

ASSIST INFORMATION SHEET:

Decontaminating microbiological equipment

Prompt and thorough decontamination of equipment used in microbiological activities is vital to protect staff, students and facilities from microbiological contamination. These procedures are suitable for the decontamination of Risk Group 1 microbes used in a Physical Containment level 1 laboratory.

Decontamination processes should commence during and immediately after a microbiology activity. Processes such as disposing of disposable items in bleach solution and wiping benches with 70% v/v ethanol after an activity is completed are implemented to contain any microbes and stop the transmission to students and staff in the following lab sessions.

Provide containers with relevant solutions and contaminated waste bags e.g. autoclave bags or oven bags for students to segregate and dispose of equipment and cultures. Each receptacle should be labelled with its function. Ensure these are removed from the laboratory at the end of the activity.

Procedure for using an autoclavable biohazard or oven bag for sterilising microbiological waste:

- **Loosely pack microbiological waste including agar plates into bags to no more than 2/3 full.** This will ensure that the steam during sterilisation will penetrate the entire load. Bags that are tightly filled to capacity will not allow effective steam penetration and the contents will not be sterilised even if all sterilisation parameters are met.
- **Make sure there are no sharp objects present** that may puncture the bag.
- **Loosely tape shut the bag leaving an opening of about 5–6cm** to allow good steam penetration. This can be done with autoclave tape or a rubber band. Never tightly close the bags as they are impervious to steam and therefore the temperature of the inside of the bag will not be sufficient for sterilisation.
- It is advisable to **place the bag into a secondary container** within the steriliser to prevent any leakage into the steriliser should the bag rupture. The container must be able to withstand the autoclaving conditions.
- **Do not overload the steriliser** with too many bags as this may block steam circulation.
- **Use a sterility compliance strip** to indicate if the correct time, temperature and pressure have been reached during the run time. These are available from scientific suppliers.
- **Sterilise at 15psi, 121°C for 15–20 minutes.**
- After sterilisation has been verified, the autoclave or oven bag containing waste items should be **disposed of by placing it into a sturdy garbage bag which is sealed for immediate disposal in industrial bins.**
- **Wear heat protective gloves** when removing waste from the steriliser.

See table over the page for suggested decontamination techniques.

References

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

| CONTAMINATED Item | Suggested decontamination technique |
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| Inoculated agar plates – plastic | Pack unopened plates loosely in an autoclave bag, leaving an opening of about 5–6cm to allow good steam penetration. Autoclave at 121°C, 15psi for 15–20mins. When cool, place unopened autoclave bag in a garbage bag and dispose in general waste. |
| Inoculated culture broth in McCartney or Bijou bottles. | Make sure that the lids are loose. Autoclave at 121°C, 15psi for 15–20mins. Empty contents in the sink with copious amounts of water. Wash in warm soapy water, rinse well and dry. <i>Resterilise</i> : Re-autoclave loosely lidded container. Store in a clean closed container. |
| Inoculated water in glass bottle | Make sure that the lids are loose. Autoclave at 121°C, 15psi for 15–20mins. Empty contents in sink. Wash in warm water and dry. <i>Resterilise</i> : Re-autoclave loosely lidded container. Store in a clean closed container. |
| Plastic dropping pipettes | 1) Soak in 0.5–1% sodium hypochlorite solution to sterilise for a minimum of 2 hours. After soaking discard into the general waste, or 2) Place directly into an autoclave or oven bag located on the student's bench space and autoclave at 121°C, 15psi for 15–20mins. When cool, place unopened autoclave bag in a garbage bag and dispose in the general waste. |
| Used swabs | 1) Soak in 0.5–1% sodium hypochlorite solution to sterilise for a minimum of 2 hours or. After soaking discard into the general waste, or 2) Place directly into an autoclave or oven bag located on the student's bench space and autoclave at 121°C, 15psi for 15–20 mins. When cool, place unopened autoclave bag in a garbage bag and dispose in the general waste. |
| Sterile 'L' spreader | 1) Soak in 0.5–1% sodium hypochlorite solution to sterilise for a minimum of 2 hours, or 2) Place directly into an autoclave resistant container and cover with foil or place into an autoclave/oven bag located on the student's bench space and autoclave at 121°C, 15psi for 15–20 mins. 3) After sterilisation, wash in warm soapy water, rinse and dry. <i>Resterilise</i> : Wrap in foil and sterilise in an autoclave or hot air oven. Store until required for re-use. |
| Sterile forceps | Carefully place into an autoclave resistant container such as a large test tube, cover with foil and autoclave. Wash in warm soapy water, rinse and dry. <i>Resterilise</i> : Wrap in foil or place inside a clean test tube, cover opening with foil and autoclave, Store until required for re-use. |
| Test tubes | Autoclave at 121°C, 15psi for 15–20mins. Empty contents in sink. Wash in warm soapy water, rinse and dry. <i>Resterilise</i> : Plug with non-absorbent cotton wool and autoclave or cover the opening of the test tubes with foil and sterilise in a hot air oven or autoclave. Store in a clean closed container. |

| CONTAMINATED Item | Suggested decontamination technique |
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| Inoculating loop | <p>Flame to red heat carefully in the blue flame of the Bunsen burner to prevent the transmission of aerosols. Cool and reuse immediately.</p> <p>Alternatively, if using disposable inoculating loops,</p> <p>1) Soak in 0.5–1% sodium hypochlorite solution to sterilise for a minimum of 2 hours.. After soaking discard into the general waste, or</p> <p>2) Place directly into an autoclave or oven bag located on the student's bench space and autoclave at 121°C, 15psi for 15–20mins. When cool, place unopened autoclave bag in a garbage bag and dispose in general waste.</p> |
| <p>Susceptibility discs</p> <p>Mastrings (set of 6 or more antibiotic discs joined together)</p> | <p>Susceptibility discs and Mastrings should remain on the agar plate after examination. The agar plate remains closed. Pack unopened plates loosely in an autoclave bag and autoclave at 121°C, 15psi for 15–20mins. When cool, place unopened autoclave bag in a garbage bag and dispose in general waste.</p> |
| <p>Paper towel exposed to contaminated areas</p> <p>Used disposable aprons/lab coats</p> <p>Used gloves</p> | <p>If not soaked in bleach or alcohol, sterilise in an autoclave or pressure cooker.</p> <p>If soaked leave for the recommended time and then dispose of into the general waste.</p> <p>An autoclave or oven bag should be placed in the laboratory for students to place these waste items directly into the bag. Do not overfill the bag. Leave an opening of about 5–6cm to allow good steam penetration and sterilise for 15–20 min at 121°C and 15psi. Place the unopened autoclave bag into a sturdy garbage bag and seal for immediate disposal in an industrial bin.</p> |
| <p>Laboratory benches</p> <p>Plastic containers used for storage and distribution of equipment</p> <p>Any other hard surface</p> | <p>Dilute disinfectant in fresh water according to the manufacturer's instructions. Use in a spray bottle.</p> <p>Dilute ethanol to 70% in fresh water, use in a wash bottle. Ethanol is flammable.</p> <p>Apply liberally to laboratory bench or other hard surface to be decontaminated. Wipe lightly with paper towel. Allow the residual to air dry.</p> |