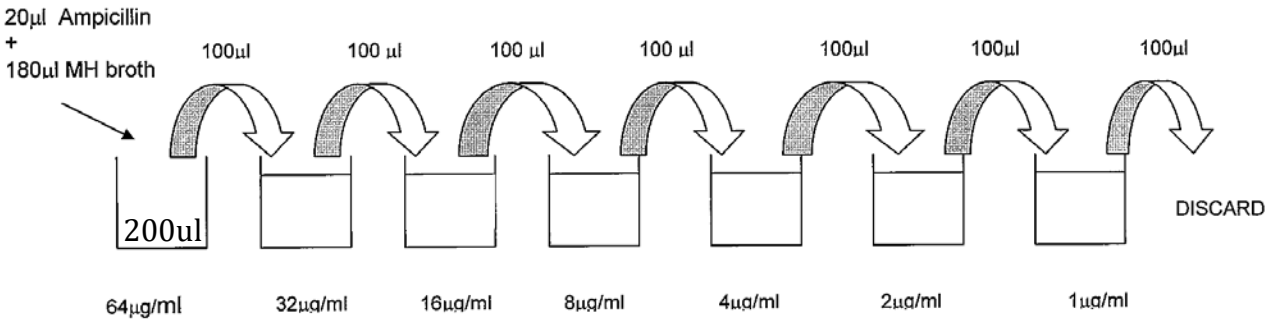


Dilutions

If you need help understanding how to calculate the value of dilution in the MIC above please watch this video and then have a go at calculating the concentrations used in your experiment.



For example for Ampicillin:



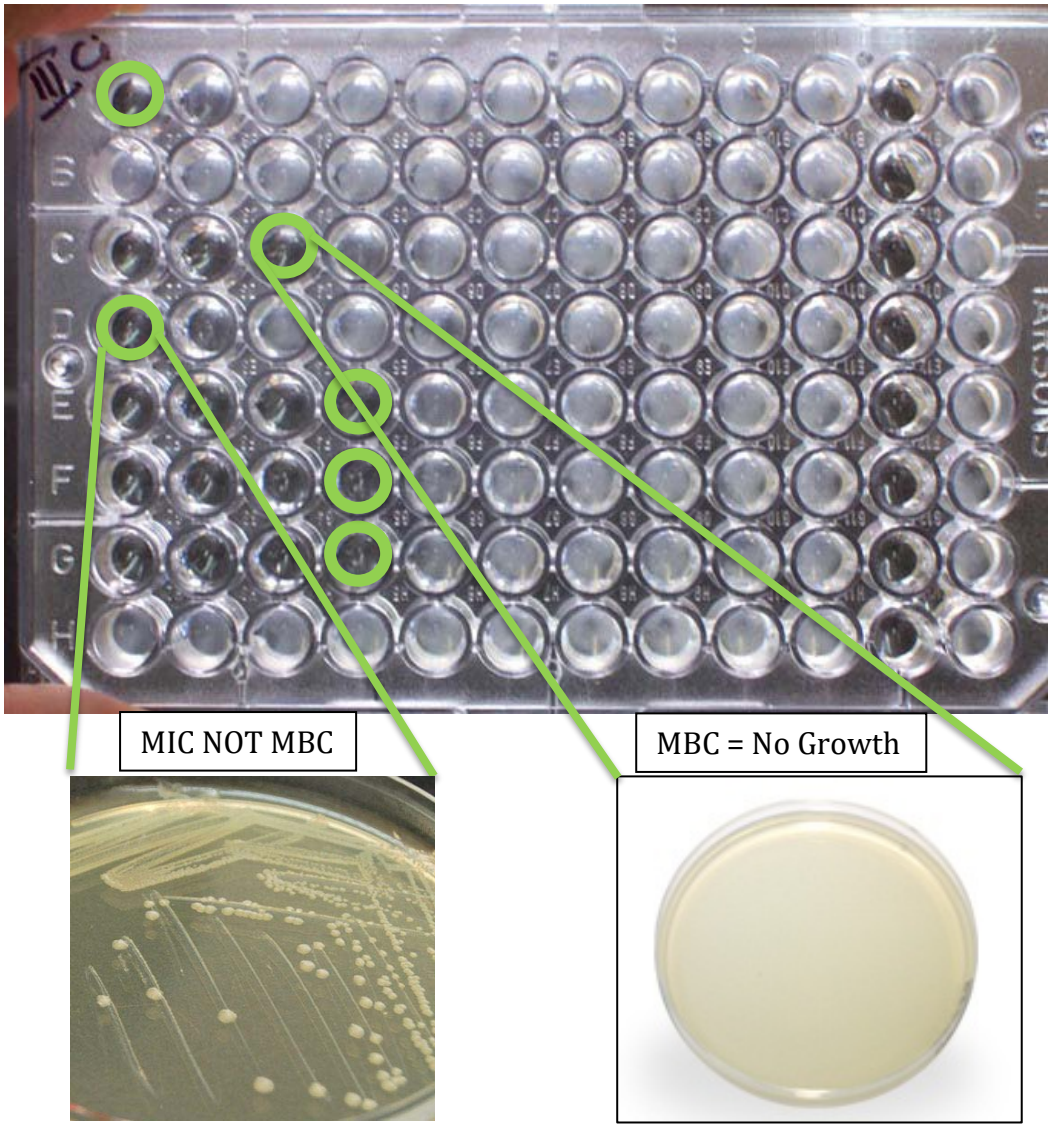
Concentration of Antimicrobial	**Well 1**	**Well 2**	**Well 3**	**Well 4**	**Well 5**	**Well 6**	**Well 7**	**Well 8**

Minimum Bactericidal Concentration (MBC)

The MBC test determines the lowest concentration at which an antimicrobial agent will kill a particular microorganism. It can be determined from broth dilution minimum inhibitory concentration (MIC) tests by subculturing to agar plates that do not contain the test agent.

The MBC is complementary to the MIC; whereas the MIC test demonstrates the lowest level of antimicrobial agent that inhibits growth, the MBC demonstrates the lowest level of antimicrobial agent that results in microbial death. This means that even if a particular MIC shows inhibition, plating the bacteria onto agar might still result in organism growing because the antimicrobial did not cause death. Please refer to *Culturing Micro-Organisms* for how to culture an organism.

Example:



Appendix

A – Recommended Readings

1. Chan, M. Combat drug resistance: no action today means no cure tomorrow, World Health Day 2011, 6 April 2011. World Health Organisation.
2. Li F, Collins JG, Keene FR. Ruthenium complexes as antimicrobial agents. Chem Soc Rev. 2015;44(8):2529-42.
3. Munro N. Antimicrobial Resistance: Thinking Outside the Box. AACN Adv Crit Care. 2015;26(3):225-30; quiz 31-2.
4. One Health Global Network: One Health: a concept that became an approach and then a movement.
5. Primrose S. Transition metal and rare earth metal complexes as potential antibacterial agents. Townsville: James Cook University; 2015.
6. Smith, R. Coast, J. The true cost of antimicrobial resistance. BMJ 2013;346:f1493.
7. Terzi HA, Kulah C, Ciftci IH. The effects of active efflux pumps on antibiotic resistance in Pseudomonas aeruginosa. World J Microbiol Biotechnol. 2014;30(10):2681-7.

B – Presentation Outline

Time: 15min
Group Presentation
Minimum 10 Slides

1. Title slide: your project, your name
2. Introduction
3. Hypothesis and Aim of your research
4. Methods
5. Results
6. Conclusions
7. The Good: tell us what went well
8. The Bad: tell us what didn't go well
9. Questions
10. Reference List & Acknowledgements (supervisors)

Awards will be present at the conclusion of all the presentations.

C – Forms

Informed Consent Forms

James Cook University

TOWNSVILLE Queensland Australia Telephone: (07) 4781 4111

INFORMED CONSENT FROM

PRINCIPAL INVESTIGATOR	Associate Professor Jeff Warner
PROJECT TITLE	*Research Immersion Program*: Antimicrobial Resistance
SCHOOL	Biomedical Sciences
CONTACT DETAILS	Biomedical Sciences Building 87 Room 107 ext. 14748

Student as asked to work with microorganism in a PC2 facility (please see research immersion student manual for full details). Time commitments for this program is 5 days. Information collected in this program is solely for the student to create a PowerPoint presentation and gain research experience.

I agree that if I have a medical condition which might be affected, exacerbated or preclude me I will inform the supervisor in charge and I shall abstain from participation. I am aware that any information I give is confidential.

Do you have or aware of any medical conditions that might be affected, exacerbated or preclude you from participating in the research immersion program (please indicate in the space provided);

Medical conditions: _____

If you have indicated a potential medical condition and you wise to participate you must inform and discuss with your academic supervisor in charge of your project before the undertaking of any task.

The aim of this project have been clearly explained to me.

I understand what is wanted of me.

I know that taking part is this is voluntary and I am aware that I need not participate and can stop at any time without affect my completion of the program.

I have read the research immersion student manual and agree to participate in all activities described in the manual.

I understand that every effort is made to keep my information confidential but this cannot be assured in every case and no names will be used to identify me with this without my approval.

Student name (*printed*):	
Student signature:	Date:
Guardian name (*printed*):	
Guardian signature:	Date:
Relationship to student:	

JAMES COOK UNIVERSITY AUSTRALIA

College of Public Health, Medical & Veterinary Sciences
James Cook University

General PC2 Laboratory Induction for Veterinary & Biomedicine Precinct

You must read and observe these instructions. Please tick beside each instruction to indicate your understanding, sign and date the form.

- ☐ All persons entering the PC2 Laboratory must act in a safe and professional manner at all times.
- ☐ **Fully enclosed shoes**, covering the dorsal surface of the foot to the ankle and heel must be worn at all times in the laboratory.
- ☐ **A laboratory gown must be worn at all times in the laboratory** as this is a requirement of the PC2 laboratory regardless of the nature of the work being completed. Lab gowns must be removed before leaving the laboratory and put on the hooks provided.
- ☐ **Cuts and abrasions**, especially on the hands, must be covered with a waterproof dressing or band-aid prior to entering the laboratory.
- ☐ Disposable gloves must be used if hazardous chemicals, animal tissue, products of animal tissues, and animal or human body fluids are used.
- ☐ Safety glasses must be worn in the PC2 lab if you are instructed to do so.
- ☐ Hair longer than shoulder length must be tied back and loose jewelry removed.
- ☐ Hands must be washed every time you leave the laboratory, except in the case of a fire.
- ☐ All equipment failures are to be reported immediately to the person in charge.
- ☐ **All incidents and hazards must be reported** to the person in charge immediately. An incident report must be generated ASAP.
- ☐ All sharps must be placed in the sharps disposal bins (yellow with red lids).
- ☐ All biohazard waste must be disposed of correctly.
- ☐ All stools must be place under a bench or out of the way when work is completed.
- ☐ If the firm alarm sounds, evacuate the laboratory immediately via the exits and assemble where instructed. Do not return to the building or wander off until advised to by the authorities that it is safe to do so.
- ☐ Be familiar with the location of the safety showers, eye wash and spill kit locations.

The following is **forbidden in the Laboratory**:

- ☐ Eating and drinking, smoking, applying cosmetics
- ☐ Use of mobile phones, calculators and timer functions
- ☐ Bags or handbags, hats

The following is **permitted on the condition** your recognize items bought into the PC2 Lab may become contaminated and used at your own risk: Laptops, IPad, tablets, or other electronic devices.

You will be instructed to leave the facilities if your conduct is deemed dangerous or disruptive.

You will comply with the instructions or direction of university staff.

Name of person inducted (*printed*):	
Signature:	Date:
Supervisor (*printed*):	
Signature:	Date:

Talent Release Form 2017

Full Name:	
Telephone contact:	
Email:	

I (name) _____, hereby consent for James Cook University to use any photograph and/or video footage taken of me or provided by me, whole or in part; recordings of my voice and/or written extraction, whole or in part of such recordings; and to use the information contained therein for any purpose in connection to learning and teaching including but not limited to: study guides, websites, social media and other forms of media.

By signing this form I agree that electronic and/or hard copy of photographic images and/or recordings of me and/or my profile are collected and stored for the purposes above.

I understand that the images and/or recordings of me and/or my profile will only be accessed by James Cook University employees, including persons acting under its permission or authority, such as commissioned agency.

I acknowledge that the information I have provided may be used to contact me; however, my details will not pass on to any third party without your approval.

I waive any right to inspect or approve of the finished product, including written copy that may appear in connection with my images and/or recordings of me and/or profile.

I understand that the use of the images and/or recordings of me and/or my profile does not give me any right to request payment and that no payment will be made to me in return for reproduction of any such image, recording or profile.

I have read and understood the terms of this release.

Student name (*printed*): _____

Student signature: _____ Date: _____

Please complete this section if the model is a minor:

I am the parent or guardian of the minor named in the release above and have leagal authority to execute the above release. I hear by approve the foregoing on behalf of the above named minor.

Guardian name (*printed*): _____

Guardian signature: _____ Date: _____

D – MSDS and SOPs (TO BE COLLATED FROM RISK WARE)

Risk Assessments
- Working in a PC1 Laboratory
- Working in a PC2 Laboratory
- Working with Bacteria or Bacterial Risk Assessment
- Working with Chemicals
- MIC & MBC experiments (MI2011, MI2021)

SOP's
- Using a micropipette
- Using a microscope
- Using a Class II Hood
- Using a Laminar Flow
- Using a Bunsen burner

E – Glossary of Terms

Antimicrobial: is an agent that kills microorganisms or stop their growth.

Antimicrobial resistance (AMR): occurs when microorganisms such as bacteria, viruses, fungi and parasites change in ways that render the medications used to cure the infections they cause ineffective.

Autoclave: a strong heated container used for chemical reactions and other processes using high pressures and temperatures, e.g. steam sterilization.

Efficacy: the ability to produce a desired or intended result.

Efflux pumps: are proteinaceous transporters localised in the cytoplasmic membrane of all kinds of cells.

Eukaryotic cell: An organism whose cells contain a nucleus surrounded by a membrane and whose DNA is bound together by proteins (histones) into chromosomes.

Gram stain: a staining technique for the preliminary identification of bacteria, in which a violet dye is applied, followed by a decolorizing agent and then a red dye. The cell walls of certain bacteria (denoted Gram-positive) retain the first dye and appear violet, while those that lose it (denoted Gram-negative) appear red.

Immunology: the branch of medicine and biology concerned with immunity.

In vitro: performed or taking place in a test tube, culture dish, or elsewhere outside a living organism.

In vivo: performed or taking place in a living organism.

Infectious diseases: are disorders caused by organisms — such as bacteria, viruses, fungi or parasites.

Inoculation loops: is a simple tool used mainly by microbiologists to retrieve an inoculum from a culture of microorganisms. The loop is used in the cultivation of microbes on plates by transferring inoculum for streaking.

Lanthanide: any of the series of fifteen metallic elements from lanthanum to lutetium in the periodic table (atomic numbers 57–71).

Ligands: an ion or molecule attached to a metal atom by coordinate bonding.

Metalloid (semi metal): an element (e.g. arsenic, antimony, or tin) whose properties are intermediate between those of metals and solid non-metals or semiconductors.

Minimum bactericidal concentration (MBC): is the lowest concentration of an antibacterial agent required to kill a particular bacterium.

Minimum inhibitory concentration (MIC): is the lowest concentration of an antimicrobial (like an antifungal, antibiotic or bacteriostatic) drug that will inhibit the visible growth of a microorganism after overnight incubation.

Murine Models: experiments conducted on rodents – rats and mice.

Mycobacteria: a bacterium of a group which includes the causative agents of leprosy and tuberculosis.

Ruthenium: the chemical element of atomic number 44, a hard silvery-white metal of the transition series.

Toxicity: the quality of being toxic or poisonous.

Zone of sterility: is a method designed to prevent contamination from microorganisms, using a Bunsen burner.