BM327 microbiology S1 lab manual

Morgan Feeney

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# Preface

## Introduction to the BM327 Microbiology Semester 1 Lab Manual

This laboratory manual contains key information about each of the five practical laboratory sessions delivered as part of BM327 microbiology semester 1.

### How to use this manual

You can navigate through this manual using the sidebar on the left.

The introductory section is a general section containing helpful information on good laboratory technique, working with microorganisms safely and aseptically, etc. - it also contains general information about the module, including the assessment. You should read through this section before the first lab and refer back to it when needed to refresh your memory.

Each of the experiment sections 1-5 contain a brief explanation of the theory underpinning the experiments we will do in the practical labs, the protocols that will be used in that lab, and an explanation of any data analysis tasks that need to be performed after the lab. Links to the protocols (handouts) for each lab are provided, but please note that you do not need to print these out - they will be provided for you in the laboratories.

### How to prepare for each lab session

You should read through the relevant theory/introductory material before coming to each practical lab session, and make sure you understand it. In some instances you may wish to review the material covered in BM330 before the lab as well (or review the underpinning microbiology theory in a textbook such as [add refs]).

* Each section will have a “To do” box like this, with a summary of the reading/tasks necessary to prepare for the lab.
* The data analysis pages will have a “To do” box too - for the tasks you need to complete after the lab.

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| Tips for success |
| * You will also find boxes throughout this lab manual with Tips, Important notes, Discussion Questions, etc (such as this one). These are intended to help you prepare for each lab (and for the assessment), so you should make sure to read through all the material. * As Benjamin Franklin said, “By failing to prepare, you are preparing to fail.” It is really important that you come prepared to each lab! A little bit of time spent reading through the material and performing any necessary tasks or calculations will really pay off. |

### How to deal with any problems, questions, or concerns

If you have any problems or questions, please feel free to speak with Dr. Feeney during the lab sessions or by e-mail.

If you are going to miss a lab session (for whatever reason), you should e-mail Dr. Feeney and also log your absence with the university using the Pegasus Personal Circumstances tool. More details can be found in your student handbook.

If you notice any errors (including typos, broken links, etc.) in this lab manual, please notify Dr Feeney, either by e-mail or by [raising an issue](https://github.com/mafeeney/BM327microS1/issues/new) on this lab manual’s github page.

# 1. Aim of the BM327 Semester 1 Microbiology laboratory sessions

The aim of this block of laboratory sessions is to introduce students to fundamental molecular biology and microbiology methods, such as UV mutagenesis, plasmid transfer, antibiotic assays and microscopy.

Within the block of five three-hour laboratory sessions, there are five distinct experimental elements. Each experimental element is linked to the others as part of an overall research project. Most of the experimental elements will take place over the course of several different laboratory sections (because of the need to incubate plates and allow microbes to grow). There are also two workshops to supplement your learning – details of these will be made available on MyPlace.

## 1.1 Microbiology Laboratories Competency Skills

Graduate recruiters look for specific job skills and competencies. Competencies are action-oriented statements of the knowledge, skills, and abilities people need to do their work successfully. They are observable and measurable. These are what you need to do the job and work in their organisation. Some are straightforward and required in many different career: communication, teamwork and problem solving are just a few. You need to show employers you have mastered essential skills such as writing, reading, numeracy, presentation, organisation and the ability to work under pressure. Graduates who demonstrate their analytical thinking during a job interview or assessment centre will stand out from other candidates. Other skills such as those gained in the laboratory are more specific and aligned to particular types of jobs e.g. ability to do a PCR.

You will be assessed for your competency in the following:

*Lab 1* \* Performing data analysis

* Performing basic lab calculations, e.g. serial dilutions
* Interpreting phenotypic and genotypic data
* Teamwork
* Keeping detailed and accurate records

*Lab 2* \* Performing data analysis

* Calculating antibiotic MBC and MIC values
* Teamwork
* Keeping detailed and accurate records

*Lab 3* \* Performing data analysis

* Using online databases
* Teamwork
* Keeping detailed and accurate records

*Lab 4* \* Performing calculations

* Performing data analysis
* Formulating hypotheses
* Using databases
* Teamwork
* Keeping detailed and accurate records

*Lab 5* \* Using appropriate software to visualize and analyze microscopy data

* Data analysis
* Data presentation
* Teamwork
* Keeping detailed and accurate records

## 1.2 Overall learning outcomes

* To understand how exposure to UV radiation can affect the ability of microorganisms to survive and produce antibiotics.
* To understand how genetic elements are transferred between bacteria.
* To understand how the susceptibility of bacteria to certain antibiotics is determined.
* To observe how bacterial cells can change their morphology as a result of certain environmental stimuli.

# 2.

**Schedule of experimental tasks**

Because of the nature of microbiology (cultures require incubation and take time to grow), many of your experiments will take place across more than one laboratory session (Figure 1). It is therefore essential that you keep careful track of all experimental tasks: e.g., carefully labelling your cultures and taking care to read all the relevant sections of the lab manual.

Before attending the practical lab, you should ensure that you have read and understood:

* The introductory sections covering [Good Lab Practice](./0goodlab.qmd) and [Aseptic Technique](./0aseptic.qmd)
* The introductory sections covering the theory on [Axenic culture](./1intro1.qmd),
* The [Protocols](./1protocols.qmd) we will be following in the lab

You also must ensure that you have read and signed the safety forms, or you will not be admitted to the lab.

# 3. Formative assessment

## 3.1 Summative assessment: Online Quiz

### 3.1.1 Contribution to overall class assessment

The BM327 semester 1 microbiology assessment comprises 25% of the overall BM327 class assessment.

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| Important |
| IMPORTANT NOTE: A link to all Safety Paperwork (eCOSHH and eRisk Assessments) for each appropriate laboratory will be sent to you in an email a few days before the session. These electronic documents MUST be read ahead of each laboratory and acknowledgement of this indicated by ticking the box that is at the end of the document. This MUST be done at least ONE day ahead of the session. Failure to do this will result in you not being allowed into the laboratory session. A link to the electronic document will also be available on the appropriate BM327 Being a Biomolecular Scientist 3 class sub-page on Myplace.  It is essential that you prepare thoroughly for these labs by reviewing each section and that you understand fully what you are doing in each lab. If in doubt, ask a member of staff. |

# 5. Good Laboratory Practice

# 6. Good Lab Practice

Many of the principles for working in a microbiology lab are the same as those used in any other biomedical research lab. These are transferable skills, which are useful for a number of different careers.

Good lab practice is a cornerstone of biomedical research: it helps to ensure that results are reproducible, and is especially crucial for any clinical or therapeutic work.

## 6.1 Keeping a Laboratory Notebook

It is essential to keep an accurate record of experiments, with details of every experiment performed, the data collected, and the data analysis that was performed. It is just as important to keep a lab notebook for experiments performed in silico as it is for experiments performed at the lab bench.

You should be able to come back to your notebook six months later, read your notes, and be able to recreate the experiment/understand the analyses that you performed. Human memory is fallible and it is important to keep clear, accurate records.

Moreover, an appropriately kept lab notebook is a legal document, important for protecting your intellectual property. You should therefore be in the habit of keeping your lab notebook up to date and accurate.

### 6.1.1 Some guidelines for keeping a good lab notebook:

* Your notebook may be kept in the form of a physical notebook (kept on paper) or an electronic notebook. The former should be written in clearly and legibly, using indelible ink. The latter should be saved often, with backup copies stored securely.
* Include the date for each entry.
* Each experiment should include a title, a hypothesis or aim, a brief description of the background, protocols and details of any reagents/equipment used, observations, and any data or statistical analysis that was performed.
* Any protocols or methods should be described in enough detail that a colleague (or you in six months’ time!) can repeat the same experiment, starting from the same dataset/materials, and reach the same results.
* Any mistakes should be crossed out and explained (not erased).
* All data should always be recorded in your lab notebook (even “failed” experiments or “outliers”).

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| Electronic Lab Notebooks |
| Increasingly, researchers are using electronic lab notebooks instead of paper ones - these are more impervious to physical destruction (e.g., chemical spills, lab fires) and often more convenient (more easily legible, searchable, etc.) However, care must be taken to ensure that the data stored in an electronic lab notebook is secure and backed up.  There are many options for lab notebooks. One popular and widely used electronic lab notebook is Benchling.   * You can join Benchling by creating an account at http://benchling.com/signup * For help with Benchling, including training kits and best practice guides, please see https://help.benchling.com/hc/en-us. |

### 6.1.2 Good Labelling Technique

It is essential that all tubes and plates be clearly and legibly labelled. You will often be working with these over a period of several weeks, and you will be unable to interpret/analyse the results from a previous week’s experiments if you have not labelled your samples correctly.

Some general guidelines for good labeling practice:

* Include your initials and the date of the experiment (if directed by your instructors, also include your bench number).
* If working with microorganisms, always include the species name (abbreviations, e.g. *E. coli* are fine).
* If labeling Petri plates, label around the outside rim of the plate (do not obscure the plate as it will make it hard to count colonies!), as shown in **?@fig-platelabelling**. You should also be sure to label the *bottom* of the plate (**?@fig-platelid**). The bottom and lid may become separated, so labeling the lid is a bad idea!

Label around the rim of the Petri plate - not across the centre Label the bottom of the Petri plate, not the lid

### 6.1.3 Other tips for good lab practice

* Read through each protocol thoroughly before beginning an experiment. Label all tubes and prepare any reagents needed before you begin. Stay organised and keep your space clean.
* Turn off your Bunsen burner (or set it to a visible orange flame) if you are not working aseptically.
* Never walk away from your bench and leave a lit Bunsen burner unattended.
* Never wear gloves when working with a Bunsen burner.
* Pipet as accurately as possible:
  + Use the correct pipet for the volume you intend to pipet and make sure it is set correctly (if uncertain, ask a demonstrator or member of staff)
  + Pipet down to the first stop when drawing up liquid into the pipet; pipet down to the second stop when expelling liquid.
  + Avoid bubbles when pipetting.
  + Only insert the very tip of the pipet into the liquid when pipetting particularly viscous mixtures (e.g., polymerase or enzyme stocks).

# 7. Aseptic Technique

## 7.1 Aseptic Technique

It is **essential** that you work aseptically when working in a microbiology lab. Microbes are ubiquitous in the environment (including in the air, and on your skin). Therefore, you must work aseptically so that your experiment contains only the organism(s) you are working with, and not unwanted contaminants!

Most of the materials you will be working with (pipet tips, microcentrifuge (Eppendorf) tubes, culture media, etc. will have been sterilised for you by autoclaving: the autoclave sterilises these items by subjecting them to pressurised steam (121°C, 15 p.s.i.).

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| Autoclaved items |
| You will be able to recognise that items such as tips or Eppendorf tubes have been autoclaved based on the use of autoclave tape on the containers for these items. Autoclave tape changes colour when subjected to high heat and pressure. It is essential that you use these sterilised items: do not take unsterilised pipet tips or microcentrifuge tubes from the shelves.   |  | | --- | | Figure 7.1: The use of autoclave tape to show that the contents of a container have been sterilised (compared to unautoclaved tape, on the right). Image credit: Wikipedia | |

One of the cornerstone principles of aseptic technique is that any objects, even those that have previously been sterile, can no longer be considered sterile if they come into contact with non-sterile objects. Be careful not to allow your pipet tips to come into contact with any non-sterile surfaces (i.e., your bench top, fingers, the lids or outsides of bottles).

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| Top Tip for Good Aseptic Technique |
| You should use good aseptic technique when working with all containers (including tip boxes, Eppendorf boxes, bottles of media, etc.), to prevent the contents from becoming contaminated.  Containers should be opened for the minimum amount of time possible (the longer they are open, the greater the chance of contamination!) Close them promptly when you have removed the tip/sample.  Tip boxes should **not** be left open. If you leave the box open, microbes present in the air can easily contaminate the tips.  A box of pipet tips that are no longer sterile. Image credit: Starlab You should therefore always: 1. Open tip box –> 2. Take pipet tip –> 3. Close tip box –> 4. Use pipet tip |

### 7.1.1 Working with a Bunsen Burner

In order to keep your work sterile and free from contamination that could interfere with your experiments, you should work near a Bunsen burner set to a blue flame ([Figure 7.2](#fig-bunsen)). The Bunsen creates an updraft of air, helping to maintain a “clean” zone around it. Having the Bunsen burner lit, but working several meters away from this clean zone, is ineffective and will likely result in contamination of your experiments.

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| Figure 7.2: A Bunsen burner set to a blue flame. Image credit: Encyclopedia Britannica |

When working with a Bunsen burner, however, it is essential that you follow the following safety guidelines, for the safety of yourself and those working near you:

* Turn off your Bunsen burner (or set it to a visible orange flame) if you are not actively using it.
* Never walk away from your bench and leave a lit Bunsen burner unattended.
* Never wear gloves when working with a Bunsen burner.

#### 7.1.1.1 Using a platinum inoculating loop

In some cases you will need to use a platinum inoculating loop, e.g. to streak bacteria for single colonies on a new Petri plate.

You must make sure to sterilise your inoculating loop by flaming it (placing the platinum wire in the flame of your Bunsen burner to sterilise it). You should flame your loop both before touching it to a sample (this prevents the transfer of contaminants into your sample) and before putting it down when you have finished using it (so that you do not transfer microbes to the lab bench/other surfaces.

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| Important |
| An inoculating loop that has just been flamed is extremely hot - you should never allow a hot loop to touch your skin. You should also avoid touching a hot loop directly to your samples, as the heat will kill the microbes you are working with. |

The procedure for flaming an inoculating loop is:

1. Place the loop in the flame of the Bunsen burner at an angle, so that the length of the loop lies along the surface between the inner and outer cones in the flame (the hottest part of the flame).
2. Hold the loop in the flame until the entirety of the loop turns cherry-red. It is not necessary to hold the loop there any longer than that: you are not trying to melt the metal!
3. Remove the loop from the flame and allow it to cool, either by holding it in the air for several seconds (in the aseptic working zone near the Bunsen burner), or by touching it to a sterile surface (i.e. the inside of a culture tube or the agar in an uninoculated Petri dish.)

### 7.1.2 Top tips for working with good aseptic technique:

* Work **near** a Bunsen burner set to a blue flame (Many students end up working miles away from their Bunsen, which is not very helpful.)
* Open all containers (including tip boxes, Eppendorf boxes, bottles of media, etc.) for the minimum amount of time possible (the longer they are open, the greater the chance of contamination!) Close them promptly when you have removed the tip/sample. Tip boxes should not be left open.
* Be careful not to allow your pipet tips to come into contact with any non-sterile surfaces (i.e., your bench top, fingers, the lids or outsides of bottles).

# 8. Using a Microscope

## 8.1 Correct use of a microscope

Bacteria are much smaller than anything else you will have looked at under a microscope; most bacterial cells are approximately 1-3 µm long. Optimal set up of the microscope is therefore essential.

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| Safety tip for working with microscopes |
| Please note that you should not wear gloves while working with a microscope: you do not want to inadvertently transfer staining reagents (or other chemicals) onto it. |

You should begin by placing a slide onto the stage and aligning the lowest power objective (usually the 10X objective) over it.

1. Raise the stage using the coarse focusing knob so that the slide is about 5 mm away from the objective.
2. Bring the slide into focus as best you can, first using the coarse focusing knob and then the fine focusing knob. You will be slowly lowering the stage so as to increase the distance between the slide and objective.
3. Once you have focussed as best you can using this objective, you should move on to the next highest power objective.

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| Finding the focal plane |
| Often the plane of focus is extremely narrow, so that small, faint objects like stained bacteria may be easily missed as the focus passes through the plane.  Thus, a good technique is to constantly move the focus up and down while also slowly moving the slide up/down or left/right on the stage. In this way, objects that flash in and out of focus tend to be more easily noticed by the eye. |

At any point while using the microscope, you may wish to:

* Adjust the “interocular separation” of the eyepieces so that they are comfortably aligned with your eyes.
* Adjust the lamp voltage at any time when using a microscope in order to get a comfortable level of light.

### 8.1.1 Use of oil with the microscope:

* The 100x objective is designed to be used with oil (it will have “oil” explicitly written on it), whereas the other objectives are not.
* Never use oil with any objective other than the 100x. Once you have added oil to your sample, never return to using a smaller magnification objective!
* Only a very small amount of oil is needed, only add a tiny drop. Don’t flood the slide!
* When you have found your focus and optimised set-up with the 40x objective, swing the objectives half-way between the 40x and 100x. DO NOT move the stage or change the focus as you do this! Then gently add a very small drop of oil onto the spot of light you can see in your sample. Gently swing the 100x objective into place.
* You will most likely have to adjust the focus a little, but only use the fine adjustment at this stage. Ask for help if you have trouble finding the focus. Under no circumstances go back to the 40x, as it does not tolerate getting in contact to the immersion oil.
* Residual oil will cling to the 100X objective after use, so make sure you clean it off with a lens tissue (specifically designed for this purpose) before putting the objective away. DO NOT use any other tissue to clean the lens.

# 9. Experiment 1 Aims and Overview

## 9.1 Aims

## 9.2 Learning Outcomes

At the end of this laboratory session, you should understand:

## 9.3 Competencies

* Performing data analysis
* Performing basic lab calculations, e.g. serial dilutions
* Interpreting phenotypic and genotypic data
* Teamwork
* Keeping detailed and accurate records

## 9.4 Importance

This laboratory session

## 9.5 Overview of Experiment 1

In this experiment, you will be :

# 10. Experiment 1 - Intro

# 11. Experiment 1 Protocols

You can view the handout for lab 1 below, or download it [here](./handouts/Lab1.pdf).

# 12. Experiment 1 - Data Analysis

After completing all of the practical lab tasks for this experiment, you should:

## 12.1 Task 1A

In the lab session,

### 12.1.1 Task 1A - Evaluating your Streak Plates

### 12.1.2 Task 1A - Quantifying the Number of Microbes in a Sample: Serial Dilutions

It is often necessary or desirable to work backwards from the number of colonies on a plate, in order to work out the number of *viable* microbes that were present in the original (undiluted) sample. (Note that any microbes present in the sample that were dead, or viable-but-not-culturable, will not give rise to colonies on the plate - so you are enumerating the number of viable microbes, not the number of cells.)

We calculate and express the number of viable microbes in the original sample in **colony forming units** (cfu) - usually in cfu/mL.

For example, if you performed a decimal serial dilution, plated 0.1 mL of the 10-4 dilution, and counted 173 colonies on the resulting plate ([Figure 12.1](#fig-serialdilplated)) - how many viable bacterial cells were present in the original culture?

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| Figure 12.1: Examples of plating and counting colonies from a decimal serial dilution |

Here, we had 173 colonies in 0.1 mL of the dilution present in the 10-4 dilution tube.

To calculate the number of cfu/mL present in the original sample, divide the number of colonies on your plate by the total dilution factor: in this case, 173 colonies/(0.1 mL \* 10-4) = 1.73 \* 107 cfu/mL.

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| Top tips for calculating cfu/mL |
| * Make sure you label your plates with the dilution factor - you will need this for the calculations! * Ideally, you want to count colonies on a plate that has 30-300 colonies. We normally consider <30 colonies to be Too Few To Count (TFTC) and more than 300 colonies to be Too Numerous To Count (TNTC). You will get more accurate estimates of your viable cell count if you use a countable plate. * Make sure you use the correct number of significant figures in your answer - you can’t be more precise than your initial measurement (number of colonies counted). * Make sure you express your answer in scientific notation (e.g., 1.73 \* 107 cfu/mL, not 17300000) and include units. * When you’ve finished your calculation, double-check that your answer makes sense! For example, you can’t have a fraction of a colony forming unit - so if your answer looks like 1.73 \* 10-7 cfu/mL, you have probably gone awry with your handling of exponents somewhere. |

## 12.2 Task 1B -

You should annotate your gel (label the DNA ladder and wells with their sample names), as for example in [Figure 12.2](#fig-annotatedgel).

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| Figure 12.2: Example of an annotated gel. |

You can find the product information sheet for the DNA ladder which we used in this lab, including the size of each band, on the manufacturer’s website.

**Questions to consider/discuss with your lab partners:**

# 13. Additional Resources/Further Reading for Experiment 1

## 13.1 Microbiology Society “Techniques” videos

These videos illustrate many of the techniques used in this lab, you may find it helpful to watch them to prepare for the lab (or as review afterwards)

* Transferring agar to a Petri dish
* Using a sterile inoculating loop
* Making a Streak Plate
* Serial Dilution
* Using a Pipette and Spreader

## 13.2 Streak Plates

* The Streak Plate Protocol (American Society for Microbiology): Some good historical background and a detailed protocol

## 13.3 Bacterial Plasmids

* Addgene: Plasmids 101: helpful introduction to the basics of plasmid biology

# 14. Experiment 2 Aims and Overview

## 14.1 Aims

## 14.2 Learning Outcomes

At the end of this laboratory session, you should understand:

## 14.3 Competencies

* Performing data analysis
* Calculating antibiotic MBC and MIC values
* Teamwork
* Keeping detailed and accurate records

## 14.4 Importance

This laboratory session

## 14.5 Overview of Experiment 2

In this experiment, you will be :

# 15. Experiment 2 - Intro

# 16. Experiment 2 Protocols

You can view the handout for lab 1 below, or download it [here](./handouts/Lab2.pdf).

# 17. Experiment 2 - Data Analysis

# 18. Additional Resources/Further Reading for Experiment 2

# 19. Experiment 3 Aims and Overview

## 19.1 Aims

## 19.2 Learning Outcomes

At the end of this laboratory session, you should understand:

## 19.3 Competencies

* Performing data analysis
* Using online databases
* Teamwork
* Keeping detailed and accurate records

## 19.4 Importance

This laboratory session

## 19.5 Overview of Experiment 3

In this experiment, you will be :

# 20. Experiment 3 - Intro

# 21. Experiment 3 Protocols

You can view the handout for lab 3 below, or download it [here](./handouts/Lab3.pdf).

# 22. Experiment 3 - Data Analysis

# 23. Additional Resources/Further Reading for Experiment 3

# 24. Experiment 4 Aims and Overview

## 24.1 Aims

## 24.2 Learning Outcomes

At the end of this laboratory session, you should understand:

## 24.3 Competencies

* Performing calculations
* Performing data analysis
* Formulating hypotheses
* Using databases
* Teamwork
* Keeping detailed and accurate records

## 24.4 Importance

This laboratory session

## 24.5 Overview of Experiment 4

In this experiment, you will be :

# 25. Experiment 4 - Intro

# 26. Experiment 4 Protocols

You can view the handout for lab 4 below, or download it [here](./handouts/Lab4.pdf).

# 27. Experiment 4 - Data Analysis

# 28. Additional Resources/Further Reading for Experiment 4

# 29. Experiment 5 Aims and Overview

## 29.1 Aims

## 29.2 Learning Outcomes

At the end of this laboratory session, you should understand:

## 29.3 Competencies

* Using appropriate software to visualize and analyze microscopy data
* Data analysis
* Data presentation
* Teamwork
* Keeping detailed and accurate records

## 29.4 Importance

This laboratory session

## 29.5 Overview of Experiment 5

In this experiment, you will be :

# 30. Experiment 5 - Intro

# 31. Experiment 5 Protocols

You can view the handout for lab 5 below, or download it [here](./handouts/Lab5.pdf).

# 32. 5data

Write your content here.

# 33. Additional Resources/Further Reading for Experiment 5