RISK ASSESSMENT

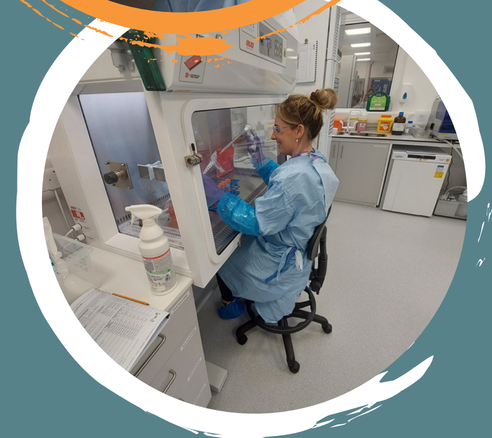
Aim: Carry out a risk assessment for a specified procedure in a scientific laboratory.

Performance criteria:

(a) Identify the main hazards present
(b) Identify the associated risks to health and safety
(c) Suggest appropriate ways to minimise risk

Activity 1

You are working as a level 2 Associate Scientist at a Life Science company in Glasgow. The project manager delivers the latest client sample, a potential vaccine against next season's influenza strain. Your job is to test the client sample for signs of microbial contamination that could cause harm to people who receive the vaccine. The vaccine cannot be released to market until all your checks have been carried out.



The first step in this process is to prepare tubes of 10 cm^3 sterile culture media. Watch the video and consider:

(a) the main hazards present
(b) associated significant risks to health and safety
(c) suggest appropriate ways that have been put in place to minimise risks.

sserc

PPE

Working in the Life Sciences

As you complete the Risk Assessment activity, look at the three images below showing the PPE used in a Life Sciences GMP (Good Microbiological Practice) laboratory. How many different items of PPE can you spot and how do these compare with what you wear for microbiology work in school?
The employer must legally supply all of these items of PPE; but the employee is also legally obliged to wear them appropriately.

MICROBIOLOGY *in the Life Sciences*

Can you work safely with microorganisms in a laboratory setting?

Performance criteria:

(a) Pour agar plates using aseptic technique
(b) Subculture microorganisms using aseptic technique
(c) Prepare wet and dry mounts to observe, using a microscope
(d) Work safely throughout, adhering to Safety in Microbiology: A Code of Practice for Scottish Schools and Colleges (SSERC, 2018).

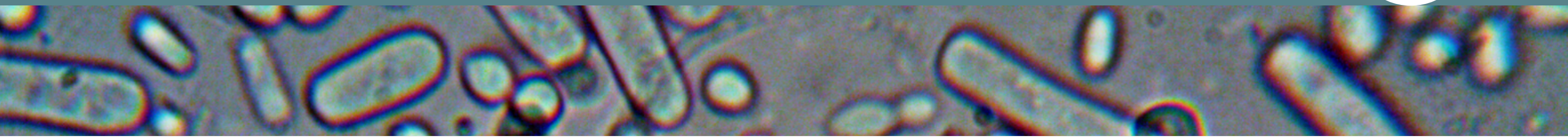
Activity 2(a) - Prepare 10 sterile Yeast Glucose Agar (YGA) plates

YGA media is prepared by combined the following reagents with distilled water in an autoclavable container.

- 2.5 g yeast extract
- 5 g agar
- 5 g glucose
- 250 cm^3 distilled water

The container of ingredients is inverted several times to mix and then autoclaved. The autoclave will run at 121°C for 15 minutes at 15 psi. This ensures the contents are sterilised. At the end of the autoclave process, the YGA media will be sterile - this means it is free from microbial contaminants and safe to use.

Cool the media to 55°C and pour ~ 20 cm^3 of sterile YGA media into 10 sterile petri dishes using aseptic technique. Watch the video on how to carry this out.



Activity (b) - Subculture microorganisms using aseptic technique

In this activity, you will subculture microorganisms, using aseptic technique, without contamination. These techniques include:

- Solid to solid (bacteria or yeast)
- Liquid to solid (bacteria or yeast)
- Solid to liquid (bacteria or yeast)
- Liquid to liquid (yeast only)

To carry out these sub-culturing techniques, there are some key aseptic techniques you need to practise first.

Watch the video and try the technique out as many time as you need to before progressing onto the sub-culturing activities.

- Dealing with a microbiological spill
- Pinkie-palm technique
- Flaming the inoculation loop

Risk Assessment

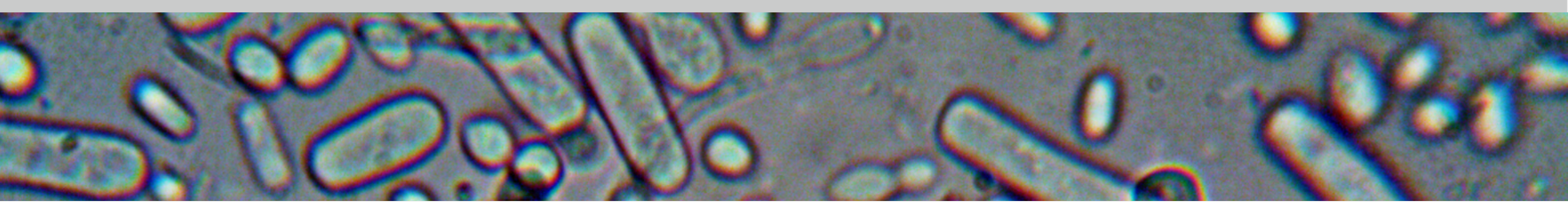
Reflect back to Activity 1 on Risk Assessment. As you prepare to carry out a range of sub-culturing techniques, consider the hazards involved, the level of risk and the control measures you will put in place to minimise the risk.

It is useful to consult the "Safety in Microbiology: A Code of Practice for Scottish Schools and Colleges" from SSERC. This provides a working risk assessment to ensure your microbiology work is safe.

HAZARD

RISK

CONTROL MEASURE



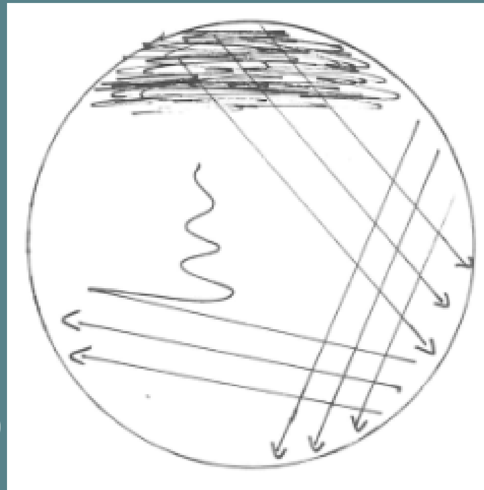
Sub-Culturing Techniques

SOLID TO SOLID TRANSFER

Watch the video on how to carry out a solid to solid transfer. In this technique, an inoculum (small sample of the microorganism) is transferred from a slope to a plate.
Microorganisms arrive to the lab from a supplier as a microbial "slope". This is usually due to easier and longer-term storage. But for use in class or a lab, the microorganism must be transferred to a sterile agar plate first.

LIQUID TO SOLID TRANSFER

Watch the video on how to carry out a liquid to solid transfer. This example is called a streak out. It is a challenging technique but the purpose is to check for the purity of the culture (are there any contaminants that you can't see in the liquid culture) and to achieve single colonies. Click to download a streak plate template to help you with this procedure. This can be printed out, laminated and then placed under your agar plate to guide your procedure.



SOLID TO LIQUID TRANSFER

Watch the video on how to carry out a solid to liquid transfer. In this technique, a sterile inoculation loop is used to transfer a small piece of a microbial colony from an agar plate to a container of sterile broth.
This might be done to determine the effect of particular treatments on microbial growth, e.g. tea tree oil. As microbial growth takes place, the sterile, clear broth becomes "turbid".

LIQUID TO LIQUID TRANSFER

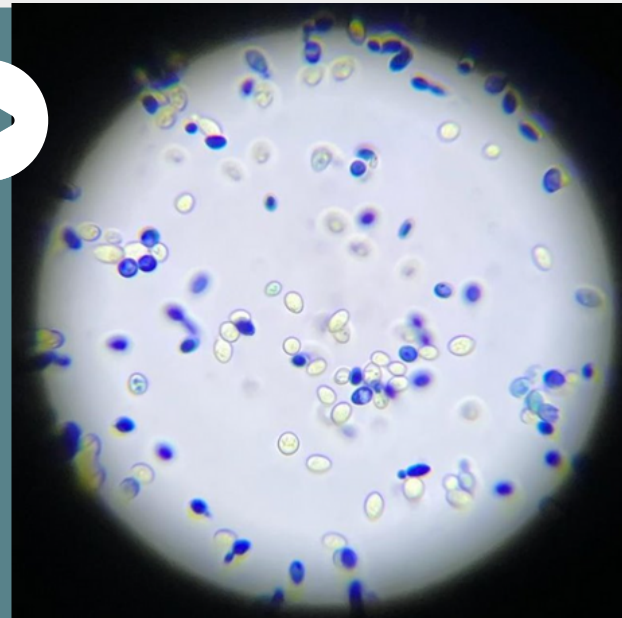
Watch the video on how to carry out a liquid to liquid transfer. In this technique, a sterile inoculation loop is used to transfer a "loop-full" of microbial culture to a sterile container of broth. This might be done as part of a serial dilution procedure, which might end with transferring the liquid culture to a sterile agar plate to perform a count of colony forming units.

Activity (c) - Prepare wet and dry mounts to observe, using a microscope

In this activity, you will prepare wet and dry mounts and then use a microscope to visualise the microorganism.

Wet Mount

Watch the video on how to carry out a wet mount with S. cerevisiae. The video shows Neutral Red being used as the stain. However, this technique works better using Methylene blue - the technique is the same. When viewing the prepared slide using a microscope, you will observe some transparent cells and some that appear blue. Methylene blue readily permeates yeast cells, but becomes colourless in living cells. The daed cells appear blue. This allows a viable cell count to be performed.

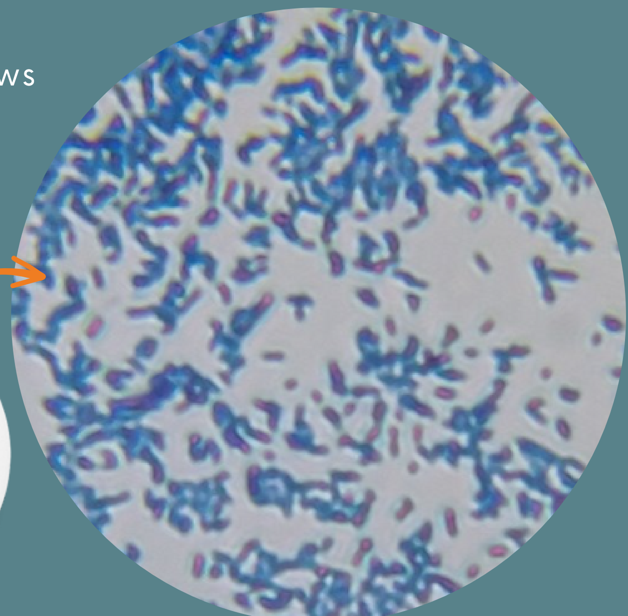


Dry Mount

For this technique, a fixed smear of the microorganism must first be carried out. This allows the cells to adhere to the slide and promotes the uptake of the stain.

Watch the video to learn how to carry out the technique.

What type of microorganism do you think this is? How can you describe the shape of the cells? Does this help you identify it?



Fixed smear

1. Label a clean microscope slide with your initials, date and microorganism.
2. Flame the loop.
3. Using aseptic technique, transfer 1 loopful of sterile water on to the centre of the microscope slide.
4. Flame the loop.
5. Using aseptic technique, transfer a very small part of a single microbial colony, e.g. M. luteus or E. coli.
6. Flame the loop.
7. Using forceps, hold the microscope slide with the smear downwards, pass the smear through the yellow flame several times to "fix" it.
8. Place on a heat-resistant mat to cool.

Simple stain using Methylene blue

1. Place the fixed smear of microorganisms on a staining rack over a staining tray.
2. Flood the smear with methylene blue.
3. Leave for 2 minutes.
4. Rinse thoroughly with water.
5. Blot dry using blotting paper.
6. Observe the cells using a microscope at x400 magnification.