UNIVERSITY OF BIRMINGHAM

GUIDANCE NOTE 6: STERILISATION AND DISINFECTION

University policy requires that:

All biological materials are disposed of safely and in accordance with University procedures;

It is important to distinguish between sterilisation and disinfection. Whereas sterilisation results in destruction of all forms of life, disinfection results in destruction of specific organisms. Microorganisms vary in their resistance to destruction by physical or chemical means. A disinfectant that destroys bacteria may be ineffective against viruses, fungi or prions. There are differences in susceptibility between gram-negative and gram-positive bacteria, and sometimes even between strains of the same species. Bacterial spores are more resistant than vegetative forms, and non-enveloped, non-lipid-containing viruses respond differently than do viruses which have a lipid coating.

Sterilisation

A variety of methods are available for the sterilisation of materials in the laboratory: these include heat, filtration and radiation.

Generally, sterilisation is best achieved in the laboratory by the use of steam or dry heat. A summary of methods employing heat can be found in Table 1.

Table 1: Sterilisation Methods Involving Heat

Method	Principle/ Conditions	Advantages	Disadvantages	Uses
Dry Heat	Thermal inactivation: destroys by oxidation	Non-corrosive Simple design and principle	Less effective than moist heat; requires longer times and/or higher temperatures	Materials that are damaged by, or are impenetrable to, moist heat
Hot Air Oven	160 -180^{o}C for 2- 4 hours	Penetrates water-insoluble materials. Less corrosive to metals and sharp instruments than steam	Slow penetration of heat. Loading and packing are critical to performance. Not suitable for reusable plastics	Anhydrous materials and powders laboratory glassware, instruments closed containers
Red-heat Flame	Oxidation to ashes (burning)	Rapid	Initial contact with flame can produce a viable aerosol. Possibility of accidental fire	Inoculating loops, needles
Incineration	Oxidation to ashes (burning) 1-60 minutes: temperatures may exceed 1000^{o}C	Reduces volume of waste by up to 95%	Improper use may lead to emission of pathogens in smoke. Requires transport of infectious waste. Excess plastic (>20%) content reduces combustibility	Decontamination of waste items prior to disposal.
Moist Heat	Irreversible coagulation of (microbial) proteins	More rapid and more effective than dry heat		
Pasteurisation	Heating to below boiling point (generally 77^{o}C for up to 30 minutes)	Can be used on heat sensitive liquids and medical devices	Not reliably sporicidal	Milk and dairy products and some heat-sensitive medical equipment
Tyndallization (Fractional Sterilisation)	Heating to 80-100^{o}C for 30 mins on successive days, with incubation periods in between	Resistant spores germinate and are killed on the second and third days	Time consuming not reliably sporicidal	Heat sensitive materials such as bacteriologic media, solutions of chemicals, biological materials
Boiling	100^{o}C 10-30 mins.	Minimal equipment required	Not reliably sporicidal.	Small instruments and equipment
Autoclaving	Steam under pressure 121^{o}C/15 psi for 15-90 mins (gravity displacement autoclave) 132^{o}C/27 psi for 4-20 minutes (pre-vacuum autoclave)	Minimal time required. Most dependable sterilant for lab use	Loading and packing critical to performance. Maintenance and quality control essential. May damage heat-sensitive items.	Sterilisation of glassware, media and instruments. Decontamination of reusable supplies and equipment. Decontamination of infectious waste

Autoclaves


Although many microorganisms are killed by comparatively low temperatures, others (e.g. bacterial spores) are much more resistant. Steam sterilisation is normally the most efficient and convenient method for equipment, media and contaminated materials. A holding time and temperature policy such as 15 mins at 121^{o}C (1.1kg cm^{-2} or 15psi) should be chosen.

To calculate the length of the cycle the time taken for the load to reach the required temperature must be added to the above holding time. These times should be determined using thermocouples placed in the coolest (normally 2/3 way down) of typical and 'worst case' loads. The temperature indicated by the autoclave thermometer (normally the drain temperature) might be very different to the load temperature.

- Autoclaves must be properly maintained and pressure vessels inspected annually by the insurance assessor.
- Autoclaves should be operated by trained individuals.
- Clear operating instructions should be displayed beside each autoclave.
- Visors, lab coats and heat resistant gloves must be worn as appropriate.
- Separate autoclaves should be used for sterilisation (media, glassware etc) and for decontamination where possible.
- Efficient steam penetration is essential and failure to displace air will lower the temperature reached for a given pressure. Lids must be removed or slackened and plastic bags undone. Autoclaves must not be overloaded. Porous load autoclaves (which remove air by creation of a vacuum) should be used for coats, dressings, etc.
- Autoclave indicator tape should be applied to distinguish processed and unprocessed items. A colour change is **not** a guarantee of sterility.
- Portable autoclaves must be used for sterilisation of media ONLY, and not for treating waste.
- Autoclaves must not be opened unless the pressure gauge indicates atmospheric pressure.
- Autoclaves containing screw capped bottles or other sealed or partially sealed containers must have warning notices and must not be opened until the **load** has cooled below 80^{o}C.
- Materials likely to explode (e.g. nitrocellulose, highly flammable liquids and oxidising agents) or produce toxic or corrosive fumes (e.g. volatile solvents, some disinfectants) must not be autoclaved.

Validation and Calibration

Autoclaves will require examination and testing as a pressure system, and the purchase of all new autoclaves must be notified to the appropriate staff in School and Colleges so that they can be added to the register for testing.

The performance of all autoclaves should be checked every 6-12 months using thermocouples placed in the load. Validation assesses the ability of the autoclave to effectively decontaminate user-defined loads and all autoclaves used to treat GM or biological waste must be validated at least annually. Those treating waste from CL3 laboratories must be validated on a 6 monthly basis as a minimum. Where the nature or composition of the load changes further validation must be carried out. During validation a worst case load is simulated and temperature probes are inserted – this is known as the 12 point thermocouple test. The thermocouples are connected to a recording device and readings are then taken during the autoclave cycle. The test must be carried out by a competent engineer using calibrated equipment in accordance with the British Standard (BS 2646: 1993).

Calibration of the autoclave is also required. This will check that the control panel of the autoclave is functioning correctly and that it is regulating the decontamination process and displaying the operating parameters accurately.

Where printers are fitted to autoclave a print-out of each run should be kept to indicate successful decontamination of the load. Spore strips containing heat resistant spores of *Bacillus stearothermophilus* or chemical or physical indicators such as Brown's tubes can also be used to test autoclaves at more frequent intervals, but these checks do not replace the use of thermocouples. Unusual loads or Group 3 hazards should be monitored on a load-by-load basis. All records of performance testing should be kept for a minimum of 5 years.

Dry heat sterilisation

Heat resistant gloves must be worn when filling or emptying hot ovens.

Ultra-violet lamps

The light (approximately 260 nm wavelength) emitted by UV lamps is germicidal, and can be used to reduce the number of microorganisms on exposed surfaces and in air. However, UV light has poor penetrating power and accumulations of dust, dirt, grease or clumps of microorganisms may shield microorganisms from the direct exposure required for destruction. UV light presents a skin and eye burn hazard, and factors such as lamp age and poor maintenance can reduce performance. The radiation produced by UV lamps should be checked regularly since the presence of blue light is not an indication of effectiveness. Exposure of the skin and eyes to UV light must be avoided and warning signs must be displayed.

Microwave Ovens

Domestic microwave ovens do not provide a method of effectively disinfecting or sterilising materials. They may be useful in warming solutions and bringing agar into solution. However, due to the nature of their operation they may generate a significant hazard in use. Vessels placed inside must not be sealed because of the explosion risk, loose non metallic caps or fresh dry cotton wool plugs (moist or used cotton wool may ignite) must be used.

When melting solidified agar there is a risk of explosion due to superheating of the centre of the media and **they should not be used for this purpose.**

The interior of microwave ovens must be kept clean as dried organic matter may ignite. When purchasing new microwave ovens stainless steel or enamel linings should be chosen to limit the spread of any fire starting in the chamber.

The Selection and Use of Disinfectants

Departments should have policies or procedures stating which disinfectants can be used for what purpose, the in-use concentration, the minimum exposure time (overnight if practicable) and the frequency of changing.

The choice of disinfectants depends on several factors including the:

- nature of the micro-organism(s)
- nature of the material being disinfected
- circumstances of disinfection
- hazards presented by the disinfectant.

Disinfectants have variable activity against different types of organism. They can be inactivated by organic material or other chemicals and need to be used at the correct dilution.

Disinfectants should be used at concentrations appropriate for the work taking into account the manufacturers' recommendations. Caution is needed when mixing disinfectants with each other (or with detergents) as some mixtures are incompatible (e.g. mixtures of formalin and hypochlorite may produce the carcinogen bis chloromethyl ether). Disinfectants should be diluted with care and not by guesswork. The effective life of diluted disinfectants varies but it is normally good practice to renew solutions regularly, often daily, and to mark and date containers. Objects usually placed in disinfectants must be completely immersed. Used disinfectants can normally be poured carefully down a sink or sluice but any solid material must be sieved out and incinerated or autoclaved and the container cleaned thoroughly before re-use.

When handling hazardous disinfectants the appropriate precautions indicated in the COSHH assessment should be used.

Disinfectant Types

Hypochlorites: Sodium hypochlorite, Presept, Chloros

- Wide range of bactericidal, virucidal and fungicidal activity, but limited activity against bacterial spores
- Inactivated by organic matter
- Corrosive to metals and may damage rubber
- Compatible with anionic and non-ionic detergents, but incompatible with cationic detergents
- Irritant
- Chlorine gas released when mixed with acids
- Carcinogenic products when mixed with formaldehyde
- Not very effective against Mycobacterium spp

Peroxygen compounds: Virkon

- Wide range of bactericidal, virucidal and fungicidal activity
- Variable activity against bacterial spores and Mycobacterium spp
- Less corrosive than hypochlorite, but does have corrosive properties
- Powder is irritant, but made up solution have low toxicity and low irritancy
- Built in colour indicator
- Stable for seven days following dilution

Alcohols: Ethanol, Methanol, Isopropanol, IMS

- Good against bacteria and fungi, but limited activity against viruses
- No activity against spores
- Recommended for limited use only – an alternative should be sought where possible
- Poor penetration of organic mater – must only be used on clean surfaces
- Alcohols must be diluted to 70 – 80% for use
- Highly flammable
- Effective against Mycobacterium spp

Aldehydes: Formaldehyde, Glutaraldehyde, Cidex

- Have irritant and toxic properties and are extremely hazardous
- May only be used for specialist applications, and not as general disinfectants
- Glutaraldehyde based disinfectants are generally used for disinfecting instruments that cannot be heat sterilised. Glutaraldehyde is a known respiratory sensitiser and should only be used under controlled conditions.
- Formaldehyde use is limited to gaseous fumigation of Microbiological Safety Cabinets and CL3 laboratories. There are alternatives, for example, Vaporised Hydrogen Peroxide.

Other disinfectants

Disinfectants such as Distel (formerly Trigene) are commonly used, and have a broad spectrum of activity. Manufacturer's instructions should always be consulted.

Disinfection of People Following Accidents

Iodine and iodophores Tincture of iodine (1% iodine in alcohol) is an effective skin disinfectant, but is irritant and may cause tissue damage, delayed wound healing or skin sensitisation. It should only be used under medical supervision. Iodophor containing surgical scrubs have a wide range of activity, are stable, have detergent properties and are non-toxic at in-use concentrations. The skin should be washed thoroughly afterwards to prevent staining.

Hypochlorite solutions are irritant and must not be used for personal decontamination unless under medical supervision.

Alcohols are non-irritant and may be used as skin disinfectants **:** a small quantity of 70% v.v. ethanol or 60-70% isopropanol should be rubbed into the skin and allowed to dry. Chlorhexidine may be added to a concentration of 0.5% w.v. Alcohols are flammable and must be stored and used with appropriate precautions. They should not be used for disinfecting large areas nor in safety cabinets.

Antiseptics (Cetavalon, Hibitane, Savlon etc.) have a limited range of activity (mainly against Gram +ve bacteria) and are inactivated by organic matter. 0.5% Chlorohexidine is useful (in alcoholic solution) as a skin disinfectant or (in aqueous solution) as a mouthwash. 2.5% Savlon is useful for wound cleaning.

Testing Disinfectants

Starch iodide papers are useful for testing hypochlorite solutions. They are turned deep blue by an available chlorine concentration of 200 ppm but are bleached by strong hypochlorite solutions. Disinfectants in use may be tested by diluting them 1:10 in diluent (nutrient broth containing 0.5% w.v. sodium thiosulphate for hypochlorites) and pipetting 10 drops (10-20μl) onto each of 2 nutrient agar plates

which are incubated for 72h at 37°C and room temperature respectively. Growth on either plate indicates a failure of disinfection. More elaborate tests can be devised to meet individual requirements.

It should be noted that there is no 'universal disinfectant' and chemical disinfection is a less reliable method of decontamination than steam sterilisation. Chemical disinfectants should be validated for the conditions of use.

Formaldehyde Fumigation of Rooms and Safety Cabinets

In certain circumstances it is necessary to fumigate microbiological safety cabinets and occasionally rooms for decontamination purposes. Formaldehyde is often used as the fumigant. It has a Workplace Exposure Limit (WEL) of 2ppm (or 2.5mg.m^{-3}), which means that exposure levels must be kept below this limit.

- **Containment level 3 labs** may be fumigated by vaporising 100ml formalin (40% formaldehyde solution) and 900ml of water for every 28.3 cubic metres. Methods involving permanganate should be avoided as potentially explosive mixtures can be made. Heating units or commercially available formaldehyde generating kits may be purchased for this purpose. Fumigation should take place if there is a significant spillage or aerosol release outside a safety cabinet. Subject to local risk assessment it may also be required before routine maintenance, or at the end of work programmes to prevent cross contamination. Fumigations must only be carried out by nominated persons, trained in the procedure. The following should be observed:
- Hydrochloric acid and chlorinated disinfectants should be removed from the room before any routine fumigation. This is to prevent the possibility of forming bis (chloromethyl) ether, which may be carcinogenic. In the event of a spillage of infectious material the laboratory should be vacated immediately, however.
- The fumigated area must be effectively sealed and room ventilation and safety cabinets must be switched off.
- People in adjoining areas, cleaning and security staff, etc. must be warned as appropriate and warning notices displayed prominently.
- Exposure should be for at least 12 hours and preferably overnight.
- The room ventilation system and safety cabinets should be remotely activated the next day. Discharged vapours must not come in contact with people or be drawn into ventilation systems.
- The area must be thoroughly ventilated after fumigation. Formaldehyde levels should be checked before anyone re-enters the laboratory. This can be achieved by sampling through a purpose made port in the door. The room may not be re-occupied if the level in any part exceeds the Workplace Exposure Limit.

Safety Cabinets should be fumigated before any maintenance work involving access to internal parts of the cabinet, such as filters or fan motor, or after any major spillage which results in contamination of inaccessible surfaces. Safety cabinets in CL3 facilities must always be fumigated before servicing.

Cabinets may be fumigated by vaporising 60ml of formalin and 60ml of water per cubic metre. Using this formula the following quantities of formalin are recommended:

Class I	20ml (plus equal volume of water)
Class II (900mm)	20ml (plus equal volume of water)
Class II (1200mm)	25ml (plus equal volume of water)
Class II (1800mm)	30ml (plus equal volume of water)

The cabinet must be sealed and after 15 minutes the fan should be switched on for 20 – 30 seconds to allow penetration of the vapour into the filters and ductwork. The cabinet must be adequately labelled during fumigation, which should last at least 5 hours and preferably overnight. Warning signs should also

be displayed on the laboratory door to indicate that fumigation is in progress. The cabinet must be thoroughly purged with air after fumigation, the airflow should be checked and surfaces washed down to remove deposited paraformaldehyde.

For recirculating Class II cabinets, flexible "elephant's trunking" should be attached to the cabinet exhaust to allow discharge of formaldehyde vapour through a ducted cabinet, fume cupboard or adjacent window. If the latter is used it is important to ensure that formaldehyde vapour will be dissipated harmlessly and not taken back into the building through windows, air inlets etc thereby exposing other people to the vapour.

Appropriate protective clothing must be worn when cleaning and fumigating.

Further information on the fumigation of microbiological safety cabinets can be found in the ACDP guidance document, "The management, design and operation of microbiological containment laboratories". **It should be noted that the use of Vaporised Hydrogen Peroxide as a fumigant is also used within Universities in the UK. At present this is not used widely within this University, but may become more popular over time. It is important to ensure that the efficacy of VHP is proven under the conditions of use.**

Decontamination of Centrifuges

Sealed buckets should be used wherever possible, and are mandatory for work at containment level 3.

Routine disinfection of clean surfaces

- Swab with a suitable non-corrosive disinfectant
- Rinse with water, dry.

Tube breakage in unsealed bucket or rotor

- If possible, leave the centrifuge closed for at least 30 minutes.
- Place all broken tubes, caps, trunnions and the rotor in a suitable disinfectant for at least 1 hour (preferably overnight) or autoclave them. Broken glass must be picked up with forceps or swabs held in forceps.
- Unbroken, capped tubes may be swabbed with disinfectant and the contents recovered.
- Swab the bowl thoroughly with disinfectant, leave overnight, then swab again, rinse with water and allow to dry.

Tube breakage in sealed buckets or rotors

- Take the bucket/rotor to a safety cabinet and open.
- Decontaminate as above.

Rotor Failure

This may lead to extensive contamination of the centrifuge. The Health and Safety Unit must be informed of these incidents.

General

- Gloves must be worn during decontamination procedures and a visor and plastic apron if there is a risk of splashing.
- All swabs and debris must be rendered safe before disposal, preferably by autoclaving and/or incineration.