PROTOCOL

INVESTIGATING MICRO-ORGANISMS

SCN 3-13b

1 Overview of Protocols

This investigation involves 3 separate protocols, which will be outlined on this page.

2 Health and Safety

Before you start, familiarise yourself with the hazards, level of risk and control measures. Review and make adjustments for your setting, taking account of the stage of your learners.

3 Materials

A list of all materials required for this investigation - and where to get them

4 Method

Step-by-step instructions on how to carry out this investigation in your setting.

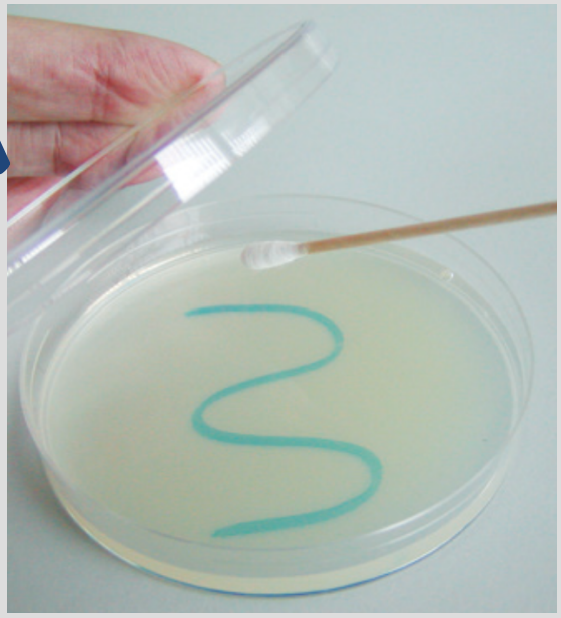
OVERVIEW

This investigation involves:

1. Carrying out an environmental swab
2. Investigating the effect of an antifungal medication on microbial growth
3. Using microscopy to observe *S. cerevisiae*

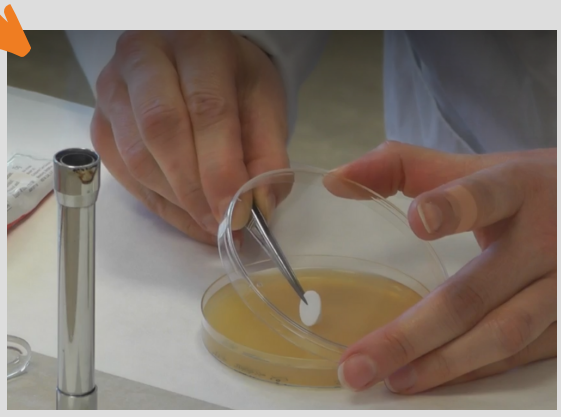
ENVIRONMENTAL SWAB

This technique involves performing a **simple streak.**



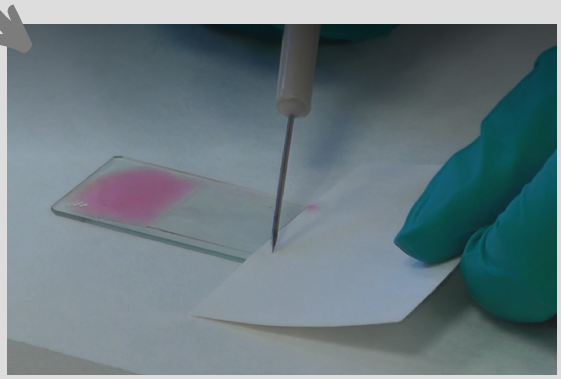
ANTIFUNGAL TREATMENT

This technique involves learners **sub-culturing** *S. cerevisiae* from a broth (liquid culture) to a Yeast-Glucose Agar plate (solid medium). Using this organism keeps this microbiological work at Level 2, according to SSERC's Code of Practice: Safety in Microbiology.



MICROSCOPY

This protocol will require learners to transfer a colony of live *S. cerevisiae* cells from a streak plate to a microscope slide. A vital stain called Neutral Red will be used to provide contrast.



HEALTH & SAFETY

This investigation involves culturing micro-organisms and, therefore, requires a Level 3-trained technician or teacher to produce sterile media, sub-culture the *S. cerevisiae* and dispose of cultures. The teacher must be trained in how to deal with a small-scale spill and feel ***competent*** in their microbiological practice. The teacher must be familiar with the Code of Practice. This section will look at the hazards associated with the 3 protocols within this investigation.

HAZARD	RISK	CONTROL MEASURES
Grown of potential human pathogens	Risk is greatest with the environmental swab protocol. Other protocols are lower risk but control measures still required.	• Approprate growth medium chosen for the organism. Media that may encourage the growth of pathogens is prohibited. • Sources of environmental samples for study are restricted (see below). • Incubation at room temperature for minimum duration. • Plates to be sealed with 2 pieces of sellotape and not reopened. • Cultures to be sterilised by autoclaving.

HAZARD	RISK	CONTROL MEASURES
Contamination of workspace, people and/or surroundings.	Risk is greatest with the environmental swab protocol. Other protocols are lower risk but control measures still required to prevent contamination	• Good microbiological laboratory practice - including thorough handwashing with water and soap, disinfection of work space with 1% hypochlorite solution. • Use of aseptic technique, including the use of sterilised apparatus. • Use of *S. cerevisiae* for sub-culturing protocols to keep work at Level 2. • Where possible, use an inoculation loop (rather than pipette) to minimise risk of spill.

CODE OF PRACTICE

This document states that all microbiological materials, cultures, media, environmental samples, etc, from whatever source should be treated as though they were a potential source of pathogens.

OTHER FACTORS TO CONSIDER

- competency of the teacher
- stage of learner

Depending on the learners in front of you, an assessment of risk must be made.

ENVIRONMENTAL SWAB RESTRICTIONS

Samples from carefully chosen areas of the environment may be used, but only to inoculate sterile solid media.

In particular, samples must not be taken for culture from:

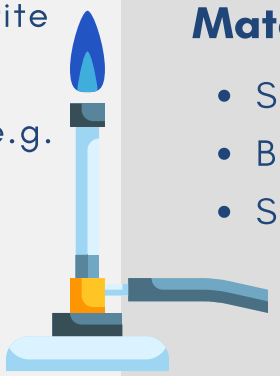
a) human [2] or other animal body surfaces;
b) body fluids and secretions;
c) animal cages or aquaria;
d) lavatories;
e) faecal material;
f) poultry, eggs or areas which have been in contact with poultry;
g) meat or meat products;
h) dead animals;
i) milk which has not been pasteurised;
j) soft, unpasteurised, cheeses;
k) water sources likely to contain faecal or sewage pollution;
l) soil fertilised by animal manure or fouled by animal faeces;
m) mud [3] (e.g. from a pond or field).

MATERIALS

The aim of this investigation is to explore the different microorganisms that can be found in the world around us and to examine how their growth can be controlled.

MATERIALS FOR ALL PROTOCOLS

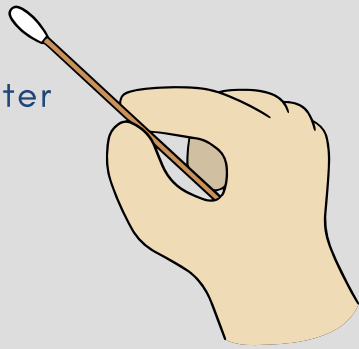
- Disinfectant (e.g. 1% hypochlorite solution) and paper towels
- Discard jar with disinfectant (e.g. 1% Virkon)
- Bunsen burner and mat
- Marker pen
- Sellotape



ENVIROMENTAL SWAB

Materials required (per pair)

- Sterile swab
- Bijou of sterile water
- Sterile YGA plate



ANTIFUNGAL TREATMENT

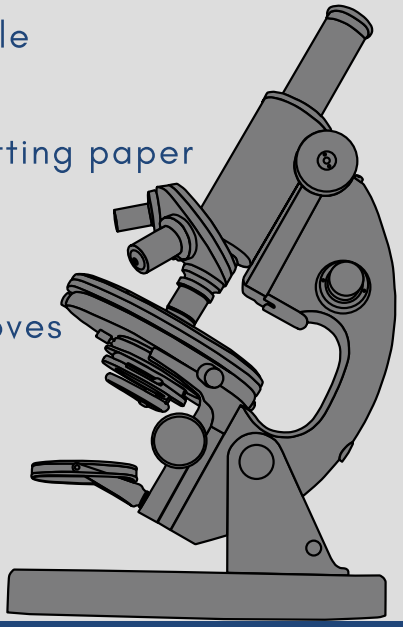
Materials required (per pair)

- Broth of *S. cerevisiae*
- Sterile YGA plate
- Sterile Pasteur pipette
- Ethanol covered in a beaker
- Spreader
- Forceps
- Filter paper discs
- Antifungal cream

MICROSCOPY

Materials required (per pair)

- Streak plate of *S. cerevisiae*
- Bijou of sterile water
- Wire loop
- Microscope slide
- Mounted needle
- Coverslip
- Fibre-free blotting paper
- Neutral red
- Microscope
- Disposable gloves
- Lens tissue



PROTOCOL 1

ENVIRONMENTAL SWAB

Check out our YouTube videos to support this protocol.

Pinkie-palm technique - This video will demonstrate a basic aseptic technique required during microbiological work.

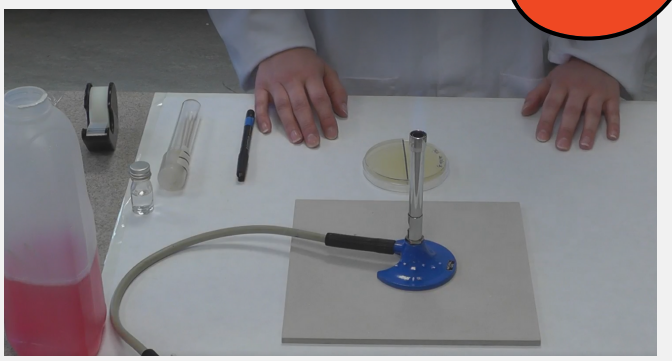
Environmental Swab protocol - this video will take you through the full protocol.

METHOD

Protocol 1: To investigate the presence of different microorganisms by taking an environmental swab. These instructions have been written for a right-handed person. Alternatively, watch the whole process here.

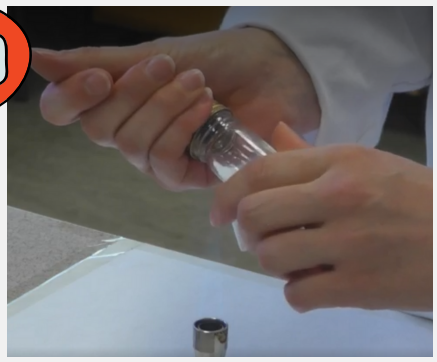
STEP 1 - PREPARE WORKSPACE

Clean your workspace with disinfectant. Set up all materials required within easy reach.



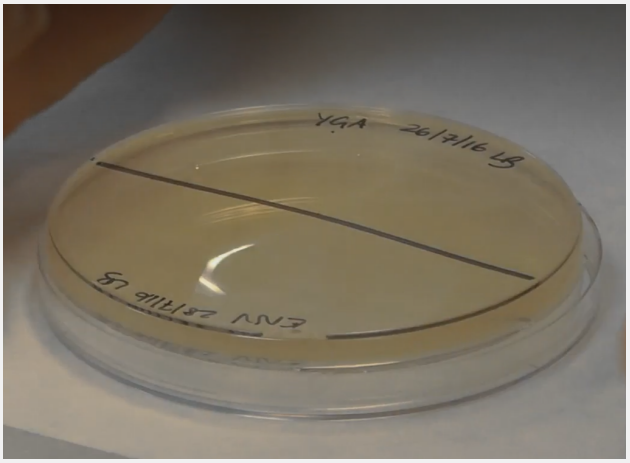
STEP 2 - ASEPTIC TECHNIQUES

Practice your pinkie-palm technique and how to flame the neck of the bottles you will be using.



STEP 3 - LABELLING YOUR PLATE

Collect your sterile YGA plate. Using a marker pen, draw a line on the base of the plate to create two halves - Write a "C" (for control) at the top of one half; write "E" (for environment) at the top of the other half. Label your plate with "Date, ENV, your initials".

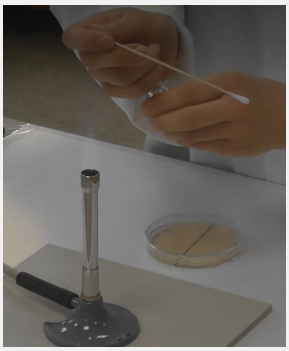


STEP 4 - READY FOR YOUR CONTROL SWAB

Taking care not to touch the cotton wool end, remove a sterile swab from the pack and hold it in your right hand.

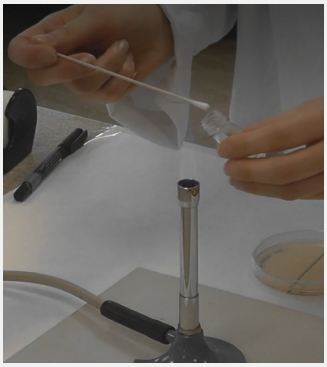
STEP 5

With your left hand, lift the bottle of sterile water (without putting down the swab) and remove the lid with your right hand. Flame the neck of the bottle.



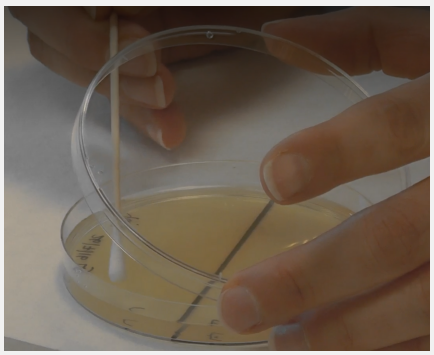
STEP 6

Insert the cotton wool bud into the sterile water and then withdraw it again. Reflame the neck of the bottle and replace the lid.



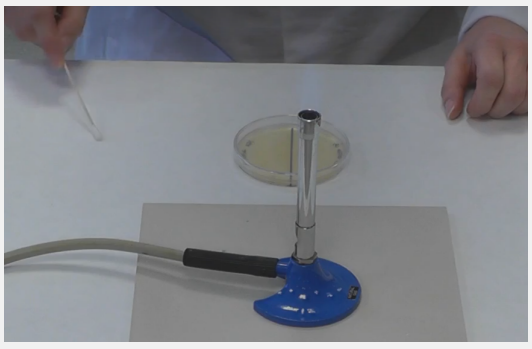
STEP 7

Lift the lid of the plate a little and gently rub the swab across the surface of the agar (on the "C" half). Take care not to break to agar surface. Replace the lid of the agar plate.



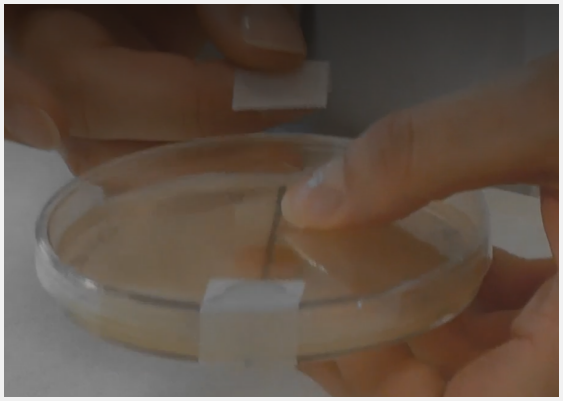
STEP 8 - THE ENVIRONMENTAL SWAB

Repeat steps 4-6 using a fresh cotton swab. Once your swab has been dipped in the sterile water, choose a suitable surface to sample by rubbing the swab over the area to be tested for 10-15 seconds.



STEP 9

Now repeat step 7, rubbing the swab over the "E" half of the plate. Finish up by adding two pieces of sellotape to the Petri dish to secure the lid to the base. This should be done as shown in the video to ensure gases can still be exchanged between the inside and outside of the plate - this prevents anaerobic conditions inside the plate.



Invert your plate and incubate at room temperature overnight.

PROTOCOL 2

THE EFFECT OF ANTIFUNGAL TREATMENT ON MICROBIAL GROWTH

Check out our YouTube videos to support this protocol.

Pinkie-palm technique - This video will demonstrate a basic aseptic technique required during microbiological work.

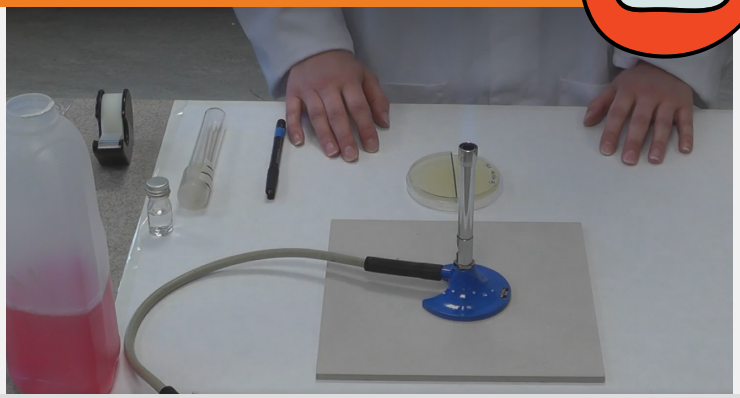
Making a Lawn Plate and adding a treatment-coated disc - this video will show you how to complete this protocol, step-by-step.

METHOD

Protocol 2: To investigate the effect of antifungal medication on microbial growth. These instructions have been written for a right-handed person. Alternatively, watch the whole process here.

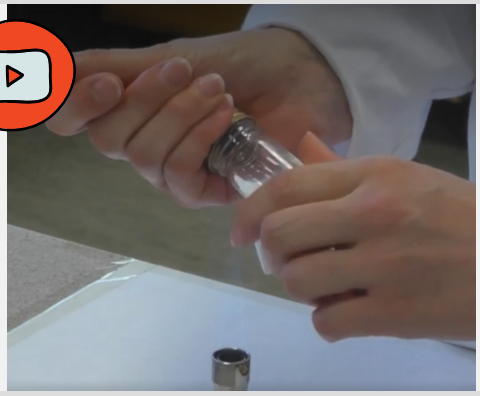
STEP 1 - PREPARE WORKSPACE

Clean your workspace with disinfectant. Set up all materials required within easy reach.



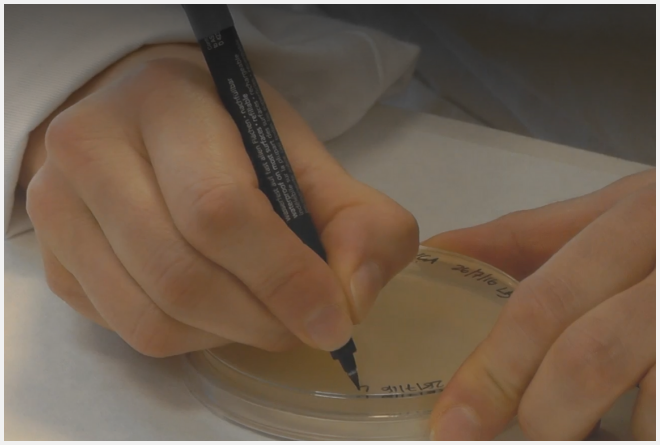
STEP 2 - ASEPTIC TECHNIQUES

Practice your pinkie-palm technique and how to flame the neck of the bottles you will be using.



STEP 3 - LABELLING YOUR PLATE

Collect your sterile YGA plate. Using a marker pen, label your plate with "Date, *S. cerevisiae*, your initials". Note the positioning of the label in the image opposite - small, neat writing around the circumference.

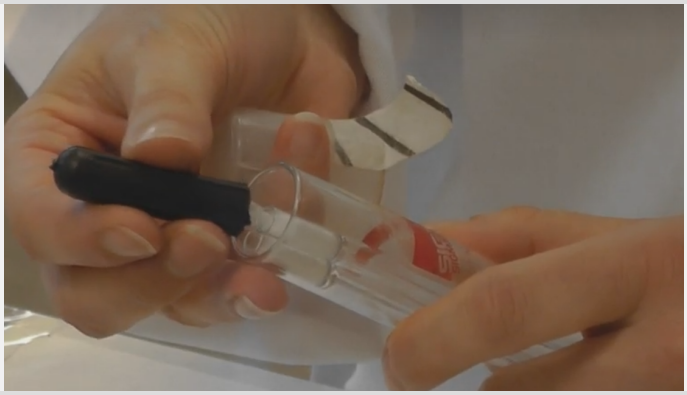


STEP 4 - PREPARING FOR LIQUID TRANSFER

Loosen the top of the bottle of *S. cerevisiae* and container of sterile pipettes so that they can be removed easily.

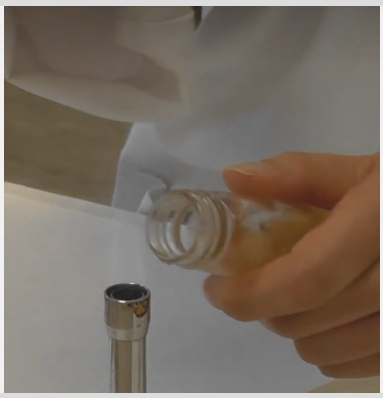
STEP 5

Remove a pipette from its sterile container, taking care not to touch the tip; attach the bulb and hold in the right hand.



STEP 6

Lift the bottle of *S. cerevisiae* and gently agitate to re-suspend. Using Pinkie-palm technique, remove the lid of the bottle and flame its neck.



STEP 7

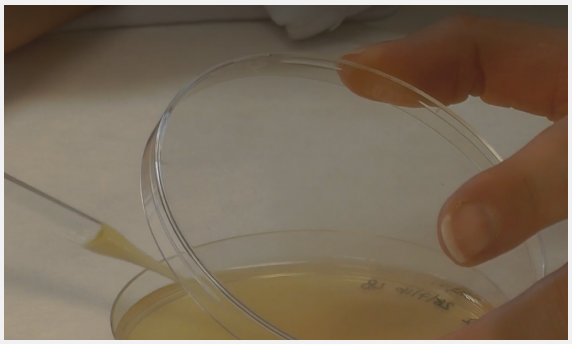
Squeeze the bulb of the pipette before it enters the broth so that it does not cause bubbles. Withdraw a little of the culture.

STEP 8

Flame the neck of the culture bottle again and replace the lid.

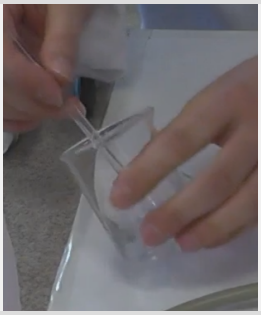
STEP 9 - MAKING THE LAWN PLATE

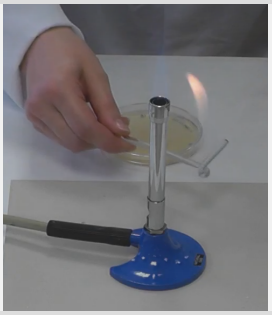
Lift the lid of the YGA plate just a little - place 10 drops of culture on the centre of the plate. Replace the lid of the YGA plate and put the pipette in the discard jar.



STEP 10

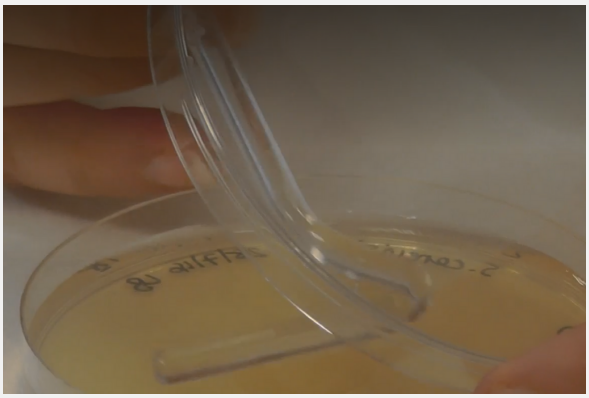
Dip a glass spreader in ethanol; flame and allow the ethanol to burn off.





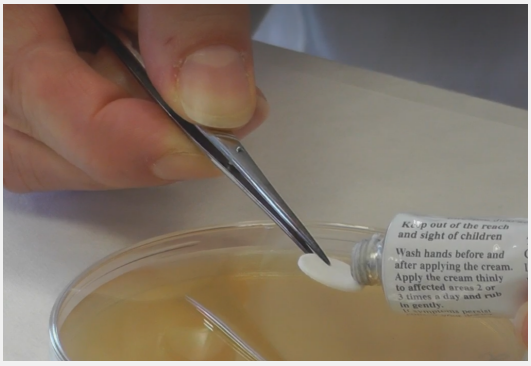
STEP 11

Lift the lid of the YGA plate and spread the culture evenly around the plate using the glass spreader. Make sure the entire surface of the agar is covered. Replace the lid of the YGA plate and flame the spreader using ethanol.



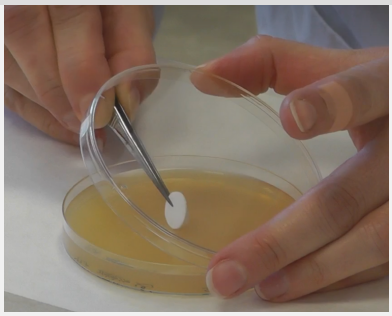
STEP 12 - ADD THE ANTIFUNGAL MEDICATION

Dip the metal forceps in ethanol; flame and allow to burn off. Pick up a sterile filter paper disc and add a small smear of antifungal cream to the disc.



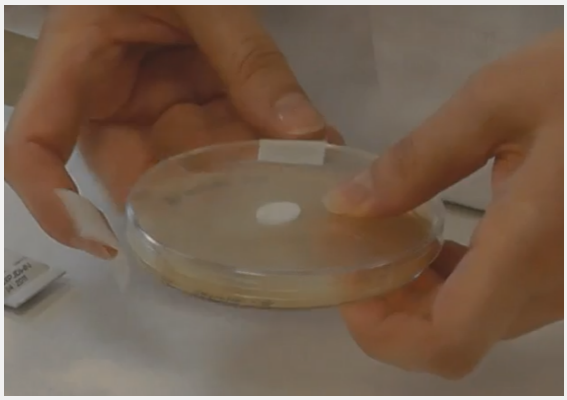
STEP 13

Lift the lid of the YGA plate a little and carefully place the disc, cream-side down, onto the centre of the agar. Dip the forceps in ethanol again, flame and allow to burn off.



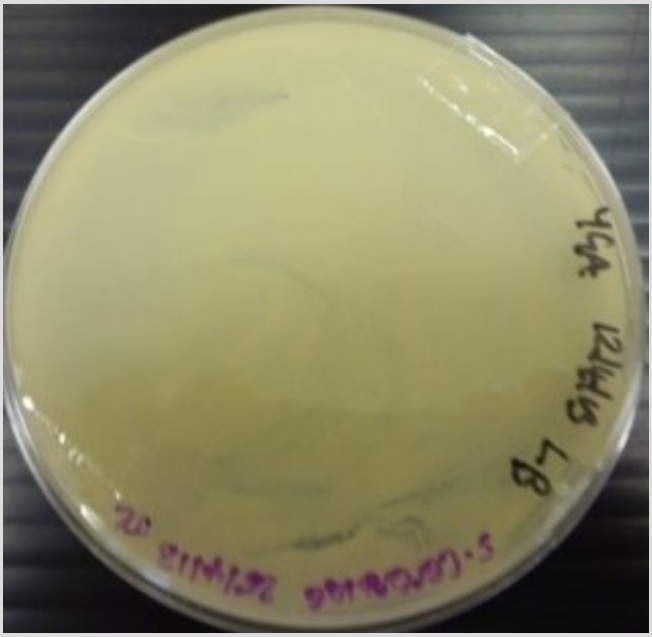
STEP 14

Seal the lid of the Petri dish to the base using two pieces of sellotape. Invert the plate and incubate at room temperature overnight.



RESULTS

This protocol could be performed with and without the antifungal treatment. Inhibition of growth can be quantified by measuring the diameter of the clearance zone. This would be an appropriate methodology at this stage of learning.



A lawn plate of *S. cerevisiae* in the absence of antifungal treatment.

A lawn plate of *S. cerevisiae* in the presence of antifungal treatment.

PROTOCOL 3

OBSERVING *S. CEREVISIAE* USING MICROSCOPY - VITAL STAINING

Check out our YouTube videos to support this protocol.

Pinkie-palm technique - This video will demonstrate a basic aseptic technique required during microbiological work.

Flaming a loop - This video will show you how to prepare a wire loop for sub-culturing techniques to reduce the risk of contamination. BE CAREFUL and WAIT FOR SUPERVISION.

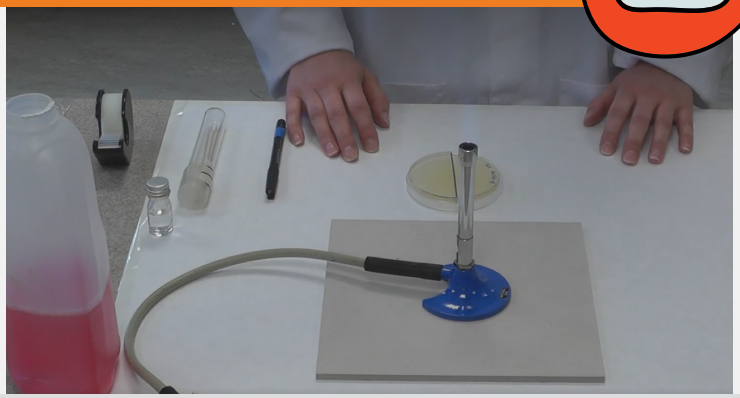
Vital staining of *S. cerevisiae* - This video will take you through the whole procedure.

METHOD

Protocol 3: To investigate the morphology of a micro-organism called *S. cerevisiae*. These instructions have been written for a right-handed person. The whole procedure can be followed using this YouTube video.

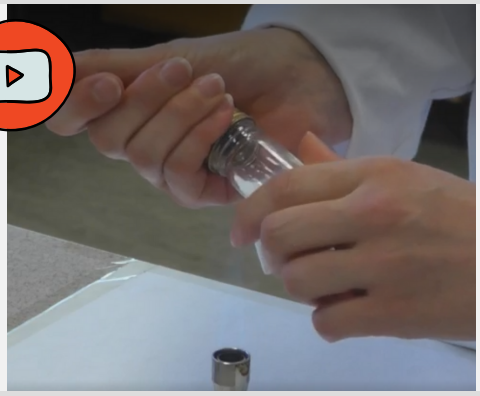
STEP 1 - PREPARE WORKSPACE

Clean your workspace with disinfectant. Set up all materials required within easy reach.



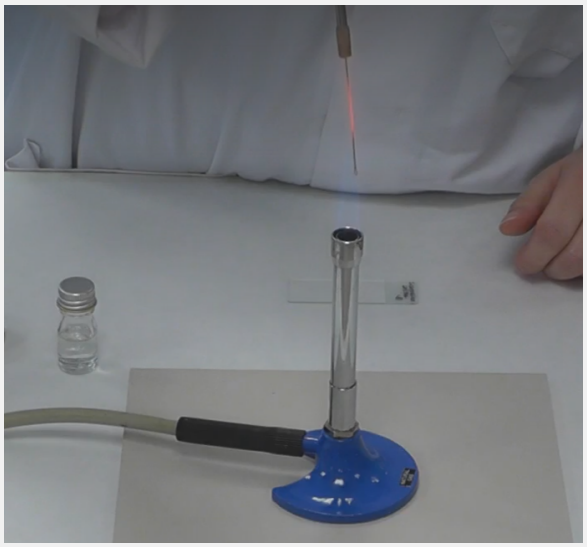
STEP 2 - ASEPTIC TECHNIQUES

Practice your pinkie-palm technique and how to flame the neck of the bottles you will be using. Click on the link to access a step-by-step video tutorial.



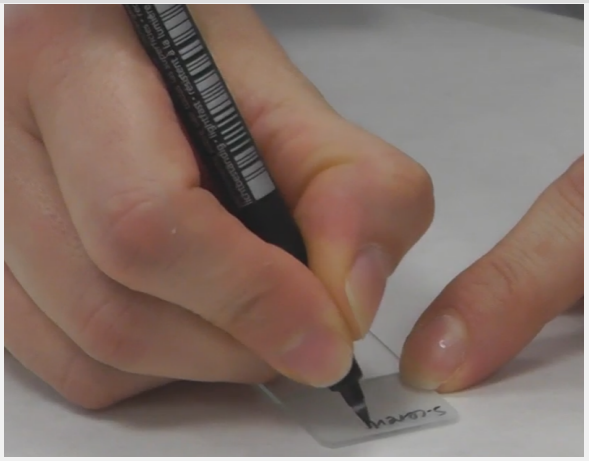
STEP 3 - FLAMING A LOOP

Practice flaming a loop by incineration using red heat. This is an important aseptic technique but be careful with your fingers! Do this with supervision.



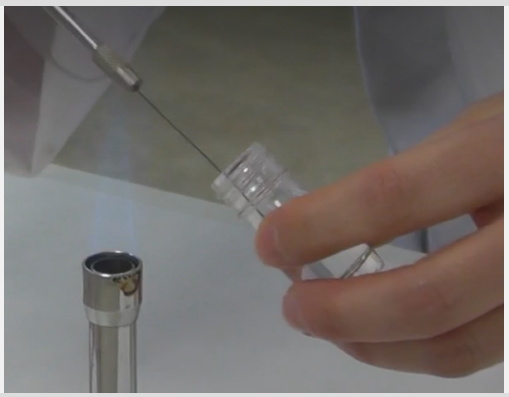
STEP 4 - ASEPTIC TECHNIQUE

Clean a microscope slide and coverslip. Label your slide with the date, *S. cerevisiae* and your initials. Flame your wire loop ready for inoculation.



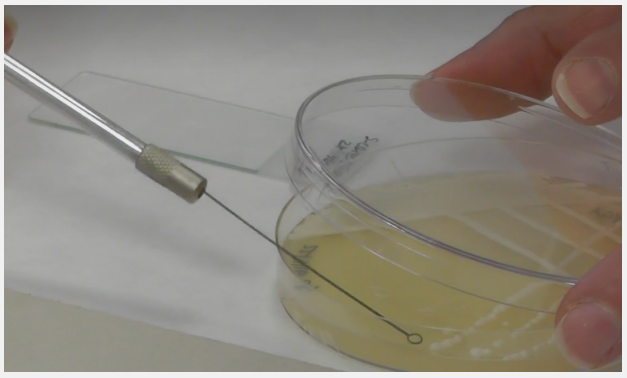
STEP 5 - TRANSFER OF WATER TO SLIDE

Using the sterile wire loop, transfer two loopfuls of sterile, distilled water to the centre of the slide.



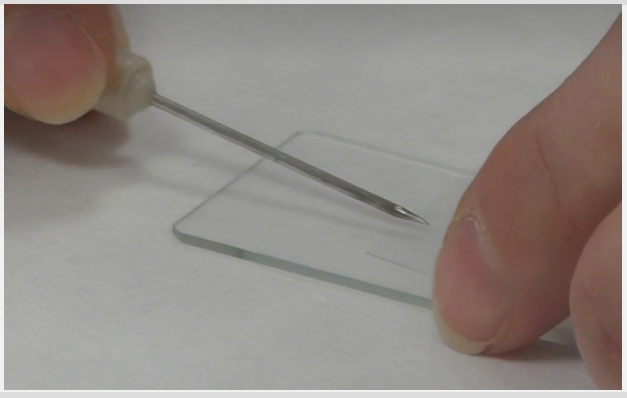
STEP 6

Reflame the wire loop and use it to transfer a small amount of yeast from a single colony into the water on the slide and mix.



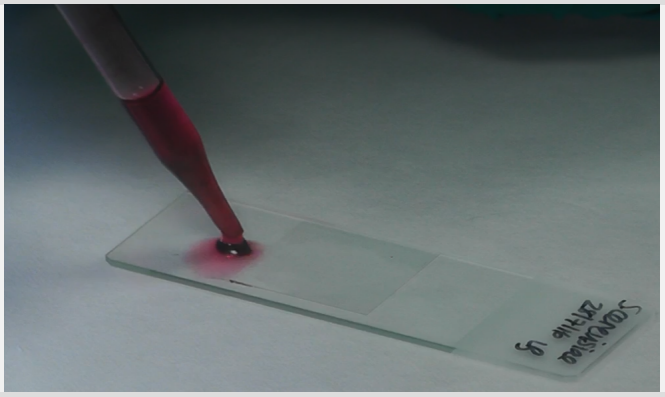
STEP 7

Carefully lower the coverslip, using a mounted needle to help reduce any air bubbles.



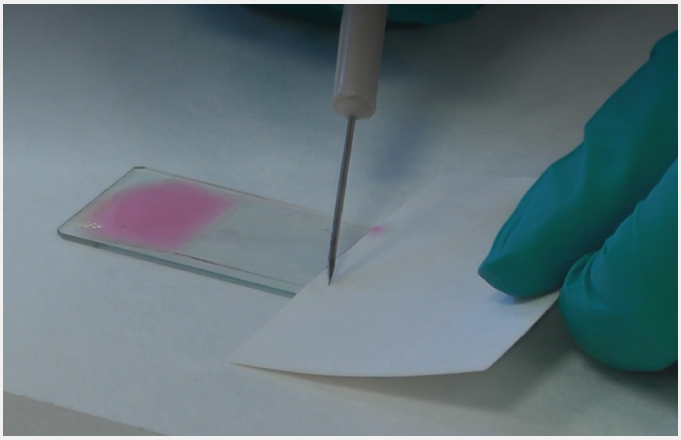
STEP 8

Using a pipette, add a drop of neutral red along one edge of the coverslip.



STEP 9

Place the edge of the blotting paper against the opposite edge of the coverslip to draw through the stain. This is important because the cells are living and not fixed to the slide.



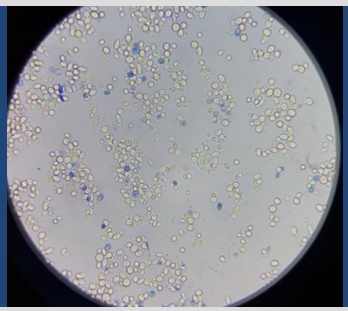
STEP 10 - OBSERVING THE CELLS

Place the slide on the microscope stage and observe under high power (x400). Record the colour of the background and the cells at 5-minute intervals for a period of 20 minutes. Living cells should take up the stain and will appear pink; dead cells will appear clear. This provides a measure of "cell viability" in your sample - what percentage of your cells are alive?

STEP 11 - SAFE DISPOSAL

When you have finished observing your cells, you should place your microscope slide in a discard jar of disinfectant, e.g. Virkon.

Alternative vital stain:
You can replace neutral red with methylene blue, another vital stain. This will be taken up by living cells over time.



CURIOSITY TAKES YOU FURTHER

Young STEM leader programme and STEM Ambassadors in Scotland

STEM AMBASSADOR HUB

STEM AMBASSADORS IN SCOTLAND

The STEM Ambassador in Scotland team are based at SSERC. Would your learners enjoy hearing from people working in the STEM sector of Scotland? Request a STEM ambassador here.

YOUNG leader led by sserc

Support your learners to **Discover**, **Create**, **Lead** and **Inspire** with a YSLP project focused on microbiology, e.g. how would they deal with a future pandemic? Raise the profile of STEM by supporting learners to consider this challenge of the future.

Micro-organisms and careers

Check out the World of Work website for information relating to careers in microbiology.

REFERENCES

SSERC (2018), Safety in Microbiology: A Code of Practice for Scottish schools and colleges.

Young STEM Leader Programme, https://www.youngstemleader.scot/

STEM Ambassadors in Scotland (SAiS), https://www.stemambassadors.scot/

STEM Learning, https://www.stem.org.uk/stem-ambassadors/schools-and-colleges

CONTACT

Any questions about this protocol? Contact Annie McRobbie (annie.mcrobbie@sserc.scot)