ASSIST AUSTRALIAN SCHOOL SCIENCE INFORMATION SUPPORT FOR TEACHERS AND TECHNICIANS

ASSIST INFORMATION SHEET:

Preparing sterile equipment for microbiology

Equipment used in microbiology should be sterile before using. This enables aseptic techniques to be used when transferring microorganisms for inoculation, sampling environmental areas, adding susceptibility discs to agar plates and Gram staining.

This equipment should be prepared before the class activity and stored in clean, lidded containers.

Equipment such as hockey stick spreaders, inoculating loops and sterile swab sticks can be purchased as single-use items from commercial scientific suppliers if the school budget allows or it is more time effective to do so.

In-house preparation of sterile items is cost effective to schools as some pieces of equipment can be repeatedly recycled. Care should be taken with ethanol as it is a flammable substance and should not be used near a naked flame.

Considerations:

- Sterilisation of equipment should be performed in a draught-free area.
- Items to be sterilised should be clean and dry, metal forceps should not be rusty, glass items should not have chips or cracks.
- Consult the planned activity or activities prior to sterilising items to ensure there is the required number of items available during the activity.
- Ensure the bench area for this purpose has been decontaminated with 70% ethanol prior to commencing.
- Soaking items in a container of 70% (v/v) ethanol for 10 minutes, disinfects/decontaminates, but does not sterilise items. Alcohols are not sporicidal.
- Aluminium foil or greaseproof paper may be used to wrap sterile items.
- Sterile items can be stored in a large lidded plastic container that has been decontaminated with ethanol and paper towel.
- **Glassware and metal instruments can be wrapped in aluminium foil and sterilised using dry heat in an oven at 160°C for 2–3 hours.**
- **All sterilising processes using an autoclave/steriliser or pressure cooker should be at 121°C for 15–20 minutes at 15psi (pounds per square inch of pressure).**
- Professional microbiologists and higher education providers promote the sterilisation technique of ‘flaming’ hockey stick spreaders and forceps prior to using by dipping in 70% ethanol and igniting it in the Bunsen flame. Incorrect techniques can encourage microbial aerosol transmission and risk the ethanol catching on fire. **Science ASSIST does not recommend this practice in the school setting, but instead recommends sterilising these items in an autoclave or an oven.**

Item	Suggested sterilising technique	Alternative technique
Sterile plastic Petri dishes	Purchase sterile, leave wrapped in original packaging until required. (Do not autoclave prior to use. Plates do not retain shape when autoclaved.)	
Sterile glass Petri dishes	Wrap glass Petri dishes in greaseproof paper or aluminium foil and sterilise in an autoclave	Wrap in aluminium foil. Sterilise using dry heat in an oven at 160°C for 2–3 hours
Nutrient agar plates	Prepare agar solution according to the manufacturer's instructions, autoclave in a heat-safe bottle with lids loose and pour plates when temperature of sterile agar is ~50^0C using aseptic technique. When set, wrap in plastic wrap. Store at 4°C until required. See ASSIST SOP: Preparing agar plates	Purchase prepared and sterile from a biological supplier
Nutrient broth	Prepare broth solution according to the manufacturer's instructions. Aliquot ~15mL into McCartney bottles (28mL capacity) keep lids loose. Autoclave. When cool tighten lids and store at 4°C until required.	Purchase prepared and sterile from a biological supplier
Sterile water	Aliquot 2mL into Bijou bottles (7mL capacity) keep lids loose. Autoclave. When cool tighten lids and store at 4°C until required.	
Sterile plastic dropping pipettes	Purchase single-use pipettes from commercial scientific, biological or medical suppliers.	
Sterile swab stick	Purchase sterile, leave wrapped in original packaging until required.	Autoclave cotton buds in foil covered beaker.
Sterile 'L' spreader	Wrap in aluminium foil and autoclave. Store until required.	Wrap in aluminium foil. Sterilise using dry heat in an oven at 160°C for 2-3 hours.
Sterile forceps	Wrap in aluminium foil or place inside a clean test tube, cover opening with aluminium foil and autoclave. Store until required.	Wrap in aluminium foil. Sterilise using dry heat in an oven at 160°C for 2-3 hours.
Sterile test tubes/ conical flasks	Cover opening with foil or plug with non-absorbent cotton wool. Autoclave.	Cover opening with aluminium foil. Sterilise using dry heat in an oven at 160°C for 2–3 hours
Inoculating loop	Flame to red heat in the blue flame of the Bunsen burner.	Purchase sterile disposable inoculating loops, leave wrapped in original packaging until required.

References

U.S. Department of Health and Human Services. 2009. *Biosafety in Microbiological and Biomedical Laboratories.* 5th edition. Centers for Disease Control and Prevention website, http://www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf

Society for General Microbiology. 2006. *Basic Practical Microbiology – A Manual.* Microbiology Online website, http://www.microbiologyonline.org.uk/file/ca2189fba3b39d24c5a44c1285d0082c.pdf

https://www.cdc.gov/HICPAC/Disinfection_Sterilization/13_10otherSterilizationMethods.html