



Preparing sterile equipment and media for microbiology

Planning

Equipment and media used in microbiology should be prepared and sterilised well in advance of the planned activity day.

Staff preparing items for microbiological activities should carefully consider the following:

- Consult the planned activity or activities prior to sterilising equipment/media to ensure there is sufficient available during the activity.
- Equipment to be sterilised should be clean, dry and in good condition, metal forceps should not be rusty, glassware should not have chips or cracks.
- Ensure the work bench has been decontaminated with 70% v/v ethanol or a hospital grade disinfectant.
- **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/ pressure cooker should be conducted at 15psi, 121°C for 15-30 minutes.**
- Some professional microbiologists and higher education providers promote the sterilisation technique of 'flaming' L-shaped spreaders (Hockey sticks) and forceps prior to use by dipping in 70%v/v ethanol and flaming in the Bunsen burner 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/pressure cooker, by using dry heat in an oven or alternatively using commercially purchased sterile disposable items.

- Some equipment such as L-shaped spreaders (Hockey sticks), inoculating loops and sterile swab sticks can be purchased as single-use sterile items from commercial scientific suppliers if the school budget allows.
- Sterile equipment can be stored for 2-3 months if the packaging is not breached.
- Prepared agar or broth should be sterilised in an autoclave/pressure cooker at 15psi, 121°C for 15-30 minutes.
- Stock sterile agar or nutrient broth can be stored for up to 6 months if they remain in the container (bottle) they were sterilised in, and the container has not been reopened.
- Poured nutrient agar plates are best prepared up to a week before an activity, and not stored for more than 4 weeks at 4°C stacked upside down in sealed plastic bags. Agar plates purchased commercially may be stored at 4°C for 4-5 weeks in their original packaging. Storing agar plates for any longer may lead to them drying out. All agar plates should be checked for contamination prior to use.
- In-house preparation of sterile items is cost effective to schools as some pieces of equipment can be repeatedly recycled.

Table showing suggested and alternative sterilising techniques.

Item	Suggested sterilising technique	Alternative technique
Plastic Petri dishes	Purchase sterile plastic Petri dishes and leave wrapped in original packaging until required. (No need to autoclave prior to use. Plates do not retain shape when autoclaved.)	
Glass Petri dishes	Wrap in aluminium foil and sterilise in an autoclave/pressure cooker.	Wrap in aluminium foil and sterilise using dry heat in an oven.
Nutrient agar plates	Prepare agar according to the manufacturer's instructions in a heat-safe bottle with the lid loose and sterilise in an autoclave/pressure cooker. Pour plates when temperature of sterile agar is ~50°C using aseptic technique. When set, wrap in plastic wrap. Store upside down at 4°C and use within 4 weeks. See <i>SOP: Preparing nutrient and plain agar plates</i> . https://assist.asta.edu.au/resource/4755/microbiology-sops-updated-school-level-2	Purchase prepared sterile nutrient agar plates from a biological supplier.
Nutrient broth	Prepare broth solution according to the manufacturer's instructions. Aliquot 10-15mL into small, lidded glass bottles such as McCartney bottles (28mL capacity) keep lids loose and sterilise in an autoclave/pressure cooker. When cool, tighten lids, store at 4°C and use within 4 weeks.	Purchase prepared sterile nutrient broth from a biological supplier.
Sterile water	Aliquot 10-15mL into small, lidded glass bottles such as McCartney bottles, keep lids loose and sterilise in an autoclave/pressure cooker. When cool, tighten lids, store at 4°C and use within 4 weeks.	Purchase sterile water in ampoules or small bottles from pharmacies or biological suppliers.
Sterile plastic dropping pipettes	Purchase sterile single-use pipettes and leave wrapped in original packaging until required.	
Sterile swab sticks	Purchase sterile single-use swab sticks and leave wrapped in original packaging until required.	Autoclave cotton buds/swab sticks wrapped in foil or in a foil covered beaker.
Sterile L-shaped glass spreaders (hockey sticks)	Wrap L-shaped glass spreaders in aluminium foil and sterilise in an autoclave/pressure cooker or using dry heat in an oven. Store until required.	Purchase sterile single-use L-shaped plastic spreaders, leave wrapped in original packaging until required.
Sterile forceps	Place forceps inside a clean test tube, cover opening with aluminium foil and sterilise in an autoclave/pressure cooker or using dry heat in an oven. Store until required.	The forceps could simply be wrapped in foil and sterilised, however, this runs the risk of perforation of the foil with the points of the forceps.
Sterile test tubes/ conical flasks/other glassware	Cover openings of glassware with foil or plug with non-absorbent cotton wool and sterilise in an autoclave/pressure cooker.	Cover openings of glassware with aluminium foil. Sterilise using dry heat in an oven
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

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