

Theme – Microscopy and developmental biology

Practical title: Developmental biology 1

Week 3 and 4

Assessment due end of Week 4

Offshore students have an online version of this prac – see Moodle prac link for all prac details.

The prac below is for on-campus students only.

PRACTICAL: Developmental Biology I

Prac Convenor: Dr Chantal Hoppe, Department of Anatomy and Developmental Biology

This practical is worth 5% of your overall grade for this unit.

Remember your lab coat, safety glasses and fully enclosed footwear, including your name-tag, face-mask and ID card.

Please BYO device (this is how you will access these prac instructions in lab). Some students may like to bring a printout of this prac instead.

Please see Below, & Moodle for pre-class reading.

Students will be allocated to a bench and demo – details will be put in an announcement start of week 3

Assessment Summary

Activity	%
Part 1 - Skills assessment: Practical skills using (& knowledge of) the light microscope, and laboratory etiquette/engagement throughout class	3
Part 2 - Online activities & quiz due 26th March 5pm	2
Total	5

Worksheet prepared by Dr Chantal Hoppe, Danielle Rhodes and Professor Helen Abud



PRACTICAL OBJECTIVES

1. Apply knowledge of the basics of light- microscopy by demonstrating sample preparation and use of a light microscope, and related activities
2. Examine sperm and identify the structure and function of different regions of mature spermatozoa.
3. Analyse a porcine semen sample and measure sperm concentration, motility, morphology and viability.

Pre-Reading & Activities

Before coming to class,

1. read the following information on How Cells are Studied (*excerpt from your Biology Bridging Course Module - details in box below*)
2. Read the article [Human Spermatozoa - revelations on the road to conception](#) found on Moodle
3. Thoroughly read the practical notes below & view your Online Quiz

Students are reminded of the Optional Online BMS Biology Bridging Course. This was designed for those students that missed out on VCE Biology, or that would like to re-familiarise themselves with basic biological concepts. This may make the transition through the biology-related units in the degree easier. This optional online course can be visited & referred to anytime during 2021 and is designed to be completed at your own pace.

It is \$14 OR free-access in this building, the BLTB. By electing to complete this course, you will be given access to a customised online textbook with interactive questions, animations and movies to familiarise yourself with key biological concepts. You will be able to check your understanding as you work through the modules.

All first year students should have received an email around O-week inviting them to the online component of the bridging course. This will include all the details for registration and access.

Alternatively you can: 1. Click on this link: <https://app.tophat.com/e/997493> 2. Sign-up for a Top Hat online account 3. Follow the prompts to access the textbook. Your course Join Code is 997493

The following is an excerpt from your Biology Bridging Course Module (TopHat Bridging Course 2021) - details above if you prefer to access online.

Introduction

Close your eyes and picture a brick wall. What is the basic building block of that wall? It is a single brick, of course. Like a brick wall, your body is composed of basic building blocks, and the building blocks of your body are cells. Your body has many kinds of cells, each specialised for a specific purpose. Just as a home is made from a variety of building materials, the human body is constructed from many cell types. For example, epithelial cells protect the surface of the body and cover the organs and body cavities within. Bone cells help to support and protect the body. Cells of the immune system fight invading bacteria. Additionally, red blood cells carry oxygen throughout the body. Each of these cell types plays a vital role during the growth, development, and day-to-day maintenance of the body. In spite of their enormous variety, however, all cells share certain fundamental characteristics.

How Cells Are Studied

By the end of this section, you will be able to:

- Describe the roles of cells in organisms
- Compare and contrast light microscopy and electron microscopy

A cell is the smallest **biological unit** of a living thing. A living thing, like you, is called an organism. Thus, cells are the basic building blocks of all organisms.

In multicellular organisms, several cells of one particular kind interconnect with each other and perform shared functions to form tissues (for example, muscle tissue, connective tissue, and nervous tissue), several tissues combine to form an organ (for example, stomach, heart, or brain), and several organs make up an organ system (such as the digestive system, circulatory system, or nervous system). Several systems functioning together form an organism (such as an elephant, for example).

There are many types of cells, and all are grouped into one of two broad categories: prokaryotic and eukaryotic. Animal cells, plant cells, fungal cells, and protist cells are classified as eukaryotic, whereas bacteria and archaea cells are classified as prokaryotic. Before discussing the criteria for determining whether a cell is prokaryotic or eukaryotic, let us first examine how biologists study cells.

Microscopy

Cells vary in size. With few exceptions, individual cells are too small to be seen with the naked eye, so scientists use microscopes to study them. A **microscope** is an instrument that magnifies an object. Most images of cells are taken with a microscope and are called micrographs.

Light Microscopes To give you a sense of the size of a cell, a typical human red blood cell is about eight millionths of a meter or eight micrometers (abbreviated as μm) in diameter; the head of a pin is about two thousandths of a meter (millimeters, or mm) in diameter. That means that approximately 250 red blood cells could fit on the head of a pin.

The optics of the lenses of a light microscope changes the orientation of the image. A specimen that is right-side up and facing right on the microscope slide will appear upside-down and facing left when viewed through a microscope, and vice versa. Similarly, if the slide is moved left while looking through the microscope, it will appear to move right, and if moved down, it will seem to move up. This occurs because microscopes use two sets of **lenses** to magnify the image. Due to the manner in which light travels through the lenses, this system of lenses produces an inverted image (binoculars and a dissecting microscope work in a similar manner, but include an additional magnification system that makes the final image appear to be upright).

Most student microscopes are classified as light microscopes (Figure 1). Visible light both passes through and is bent by the lens system to enable the user to see the specimen. Light microscopes are advantageous for viewing living organisms, but since individual cells are generally transparent, their components are not distinguishable unless they are coloured with special stains. Staining, however, usually kills the cells.

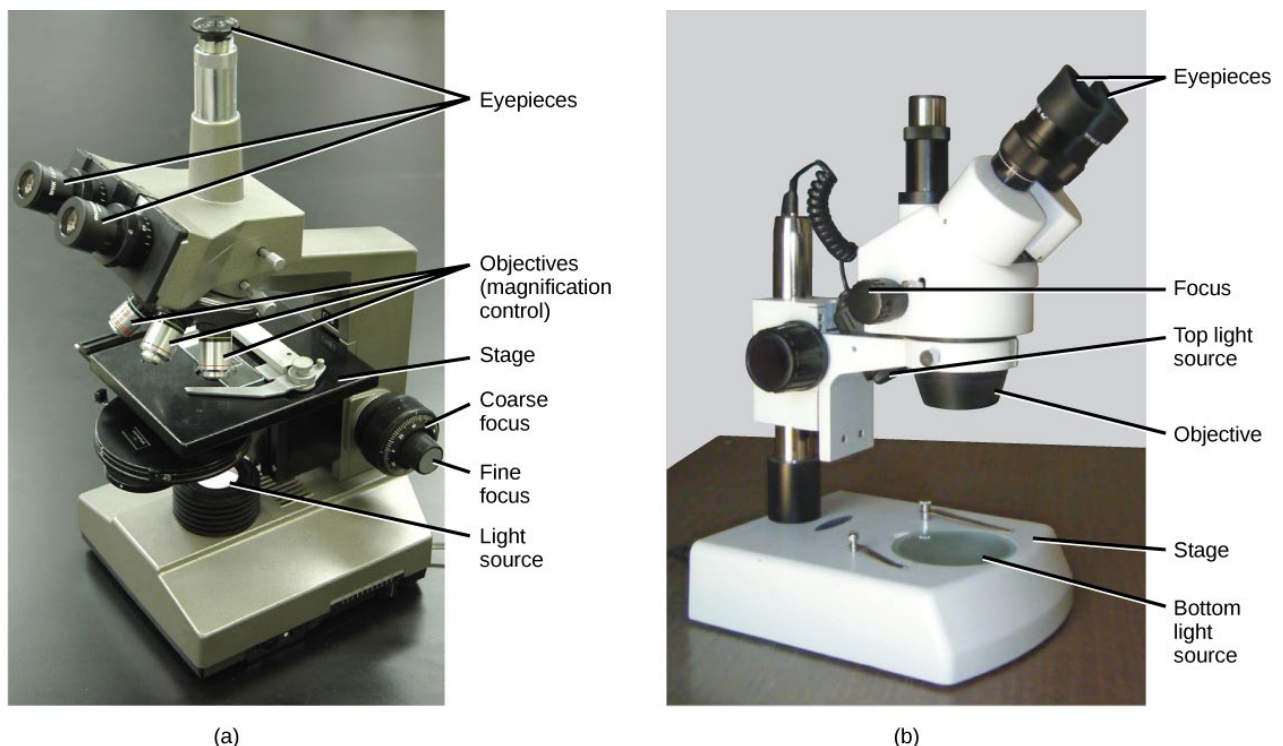


Figure 1 (a) Most light microscopes used in a college biology lab can magnify cells up to approximately 400 times. (b) Dissecting microscopes have a lower magnification than light microscopes and are used to examine larger objects, such as tissues.

Light microscopes commonly used in the undergraduate college laboratory magnify up to approximately 400 times. Two parameters that are important in microscopy are **magnification** and **resolving power**. Magnification is the degree of enlargement of an object. Resolving power is the ability of a microscope to allow the eye to distinguish two adjacent structures as separate; the higher the resolution, the closer those two objects can be, and the better the clarity and detail of the image. When oil immersion lenses are used, magnification is usually increased to 1,000 times for the study of smaller cells, like most prokaryotic cells. Because light entering a specimen from below is focused onto the eye of an observer, the specimen can be viewed using light microscopy. For this reason, for light to pass through a specimen, the sample must be thin or translucent.

Dissecting Microscopes

A second type of microscope used in laboratories is the dissecting microscope (Figure 1 b). These microscopes have a lower magnification (20 to 80 times the object size) than light microscopes and can provide a three-dimensional view of the specimen. Thick objects can be examined with many components in focus at the same time. These microscopes are designed to give a magnified and clear view of tissue structure as well as the anatomy of the whole organism. Like light microscopes, most modern dissecting microscopes are also binocular, meaning that they have two separate lens systems, one for each eye. The lens systems are separated by a certain distance, and therefore provide a sense of depth in the view of their subject to make manipulations by hand easier. Dissecting microscopes also have optics that correct the image so that it appears as if being seen by the naked eye and not as an inverted image. The light illuminating a sample under a dissecting microscope typically comes from above the sample, but may also be directed from below.

Electron Microscopes

In contrast to light microscopes, electron microscopes use a beam of electrons instead of a beam of light. Not only does this allow for higher magnification and, thus, more detail (Figure 2), it also provides higher resolving power. Preparation of a specimen for viewing under an electron microscope will kill it; therefore, live cells cannot be viewed using this type of microscopy. In addition, the electron beam moves best in a vacuum, making it impossible to view living materials.

In a scanning electron microscope, a beam of electrons moves back and forth across a cell's surface, rendering the details of cell surface characteristics by reflection. Cells and other structures are usually coated with a metal like gold. In a transmission electron microscope, the electron beam is transmitted through the cell and provides details of a cell's internal structures. As you might imagine, electron microscopes are significantly more bulky and expensive than are light microscopes.

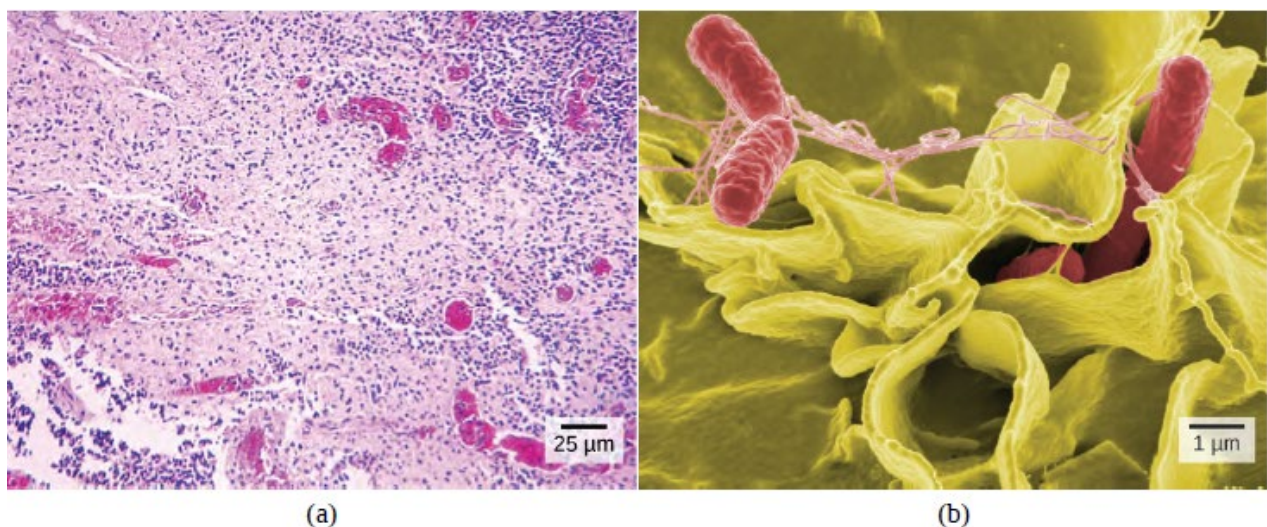


Figure 2 (a) *Salmonella* bacteria are viewed with a light microscope. (b) This scanning electron micrograph shows *Salmonella* bacteria (in red) invading human cells. (credit a: modification of work by CDC, Armed Forces Institute of

PRACTICAL CLASS OVERVIEW

EXPLORING THE BASIS OF MALE INFERTILITY

Infertility or subfertility is a common problem in Australia, with approximately 15% of couples experiencing difficulties conceiving. This has been addressed by the introduction of artificial reproductive technologies (ART) including *in vitro* fertilisation (IVF) and intracytoplasmic sperm injection (ICSI). In Australia, more than one percent of births are now the result of ART. However, these techniques cannot address all causes of infertility, particularly in cases where the underlying defect is unknown. Further research is required to identify the underlying causes of infertility and develop new individualised treatment options.

Male fertility is also an important consideration for industries where breeding of animals is important either for agricultural purposes or for other purposes (for example selective breeding). Artificial insemination can be used which requires the assessment of the quality of semen.

In this practical we will examine the process of spermatogenesis, which is responsible for the continual production of sperm, and the characteristics of sperm that are often examined in fertility testing.

- *Students will rotate between activities at designated times as instructed by the TAs*
- *You should work in pairs for this activity*
- ***The “Skills Assessment” component of the assessment will be conducted throughout and particularly toward the end of class and will be an individual assessment, conducted by your demonstrator.***

General Instructions for Microscopy

1. Always start a microscope session with the **lowest power objective** (the x4 objective). With the x4 objective in position, use the coarse focus knob to position the microscope stage to the lowest position below the objective, then place the slide on the microscope stage. Position the slide, using the stage adjustment knobs, so that the specimen (e.g. *spermatozoa*) is in the middle of the beam of light shining through the hole in the stage.
2. Slowly raise the stage to its upper limit. Watching from the side you should be able to see that there is still space between the objective and the specimen since most microscopes have ‘stops’ built into their arms to ensure you do not crush the slide between the objective and the stage. If you are not sure if you are too close to the specimen, ask your TA.
3. Now, looking through the eyepieces:
 - a) Slowly lower the stage using the coarse-focusing knob until the specimen comes into focus. Finalise the focus with the fine focusing knob.
 - b) Using the stage adjustment knobs, adjust the position of the slide until the specimen is in the centre of your field of view.

Important reminder regarding safety considerations: As you are working with biological material (should always be treated as potentially infectious) and trypan blue (may cause eye and skin irritation), you **MUST** wear personal protective equipment (PPE) when conducting this part of the practical - this includes gloves (supplied), a lab coat, safety glasses and fully enclosed shoes. All samples must be handled with care. Please be mindful that once you begin handling samples with your gloves, they **WILL** be contaminated so **DO NOT** touch your face, phone etc. as they will also become contaminated in the process. All waste (sharps and biohazard) will need to be disposed of in the correct bins provided. Please consult staff or assistants if you are unsure.

You must wash your hands thoroughly before leaving the laboratory.

“Skills Assessment”

This component of the assessment will be conducted throughout class, and will be an individual assessment carried out in front of your demonstrator.

It will involve demonstrating your knowledge of, and your practical skill (includes general engagement/etiquette throughout class) in using the light microscope.

Your demonstrator may ask you questions throughout this exercise, and grade you according to the following (includes observations throughout the practical class):

1. Working in a team (i.e. being an active team member, helping others, demonstrating respectful behaviour)	1 mark
2. Scientific accuracy and proper use of equipment (i.e. recording and analysing own results carefully, handling equipment with care)	2 marks
3. Engagement and Lab etiquette (willingness to try, positive attitude & appropriate pack up at end of prac)	1 mark
4. Correct handling of the spermatozoa & microscope setup	1 mark
5. Ability to focus on spermatozoa and/or trouble-shooting	1 mark
6. Demonstrated knowledge of the light microscope (can be via answering of questions)	2 mark
7. Explanation & understanding of results	2 marks
	/10 Marks

Per category: 0 marks if not evident, minus 0.5 – 1.5 marks if evident but needs improvement (depending on category), full marks if demonstrated well

Please note: students may need some practice with specific parts of the practical – this is OK, and students should ask for help. The assessment in-class is not based on avoidance of mistakes but rather your attitude, and understanding of practical components (see criteria above).

Please note limited verbal feedback will be given in-class regarding your assessment. *Grades will be uploaded onto Moodle.*

***Before you leave the prac, make sure you have checked with your demonstrator that they have your assessment completed.**

Porcine Semen Analysis

Semen analysis is the most important test for evaluating male fertility. Semen quality is assessed by measuring sperm concentration (sperm count), motility (ability to move), morphology (size and shape) and viability (ability to live and endure). Low quality semen is associated with reduced fertility and poor reproductive outcomes, while high quality semen is associated with reproductive success.

Semen analysis is commonly performed in fertility clinics to diagnose male infertility and determine what treatment options are available to infertile couples. The agricultural industry also utilises semen analysis to identify fertile animals for breeding programs.

Artificial Insemination Program

A pig farmer is establishing a new artificial insemination program and would like you to analyse a semen sample from one of his stud males. The outcome of artificial insemination largely depends on semen quality, therefore the semen sample must meet the minimum quality control standards outlined in the table below:

Sperm Concentration	20 million per mL
Sperm Motility	>70% normal motility
Sperm Morphology	<20% abnormal morphology
Sperm Viability	>70% viable

Perform the following semen assessments and based on your results conclude if this boar is fertile or infertile *and* provide a recommendation to the farmer.

1. SPERM MOTILITY AND HYPERACTIVATION

Sperm motility is an essential aspect of male fertility. The number of sperm with forward motility must be greater than 70% to reach the minimum quality standard. When sperm are first deposited in the female reproductive tract the flagellum beats in symmetrical waves that propel the sperm forward in a straight line (progressive motility). When sperm reach the oviduct the flagellum begins to beat in asymmetrical waves of increasing height. This causes the sperm to swim rapidly in zig-zags (hyperactive motility) and helps them to swim through the dense mucous of the oviduct. Both of these types of motility are normal for sperm.

Assess the motility of porcine sperm in untreated and hyperactivated samples.

Materials	
1 x porcine semen sample	1 x eppendorf tube of 40mM caffeine
2 x plastic transfer pipettes	2 x microscope slides
2 x large rectangle coverslips	

1. Collect the required materials. These will be on the white trays at your bench or at the back or side benches. Your TA will bring a sample of porcine semen and caffeine to each pair.

Note: The semen and caffeine have been heated to 37°C to replicate conditions within the female reproductive tract. Ensure you work quickly as sperm motility will decrease as the sample cools – it may be useful to hold the tubes in the palm of your hand to keep them as close to 37°C as possible.

2. Gently invert the semen sample 2-3 times to resuspend the sperm.
3. Using a plastic pipette place a small drop of semen onto a microscope slide. Carefully place a large rectangle coverslip over the sample and allow the cells to settle for 1 minute.
4. Place the slide on the microscope stage and focus on the sperm using the x40 objective. You should begin this process from the x4 objective and progress to the x40 objective.

Note: The sperm are almost transparent and may be difficult to focus on. You will need to adjust the brightness and contrast of your microscope to observe them. Please refer to Practical 1 (Microscopy) for a refresher if required.

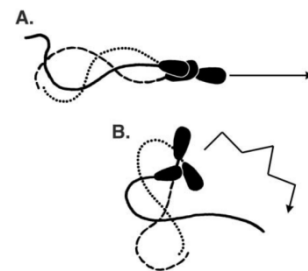
5. Observe 20 sperm and score them based on the following motility criteria. Record your observations in the results table below and indicate the number of sperm in each category.

A. Progressive Motility Sperm move forward in a straight path

B. Hyperactivated Motility Sperm move rapidly in zig-zags or circles

C. Irregular Motility Sperm movement is jerky and irregular

D. Non Motile Sperm do not move



	Untreated Semen	Hyperactivated Semen
Progressive Motility		
Hyperactivated Motility		
Irregular Motility		
Non Motile		

Note. Remember that both progressive and hyperactivated motility are normal forms of motility for sperm

6. Place this prepared slide to the side in a place on your workbench where you will not knock it. You will be using this slide to complete the sperm morphology assessment in Activity 2.
7. Using a plastic pipette place a small drop of caffeine onto a fresh microscope slide. Then add a small drop of semen on top of the drop of caffeine. Carefully place a large rectangle coverslip over the sample and allow the cells to settle for 1 minute.

Note: Caffeine treatment will induce hyperactivation in the sperm.

- Once again, use the x40 objective to observe the motility of 20 sperm and record your observations in the table above.

Q. Calculate the percentage of normal motile sperm in the untreated and hyperactivated samples using the formula below.

$$\text{Percent motility} = \frac{(\text{number of sperm progressive motility} + \text{number of sperm hyperactivated motility})}{\text{total number of sperm}} \times 100$$

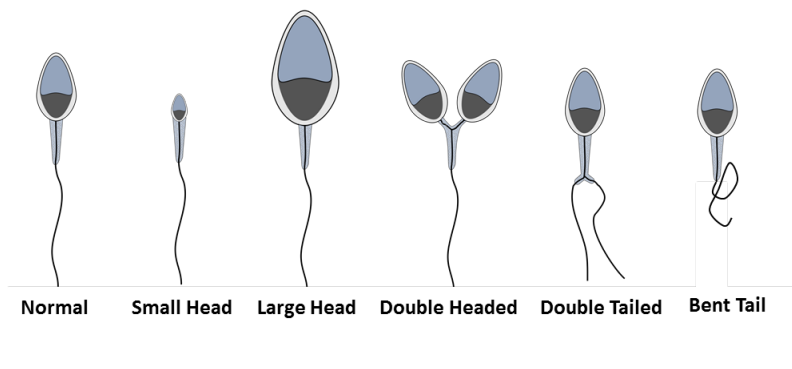
Untreated Semen	
Hyperactivated Semen	

2. SPERM MORPHOLOGY ASSESSMENT

The morphology (size and shape) of sperm is an essential aspect of male fertility that determines whether or not sperm are capable of fertilisation. Sperm morphology varies between species, with human sperm developing conical shaped heads and porcine sperm developing heads that are paddle shaped. Even within semen samples from fertile men/animals, a significant percentage of sperm may be morphologically abnormal. In porcine samples the number of sperm with abnormal morphology must not exceed 20% to meet the minimum quality standard.

Assess the morphology of sperm in the porcine semen sample.

- Get a new porcine semen sample and place on microscopy slide. Place on the microscope stage and focus on the sperm using the 40x objective. *Note:* The sperm will no longer be motile, hence it will be easier to observe their morphology.
- Observe 20 sperm** and score them based on the following morphological criteria. Record your observations in the results table on the next page and indicate the number of sperm in each category.



A b n o r m a l	Morphology	
	Normal	
	Small Head	
	Large Head	
	Double Headed	
	Double Tailed	
	Bent or Broken Tail	

Q. Calculate the percentage of sperm with abnormal morphology.

Percent abnormal sperm = (number of sperm with abnormal morphology / total number sperm) x 100

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3. Discard your used slides and coverslips in the sharps bin and any biologically contaminated material in the biohazard bin. Please check with your TA if you are unsure.

3. SPERM CONCENTRATION AND VIABILITY ASSESSMENT

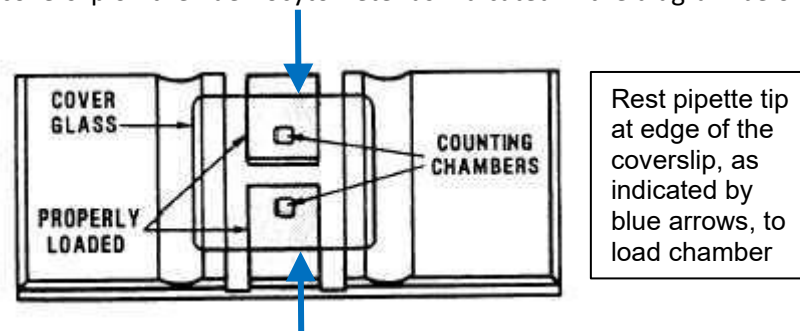
Sperm concentration refers to the number of sperm cells present within a semen sample. The concentration of sperm varies greatly between species and individuals, with porcine samples requiring a minimum concentration of 20 million sperm per mL to meet quality standards. Concentration is measured using a haemocytometer, which is a glass counting chamber similar to a microscope slide.

Sperm viability refers to the percentage of live sperm within a semen sample. Live and dead sperm are differentiated using a dye called *trypan blue*. Live sperm have an intact cell membrane that will exclude the dye leaving them colourless. Dead sperm will have a compromised cell membrane that allows dye to enter the cell and consequently will stain bright blue. Sperm viability must be greater than 70% to reach the minimum quality standard. In fertility clinics assessing sperm viability is particularly important in samples with low motility, in order to distinguish between dead sperm and live sperm that are immotile.

Determine the concentration and viability of sperm in the semen sample.

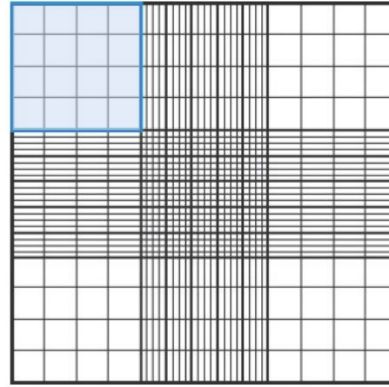
Materials	
1 x <i>stained</i> (with trypan blue) porcine semen sample	1 x haemocytometer (to be returned) plus instruction sheet
1 x plastic transfer pipette	1 x small square coverslip

1. Collect the required materials. These will be on the white trays at your bench or at the back or side benches.
2. Place a small square coverslip on the haemocytometer as indicated in the diagram below.



3. Gently invert the trypan blue-stained semen sample 2-3 times to resuspend the sperm.
4. Using a plastic pipette, carefully fill one chamber of the haemocytometer by gently resting the tip of the pipette at the edge of the chamber (see diagram above, instruction sheet at your workstation or short video on Moodle). The sample will be drawn out of the pipette by capillary action. Take care not to overfill the chamber.
5. Allow the cells to settle for 1 minute.

6. Place the slide on the microscope stage and focus on the haemocytometer grid lines using the x10 objective. Locate and focus on the 16 squares that comprise the top-left hand corner of the grid, as indicated in the figure on the right.



7. Increase the magnification by moving to the x40 objective and count the number of sperm that are present in the 16 squares. Record the number of sperm that are live and viable (unstained by trypan blue) and the number of sperm that are dead (stained with trypan blue) in the table below.

Total Sperm	Viable Cells (Unstained)	Dead Cells (Stained Blue)

Q. Determine the concentration of sperm in the sample.

Note: The sample was diluted 1/20 before being distributed to students. The haemocytometer is designed so that the number of cells counted in one set of 16 corner squares is equivalent to the number of cells in the sample $\times 10^4/\text{mL}$.

Number of sperm per mL = total number sperm counted $\times$ dilution factor $\times 10^4$

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Q. Calculate the percentage of viable sperm in the sample.

Percent viability = (number of viable cells / total number sperm counted) $\times 100$

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PORCINE SEMEN ANALYSIS CONCLUSION

Based on your results, and the minimum quality control standards outlined earlier, would you recommend that the pig farmer uses this boar for artificial insemination? Why / Why not?

Practical skill development

Practical skills acquired in this session:	Further use and development of this skill:
Use of a light microscope	Practical (Introduction to Histology) Practical (Immunology) Practical (Microbiology) BMS2052 (Microbes in health and disease)
Preparation and staining of a biological sample	Practical (Microbiology), BMS2052
Basic scientific calculations (e.g. transforming data, concentrations) and data analysis	Practicals (Metabolism, Immunology) BMS1052 (Human Neurobiology) BMS1062 (Molecular Biology) BMS2031 (Body systems) BMS2042 (Human genetics)
Observation and/or identification of organisms, cells or structures	All practicals in BMS1021 BMS2011 (Structure of the human body, an evolutionary perspective) BMS2042, BMS2052