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Safety in Microbiology for schools

Practical laboratory manual

CPD for technical support staff in schools

STAC
Scottish Technicians' Advisory Council

Contents

Preparation of disinfectants

1

MATERIALS

- Eye protection
- Disposable gloves
- Hypochlorite or concentrated bleach (Domestos)
- Virkon™
- Measuring cylinder
- Bottle
- Discard jar
- Tap water

INSTRUCTIONS

1) Wear a lab coat and use eye protection and gloves when dispensing disinfectant.

2) Prepare 200 cm^3 of a 1% hypochlorite/Domestos solution, pour into a stoppered bottle and retain for disinfecting work surfaces.

3) Make up 500 cm^3 Virkon™ according to manufacturer's instructions and place in clean discard jar with a lid. Label and retain as discard jar for lab work.

Note
Add Virkon™ to water to prevent excess frothing.

4) Record brief description of the procedure you have carried out in your lab book.

Preparation of spills kit

2

MATERIALS

- Plastic tub
- Disposable plastic gloves (2 pairs)
- 500 cm^3 measuring cylinder
- Virkon™
- 500 cm^3 glass bottle with lid
- Marker pen
- Label
- Paper towels
- Small plastic dustpan and brush
- Plastic tongs
- Autoclavable waste disposal bag

INSTRUCTIONS

1) Wear a lab coat, use eye protection and gloves when dispensing disinfectant.

2) Label tub 'SPILLS KIT'.

3) Measure 500 cm^3 water into the glass bottle and mark the level.

4) Pour out the water.

5) Place a label on the outside of the bottle, the top being level with the mark you have made.

6) Record date and type of disinfectant on label.

7) Measure 5 g Virkon™ into the bottle and replace lid.

8) Place in tub along with paper towels, dustpan and brush, tongs and autoclave bag.

9) Keep spills kit in a conspicuous location.

10) Record briefly the procedure you have carried out in your lab book.

3 Preparation of workspace

MATERIALS
- Benchkote and non-absorbent tape if required
- Disinfectant and paper towel
- Discard jar with disinfectant
- Bunsen burner
- Wire loop/inoculating instrument
- Cultures and media

INSTRUCTIONS
The following applies to right-handed operators. Left-handed people should reverse the arrangement on the bench.

1) Tie back long hair.

2) Wash and dry hands thoroughly.

3) Cover any cuts/grazes with waterproof plaster.

4) Put on lab coat.

5) Collect materials.

6) Attach benchkote to the bench if necessary using sticky tape.

7) Swab the surface of your work space with 1% hypochlorite or 1% bleach using the paper towel. Discard paper towel.

8) Keep work space clear of non-essential items.

9) Place the wire loop/inoculating implement to the right of the bench so that you can reach it with ease.

10) Place the Bunsen burner on a heat resistant mat centrally so that you can reach it with ease but not so close that you are likely to burn yourself.

11) Place cultures and media to the left but still within easy reach. Place discard jar containing Virkon™ to the right within easy reach.

Record brief description of the procedure you have carried out in your lab book.

The bench must be set up in this way each time microbiology practical is carried out.

Media preparation: preparation of nutrient broth

4

MATERIALS

- Beaker
- Nutrient broth powder or YGA ingredients
- Spatula
- Weighing boat
- Weighing scales
- Distilled water
- Measuring cylinder
- Magnetic stirrer/stirring rod
- 10 cm^3 pipette/syringe
- 15 universal bottles and lids
- Autoclave tape
- Water bath
- Marker pen

METHOD

1) Label 15 universals.

2) Add the appropriate quantity of powder to 150 cm^3 deionised water (see manufacturer's instructions).

3) Dissolve powder (use a magnetic flea if possible).

4) Dispense 10 cm^3 volumes into universals.

5) Tighten caps fully then loosen 1/2 turn.

6) Place in container for autoclaving to prevent tipping and spilling.

7) Autoclave at 121° C for 15 minutes.

8) Tighten caps when cool and leave at room temperature.

9) Record briefly the procedure you have carried out in your lab book.

After 24-48 hours, examine your broths.

Record your observations in your lab book in a table such as the one below. Show the results and table to the tutor.

Broth no.	Clear / Turbid	Sterile / Contaminated
No of sterile broths:		
Signature of tutor:		

Preparation of solid media: Preparing the agar

5

Supplier's instructions regarding quantities are found on the containers. Solid media can also be made by adding agar to broth.

MATERIALS

- Nutrient agar powder or YGA ingredients
- Spatula
- Weighing boat
- Weighing scales
- Distilled water
- Measuring cylinder
- Conical flask
- Heat resistant gloves
- Syringes or pipettes
- Cotton wool
- Aluminium foil
- Bunsen burner, tripod and mat
- 15 universal bottles and lids
- Autoclave tape
- Water bath
- Marker pen
- Bottle for large volumes

METHOD 1

For pouring large numbers of plates

1) Measure out the required weight of nutrient agar powder or YGA ingredients to make up 200 cm^3 'solution' (see container to calculate correct weight).

2) Add agar powder to 200 cm^3 of distilled water in an appropriate bottle.

3) Tighten caps fully then loosen 1/2 turn.

4) Autoclave at 121° C for 15 minutes.

5) Cool to 55° C in a water bath.

6) Using aseptic technique, dispense into 10 sterile plastic Petri dishes (see 'pouring plates').

METHOD 2

For student plate and slope preparation

1) Wear eye protection and use heat resistant gloves.

2) Label 10 McCartney bottles (for slopes) with name, date and type of medium (use autoclave tape).

3) Label 5 universal containers (for plates).

4) Measure out the required weight of nutrient agar powder to make up 220 cm^3 'solution' (see container to calculate correct weight).

5) Add agar powder to 220 cm^3 of distilled water in conical flask and swirl around.

6) Make cotton wool stopper.

7) Place stopper in neck of conical flask and cover with aluminium foil (this prevents the cotton wool catching fire when over a Bunsen flame).

8) Place the conical flask containing the agar in a water bath or on a magnetic heater.

9) Heat over a Bunsen flame, gently swirling occasionally (use a heat resistant glove) until it dissolves fully. (N.B. The solution must be heated until the liquid is transparent, otherwise the agar will not set properly - check with tutor).

10) Dispense 12 cm^3 molten agar into each McCartney bottle.

11) Pour molten agar into the universal containers leaving a space of 2-3 cm at the top.

12) Tighten caps fully then loosen 1/2 turn.

13) Place in basket for sterilisation.

14) Autoclave for 15 minutes at 121° C.

15) Cool McCartney bottles tighten lids then lie bottle at an angle against a marker pen or similar. Allow to solidify to form the agar slope.

16) Cool the universals to 55° C then use each universal to pour one plate. Alternatively, allow to solidify and tighten lids of universals when cool. The agar can be stored in this form for several months.

When required, lids of universals should be loosened slightly and agar heated to 100° C until melted then placed in a water bath at 55° C. It is important that the water level in the water bath lies above that of the agar to prevent solidification.

Pouring plates

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MATERIALS

- 5 bottles of sterile nutrient agar
- 15 sterile plastic Petri dishes
- Bunsen burner and mat

METHOD

This method is written for right-handed people. If you are left handed, please reverse handling instructions.

1) Wear a lab coat and use eye protection.

2) Collect the Petri dishes, Bunsen burner and mat.

3) Label the empty sterile Petri dishes on the base with name, date and type of agar. (N.B. if the lid comes off, the plate is no longer sterile and you must discard it).

4) Light the Bunsen burner.

5) Collect one bottle of sterile molten agar from the water bath. Check it is not too hot and that it has not started to solidify.

6) Place a labelled Petri dish right way up on the bench.

7) Check that the top of the bottle of agar is loose (next page).

8) Hold the bottle of agar in the left hand.

9) Unscrew and remove the cap of the bottle with the little finger of the right hand.

10) Pass the neck of the bottle backwards and forwards through a blue Bunsen flame.

11) With the right hand lift the lid of the Petri dish a little and gently pour in the molten agar.

12) Replace the lid of the Petri dish.

13) Replace the cap of the bottle and put it down.

14) Swirl the plate very gently to distribute the agar evenly. (N.B. The base of the plate must be covered, agar must not touch the lid of the plate and the surface must be smooth with no bubbles).

15) Repeat for the other bottles and plates.

16) Leave the agar to solidify.

17) Once cool, turn the plates upside down.

Record brief description of the procedure you have carried out in your lab book.

After 24-48 hours, examine your plates. Record your observations in your lab book in a table such as the one below. Show the results and table to the tutor.

Note
The plates may be stored at room temperature or in the fridge for several weeks depending upon the depth of the agar. Before use, plates which have been stored must be examined very carefully for contamination. Any contaminated plates must be autoclaved then discarded.

Plate no.	Plate characteristic			
	Smooth surface	Even layer of agar	Base covered	Contamination
No of sterile plates:				
Signature of tutor:				

Treatment of spillages

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SPILLAGE KIT CONTENTS

- Disposable plastic gloves
- Bottle of Virkon™ marked with a dilution line
- Disposable cloths or paper towels for soaking with disinfectant and applying to spill
- A small plastic dustpan and paper towels
- Autoclavable plastic tongs
- An autoclavable waste disposal bag

TO DEAL WITH A SMALL SCALE SPILL

1) Wear a lab coat, disposable plastic gloves and use eye protection.

2) Cover spillage with paper towels.

3) Dilute the disinfectant to the recommended concentration.

4) Pour a ring of freshly made up disinfectant around the spill and over the paper towels covering the spill.

5) Leave for at least ten minutes.

6) Using paper towels, sweep the debris on to the plastic dustpan.

7) Using tongs if necessary, place the debris into an autoclavable disposal bag (broken glass should be placed in a separate solid autoclavable container or 'double-bagged').

8) Autoclave the debris.

9) Place in a bin bag and dispose of with domestic waste.

10) Autoclave the dustpan and tongs. If not autoclave safe, immerse in Virkon™ for at least 24 hours.

Record briefly the procedure you have carried out in your lab book.

Sterilistation using a pressure cooker

8

MATERIALS

- Protective gloves
- Pressure cooker/autoclave
- Water

MATERIALS TO BE STERILISED

METHOD

1) Wear a lab coat and use eye protection. Wear protective gloves when handling hot equipment.

2) Add water to the recommended depth (3 cm to the base of the pressure cooker).

3) Place the trivet/stand in the base if required.

4) Loosen caps slightly (tighten then loosen a half turn) on bottles and place the materials to be sterilised in cooker/autoclave. Note that bottles should contain a maximum of 500 cm^3 of medium and there should be space for expansion above the medium in the bottle.

5) Place Browne's tube/test strip as close as possible to centre of materials to be sterilised.

6) Secure the lid of the pressure cooker/autoclave according to the manufacturer's instructions.

7) Heat on an electric or gas ring till steam issues evenly or switch on if the heater is integral.

8) Place on the valve (pressure cooker and some autoclaves).

9) When cooker begins to 'hiss' evenly, turn down heat.

10) Continue heating gently for 15 minutes. Note that steam should continue to issue gently from the valve - if it does not, pressure and temperature are likely to have fallen.

11) Turn off heat and allow to cool. Do not attempt to speed up cooling - this can distort the pressure cooker and can cause media to boil over.

12) Take care when opening lid that steam does not issue towards the operator.

13) Allow sterilised materials to cool before removing from the vessel.

Record briefly the procedure you have carried out in your lab book.

Sub-culturing

9

Subculturing involves removal of an inoculum from a source such as an agar plate, broth or test surface and the inoculation of medium such as broth or agar.

Note
Instructions for sub-culturing techniques are intended for right-handed operators. If you are left-handed, please reverse the instructions accordingly.

Carry out different combinations of the following techniques. Instructions are given on the following pages.

- For each technique, record briefly the procedure you have carried out in your lab book.
- After 24-48 hours, examine your cultures. Record your observations in a table. For each broth, record whether it is turbid (cloudy) and therefore there is growth.
- For each plate, record in a table whether there is growth, single colonies if appropriate and contamination.
- Example tables are shown below.

Show your cultures to a tutor and ask them to sign your lab book if appropriate.

- Organism
- Turbidity
- Growth
- Signature

MATERIALS

- Lab coat
- Eye protection
- Disinfectant and paper towels
- Discard jar with disinfectant
- Bunsen burner and mat
- Sterile swabs
- Bijoux bottle of sterile water
- Wire loop
- Forceps
- Scalpel
- Cork borer
- Sterile Pasteur pipettes
- Ethanol in a covered beaker
- Inoculate - plate and slope cultures
- Inoculate - broth cultures/sterile water
- Sterile agar plates
- Sterile agar slopes
- Sterile broths
- Sellotape

Note
The instructions are for right-handed people. If you are left-handed, please reverse the instructions accordingly.

Organism	Turbidity	Growth	Signature

Organism	Growth	Single colonies	Contamination	Signature

From environment using sterile swab

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

2) Lift a bottle of sterile water in the left hand. Do not put down swab.

3) Remove the lid of the bottle with the little finger of the right hand, which is still holding the swab.

4) Flame the neck of the bottle.

5) Insert and withdraw the cotton wool bud.

6) Flame the neck of the bottle and replace the lid.

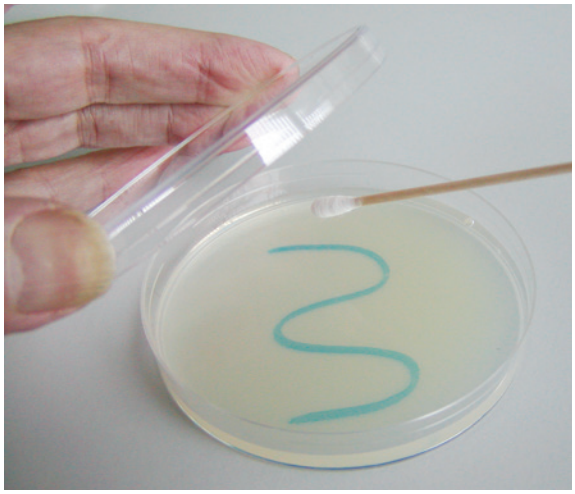
7) Rub the swab on the area to be tested for 10-15 seconds.

To plate using swab or wire loop

1) Partially lift the lid of the agar plate and gently rub the swab across the surface of the agar (see pictue below). Take care not to break the agar surface.

2) Replace lid of Petri dish.

3) Sellotape lid to base of plate (two diametrically opposed pieces).



Plating out using a swab.

From slope

1) Loosen the top of the bottle containing the slope culture so that it can be removed easily.

2) Hold the loop in the right hand.

3) Flame the loop and allow to cool. Do not put down loop or wave it around.

4) Lift the universal containing the inoculum with the left hand.

5) Remove the lid of the universal with the little finger of the right hand. (Turn the bottle, not the lid). Do not put down the lid.

6) Flame the neck of the universal.

7) Bringing the bottle to the loop, insert the loop into the bottle, touch a small area of growth and withdraw loop.

8) At all times, hold the loop as still as possible.

9) Flame the neck of the universal.

10) Replace the lid on the universal using the little finger, turning the bottle not the lid.

11) Place slope on bench.

To agar slope

1) Lift a sterile agar slope with the left hand.

2) Remove the lid of the bottle/tube with the little finger of the right hand which still holds the charged loop.

3) Flame the neck of the bottle/tube.

4) Streak the loop backwards and forwards across the surface of the agar slope and remove the loop.

5) Flame the neck of the bottle/tube, replace the lid and place the bottle/tube in rack or on bench.

6) Flame the loop and place on a heat resistant mat.

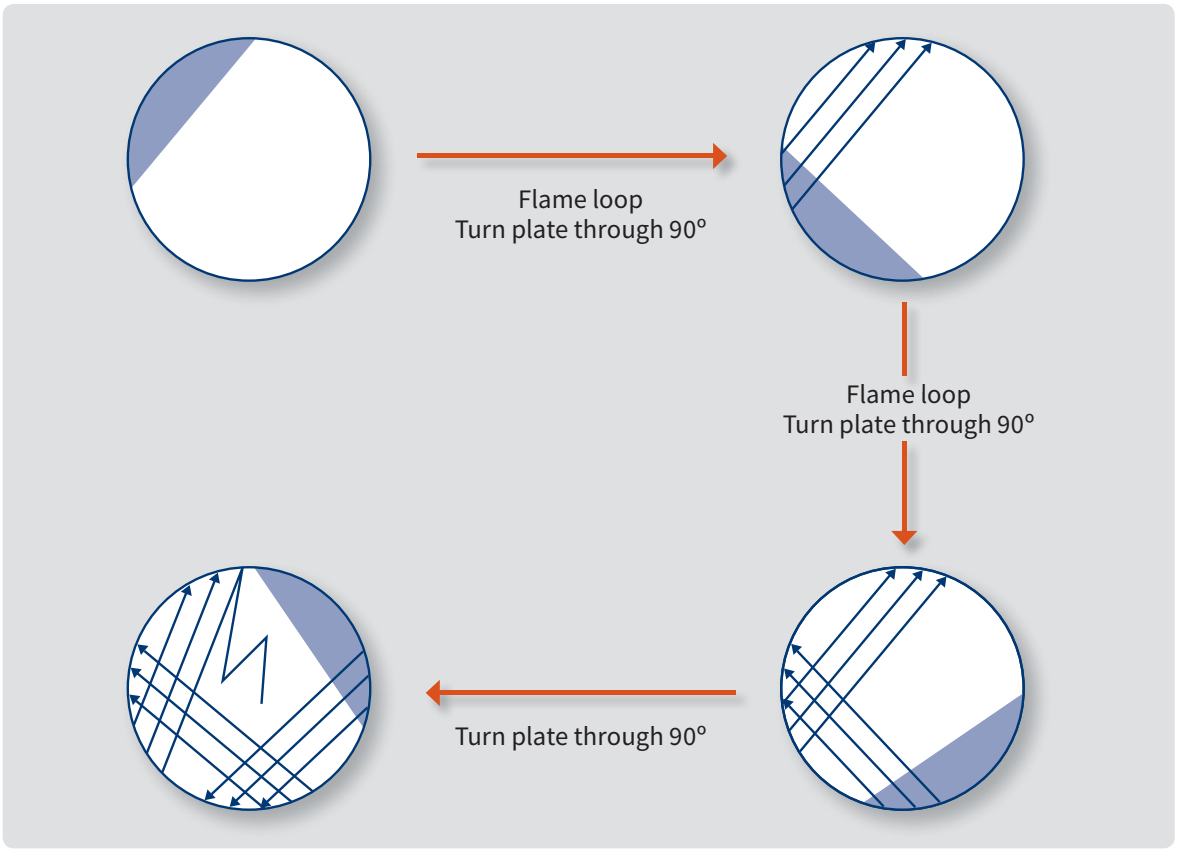
From plate

1) Hold the loop in the right hand.
2) Flame the loop and allow to cool. Do not put down loop or wave it around.
3) With the left hand, lift the lid a little of the Petri dish containing the inoculum.
4) Touch a single colony with the wire loop.
5) Withdraw loop carefully without touching plate. Do not put down loop or wave it around.
6) Replace lid of Petri dish.

To plate (streak)

See diagram below.

1) Partially lift the lid of the Petri dish containing the solid medium.
2) Holding the charged loop parallel with the surface of the agar, smear the inoculum backwards and forwards across a small area of the medium (streaked area = A).
3) Flame the loop and allow to cool. Turn dish and streak loop from region A across the surface of the agar in three parallel lines (to B).
4) Flame the loop and allow to cool. Turn dish and streak loop from B across the surface of the agar in three parallel lines (C).
5) Turn dish and streak loop from C to D across surface of agar.
6) Replace lid of Petri dish.
7) Flame loop and place on heat resistant mat.



From liquid

1) Loosen the tops of the universals containing the broth cultures so that they can be removed easily.

2) Hold the loop in the right hand.

3) Flame the loop and allow to cool. Do not put down loop or wave it around.

4) Lift the universal containing the inoculum with the left hand and gently agitate to resuspend.

5) Remove the lid of the universal with the little finger of the right hand. (Turn the bottle, not the lid). Do not put down the lid.

6) Flame the neck of the universal.

7) Bringing the bottle to the loop, insert the loop into the culture broth and withdraw.

8) At all times, hold the loop as still as possible.

9) Flame the neck of the universal.

10) Replace the lid on the universal using the little finger, turning the bottle not the lid.

11) Place universal on bench.

To liquid

1) Lift a universal of sterile nutrient broth in the left hand.

2) Remove the lid of the universal with the little finger of the right hand which still holds the charged loop or pipette. Do not put down the lid.

3) Flame the neck of the universal.

4) Insert the loop charged with inoculum into the sterile broth. Touch on the inside of the universal and withdraw.

If using a pipette, gently release the required number of drops from the pipette into the culture and withdraw pipette.

Do not agitate or cause bubbles in the liquid medium.

5) Flame the neck of the universal, replace lid and place the universal on the bench.

6) Flame the loop and place on heat resistant mat place pipette in discard jar.

7) Tighten lid of universal to make secure but do not over tighten.

From liquid medium using a Pasteur pipette

1) Loosen the tops of the universals containing the broth cultures so that they can be removed easily.

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

3) Lift the universal containing the inoculum with the left hand and gently agitate to re-suspend.

4) Remove the lid of the universal with the little finger of the right hand. (Turn the bottle, not the lid). Do not put down the lid.

5) Flame the neck of the universal.

6) Squeeze the teat of the pipette before it enters the broth so that it does not cause bubbles and possible aerosols and withdraw a little of the culture.

7) At all times, hold the pipette as still as possible.

8) Flame the neck of the universal.

9) Replace the lid on the universal using the little finger, turning the bottle not the lid.

10) Place universal on bench.

To plate (lawn)

1) With the left hand, partially lift the lid of the Petri dish containing the solid medium.

2) Take care not to drip culture from the end of the pipette, place ten drops of culture to the centre of the plate or enough to cover a 5 pence piece.

3) Replace the lid of the Petri dish.

4) Place pipette in discard jar.

5) Dip glass spreader in ethanol. Flame and allow the ethanol to burn off.

6) Lift the lid of the Petri dish to allow entry of spreader.

7) Spread drops of liquid culture evenly around plate, rotating plate with left hand if this feels comfortable. Make sure the entire agar surface is covered.

8) Replace the lid of the Petri dish.

9) Flame spreader using ethanol.

From mould plate

Using:

i) scalpel
ii) forceps
iii) cork borer (or blunt end of Pasteur pipette)
iv) mounted needle

1) Dip the implement in ethanol, place in Bunsen flame briefly and allow ethanol to burn off. Do not put it down.

2) Lift the lid a little of the Petri dish containing the inoculum with the left hand.

3) Hold the inoculating implement in the right hand.

4) Keep the ethanol at a safe distance from the Bunsen flame.

5) i) with scalpel, cut a square shape in the fungal mycelium;
ii) with forceps, pick up a little piece of mycelium;
iii) with cork borer or pipette, cut a core of mycelium.

(If the centre of the colony is producing spores, remove the inoculum from the edge of the colony).

6) Using the appropriate implement, lift a cube, strands or core of agar with fungal mycelium.

7) Withdraw implement from Petri dish.

8) Replace lid of Petri dish.

9) At all times, hold the inoculating implement as still as possible.

To mould plate

Using:

i) scalpel
ii) forceps
iii) cork borer (or blunt end of Pasteur pipette)
iv) mounted needle

1) Partially lift the lid of the Petri dish containing the sterile medium.

2) Place the cube/core of agar or fungal mycelium on to the centre of the sterile agar. Use a mounted needle, which has been flamed in ethanol to assist with the transfer process if necessary.

3) Withdraw implement.

4) Dip the implement in ethanol, flame as above and place on heat resistant mat.

Preparation of a smear of bacteria or yeast from a solid culture

10

MATERIALS

- Lab coat
- Eye protection
- Benchkote if necessary
- Disinfectant and paper towels
- Discard jar with disinfectant
- Bunsen burner and mat
- Glass slide
- Lens tissue
- Labels
- Forceps
- Loop
- Bottle of sterile water (bijoux or universal)
- Plate cultures of yeast and named bacteria
- Blotting paper

INSTRUCTIONS

1) Wear a lab coat and use eye protection.

2) Collect materials.

3) Clean slide thoroughly using lens tissue. If necessary pass it through a hot Bunsen flame to remove grease.

4) Label one end of the slide with initials, date and organism (use a self adhesive label, wax pencil or glass marker).

5) Flame loop.

6) Using aseptic technique, transfer a loopful of sterile water on to the centre of the slide.

7) Flame loop.

8) Using aseptic technique, transfer a very small part of a single colony from the plate culture into the water and mix well, making sure that the smear is not too thick.

9) Flame loop and place it on a heat resistant mat.

10) Using forceps to hold the slide and with the film downwards, pass the smear through a yellow/blue flame several times to ‘fix’ it.

11) Place on a heat resistant mat to cool.

The smear is now ready to be stained.

Simple staining of a smear preparation

11

MATERIALS

- Lab coat
- Eye protection
- Disposable gloves
- Benchkote if necessary
- Disinfectant and paper towels
- Discard jar with disinfectant
- Fixed smears of bacteria or yeast
- Fibre free blotting paper
- Labels
- Forceps
- Staining rack and dish
- Distilled water bottle
- Methylene blue stain

INSTRUCTIONS

1) Wear a lab coat, disposable gloves and use eye protection.

2) Place the fixed smears of bacteria on a staining rack over a sink or staining tray (pie dish).

3) Flood with methylene blue and leave for 2 minutes.

4) Hold the slide at a 45° angle over the sink or staining tray, use the wash bottle to rinse the smear well with water.

5) Blot dry between two layers of fibre free blotting paper, taking care not to rub off the cells and allow to dry in air.

6) Examine under oil immersion if possible. Otherwise, under x600 (x15 eyepiece, x40 objective).

7) Record shape (rod, spherical or spiral) and arrangement (clusters, chains or pairs) of the bacteria examined.

8) When finished, dispose of slides into discard jar.

9) In your lab book, record brief description of the procedure and draw diagrams of the organisms you examine. Make sure that you record the name of the organism and the magnification used.

N.B. This method can be used for other simple stains.

Negative stain

12

MATERIALS

- Lab coat
- Eye protection
- Disposable gloves
- Disinfectant and paper towels
- Discard jar with disinfectant
- Glass slide
- Lens tissue
- Wire loop Labels
- Small beaker
- Nigrosin stain
- Live yoghurt
- Sterile water

INSTRUCTIONS

1) Wear a lab coat, disposable gloves and use eye protection.

2) Mix a little yoghurt with an equal quantity of distilled water in a beaker.

3) Flame loop.

4) Place a loopful of the mixture at one end of a glass slide.

5) Flame loop.

6) Add one loopful of nigrosin stain and mix thoroughly using the wire loop.

7) Flame loop.

8) Prepare the film by dragging the yoghurt/stain mixture along the slide with the short edge of another slide. Do not fix with heat.

9) When absolutely dry, examine under oil immersion if possible. Otherwise, under x 600 (x15 eyepiece, x40 objective).

10) When finished, dispose of slides into a discard jar.

In your lab book, record brief description of the procedure and draw diagrams of the organisms you examine.

Make sure that you record the name of the organism and the magnification used.

Vital stain

13

MATERIALS

- Lab coat
- Eye protection
- Disposable gloves
- Benchkote if necessary
- Disinfectant and paper towels
- Discard jar with disinfectant
- Bunsen burner and mat
- Microscope
- Lens tissue
- Glass slide and coverslip
- Wire loop
- Distilled water
- Plate culture of yeast
- Neutral red
- Fibre free blotting paper

INSTRUCTIONS

1) Wear a lab coat, disposable gloves and use eye protection.

2) Clean slide and coverslip.

3) Using aseptic technique, transfer two loopfuls of distilled water to the centre of the slide.

4) Using aseptic technique, transfer a small amount of yeast from a single colony into the water on the slide and mix.

5) Carefully lower the coverslip.

6) Using the Pasteur pipette, draw up a little neutral red.

7) Slowly release the stain along one edge of the coverslip.

8) Place the edge of the blotting paper against the opposite edge of the coverslip to draw through the stain.

9) Observe under high power (x40) of the microscope.

10) Record the colour of the background and the colour of the cells at five minute intervals for a period of twenty minutes.

In your lab book, record brief description of the procedure and draw diagrams of the organisms you examine. Make sure that you record the name of the organism and the magnification used.

N.B. This method can be used with other vital stains and micro-organisms.

The Gram's stain technique

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MATERIALS

- Lab coat
- Eye protection
- Disposable gloves
- Benchkote if necessary
- Disinfectant and paper towels
- Discard jar with disinfectant
- Fixed smears of bacteria
- Staining rack
- Crystal violet
- Gram's iodine
- 95% ethanol
- Safranin
- Labels
- Forceps
- Fibre free blotting paper
- Distilled water bottle
- Bunsen burner and mat

INSTRUCTIONS

1) Wear a lab coat, disposable gloves and use eye protection.

2) Place the fixed smears of bacteria on a staining rack over a sink or staining tray (pie dish).

3) Flood with crystal violet and leave for 1 minute.

4) Flood with Gram's iodine for 1 minute. (The iodine acts as a mordant i.e. a substance which increases the affinity of the bacterial cell for the dye).

5) Hold the slide at a 45° angle and use the wash bottle to rinse the slide well with water. Drain.

6) Hold the slide at a 45° angle over the sink or staining tray, apply the alcohol at the top of the slide with the dropper and allow the decolouriser to run down the slide over the smear, then rinse immediately with water.

7) Flood with safranin for 1 minute.

8) Wash well with water.

9) Blot and allow to dry in air.

10) Examine under oil immersion if possible. Otherwise, under x600 (x40 objective, x15 eyepiece).

11) Record the Gram reaction (positive or negative), shape (rod, spherical or spiral) and arrangement (clusters, chains or pairs) of the bacteria examined.

12) When finished, dispose of slides into a discard jar.

Lactophenol blue staining of a filamentous fungus

15

MATERIALS

- Lab coat
- Eye protection
- Disposable plastic gloves
- Benchkote if necessary
- Disinfectant and paper towels
- Discard jar with disinfectant
- Bunsen burner and mat
- Lens tissue
- Glass slide and coverslip
- Forceps and mounted needle
- Covered beaker containing ethanol
- Plate culture of mould fungus
- Lactophenol blue (keep lactophenol off skin)
- Microscope

INSTRUCTIONS

1) Wear a lab coat, disposable gloves and use eye protection.

2) Clean slide.

3) Place a drop of lactophenol blue in middle of slide.

4) Flame forceps with ethanol and replace lid.

5) Using aseptic technique, partially lift the lid of the Petri dish and use forceps to remove a small piece of the fungal colony.

6) Replace lid.

7) Place fungus in lactophenol blue and tease out well using forceps and needle.

8) Flame forceps and needle with ethanol.

9) Using the mounted needle to support the coverslip, carefully lower it over the preparation. Take care to avoid producing bubbles.

10) Examine under the microscope using the x40 objective lens.

In your lab book, record brief description of the procedure and draw diagrams of the organisms you examine. Make sure that you record the name of the organism and the magnification used.

Setting up a microscope

16

MATERIALS

- Microscope
- Prepared slides of micro-organisms
- Lens tissue

INSTRUCTIONS

1) Clean the lenses and all other glass surfaces on the microscope with lens tissue to remove dirt and greasy marks as you have been shown.

2) Feel beneath the stage for the iris diaphragm control and set it to about half open.

3) Turn the condenser focus control and note the movement of the condenser. Raise the condenser fully, then lower 1-2 mm. Focus the condenser as shown by the lecturer.

4) Place the slide on the stage, specimen uppermost and manoeuvre the slide until the specimen is above the light source.

5) Turn the x 10 objective lens till it clicks into place above the slide.

6) Turn the lamp control to a low setting and switch it on.

7) Turn the rough (coarse) focus knob till the objective lens is as close as possible to the stage.

8) Look down the eyepiece lens and slowly turn the rough (coarse) focus knob till the material on the slide comes in to focus.

9) Turn the fine focus knob till the image becomes sharp. The specimen should now be sharply focused but may be unevenly illuminated.

10) Look down the eyepiece and move the mirror till the field of view becomes brightly and evenly filled with light. The specimen should now be in sharp focus and evenly illuminated but may be too bright.

11) Remove the eyepiece, look down the body tube and adjust the iris till the disc of light takes up 50% of the circle.

12) Replace the eyepiece. The microscope should now be set correctly under x10 magnification.

13) Manoeuvre the slide until the desired part of the specimen is in view.

14) Adjust the fine focus if necessary.

15) If appropriate, make a labelled diagram. Include a heading and magnification.

16) Rotate the turret to bring the x40 objective lens above the slide.

Do not further adjust the coarse focus control!

17) Remove one eyepiece, look down the body tube and adjust the iris till the disc of light takes up 50% of the circle. Replace the eyepiece.

18) Using only the fine focus knob, make the image sharp.

19) Record brief description in your lab diary/ notebook of the procedure you have carried out and make drawings of the specimen. Make sure your diagrams have headings and that the magnification is included.

The following applies only if you are using x100 objective:

20) Turn the turret till the x40 and x100 lenses are at equal distances from the slide.

21) Carefully place a single drop of immersion oil on the slide. If immersion oil drips on to any other part of the microscope, wipe it off immediately and inform the lecturer or the technician.

22) Rotate the turret to place the x100 objective lens in the oil on the slide.

Do not adjust the coarse focus control!

23) Remove one eyepiece, look down the eyepiece tube and adjust the iris till the disc of light takes up 50% of the circle. Replace the eyepiece.

24) Using the fine focus control make the image sharp. Make drawings of the specimen.

25) When you have finished working with the microscope, clean the lenses thoroughly before putting it away.

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