BS Section 3.7
Treatment of Biological Waste and Spill Cleanup

3.7.1 Biological Waste, Types and Initial Sorting

(See also GCS19.b Biological and Clinical Waste Disposal)

Waste collection treatment and disposal is covered in AS 2243.3, Section 7. This is an important subject as it covers the time when material leaves the control of the laboratory worker until disposables are rendered safe by sterilisation, incineration or burial, and recyclables are made safe by sterilisation and rendered safe by sterilisation and washed for re-use. Since the problems in waste disposal present themselves in different combinations, each laboratory may have to devise particular routines to try and make the safest practice the simplest and easiest. There are some clear priorities in sorting and segregation which will be common to most laboratories.

a. Sharps

Sharps, syringes with needles, scalpel blades, pasteur pipettes and broken glass etc., cause most injuries and infections. They should be placed in a rigid puncture-proof container immediately after use, and prior to any other treatment.

b. Large liquid cultures

Large liquid cultures should be killed in their growth or separation containers by autoclaving or chemical disinfection.

c. Radioactive material

Radioactive material is usually unacceptable in autoclaves. Use chemical disinfection and set aside, if necessary in a shielded area until radioactivity levels are acceptable for standard disposal.

d. Instruments and glassware

Instruments and glassware for recycling should be kept separate from material to be discarded.

e. Other infectious material

Other infectious material is easiest to accumulate according to its destination; typically autoclaving followed by incineration. Note that material treated with hypochlorite (chlorine bleach) should **NOT** be autoclaved.

f. **Non-infectious material**

Non-infectious material such as paper and plastic should be accumulated in a container, e.g. a plastic bag lined bin, which is clearly distinct from other waste. A breakdown in segregation of infectious from non-infectious waste presents a very real physical risk to workers and has a disastrous effect on morale and on personal relations.

g. Safe Collection and Labelling

Security of containment and clarity of information are the priorities. The standard biohazard emblem should be displayed on containers of all material accumulated or packed for transport. Colour-coding is usual. For cultures and glassware etc. awaiting transport to the autoclave, stable bins or buckets with lids should be kept in consistent locations. Disposable infectious material should be collected in a double-layer robust autoclavable plastic bag, securely supported, with a non-sealing lid. Killed material for incineration should be sealed in marked plastic bags. Laboratory staff should always be aware that others are highly dependant on good practice and clear information from the laboratory of origin. The purpose of the label is to provide information is to allow others to handle the material safely, to deal rationally with a spill and to obtain further useful information quickly. The responsibility is clearly with the dispatcher to do this adequately.

h. Treatment, Washing and Recycling

Treatment strategy is influenced by the priorities of initial sorting and options for final disposal.

i. **Sharps**

Sharps are best incinerated in their puncture-proof containers with no further handling, but if it were necessary to deal with reusables sharps (highly discouraged), or extremely infectious material then autoclaving in a suitable container should be considered in preference to chemical disinfection.

j. **Autoclaving**

Autoclaving in open-topped containers is preferred treatment for most other waste. Materials requiring particular care. Following autoclaving, cultures containing bulk amounts of genetic material demanding particular caution such as toxin genes and activated oncogenes may be treated with an aliquot of 5M HCl sufficient to lower the pH to 2, producing acid depurination of nucleic acids.

k. Chemical disinfection

Chemical disinfection with hypochlorite solution to give a final concentration of 5000 mg/L available chlorine (= a fresh 1 in 10 dilution of commercial chlorine bleach) for at least 30 minutes is often used for liquid cultures and glassware. Other chemicals such as 50-95% ethanol and/or hibitane or detergents may be used to disinfect materials such as metals sensitive to hypochlorite. Treatment should be complete before material is submitted to a washing and recycling system. After sterilisation, microbiological waste must be disposed of according to local

regional regulations. In general, killed microbiological liquid waste may be disposed of to the sewer. Solid material may be sent to landfill or incinerated if flammable. In conclusion,

- The generator of infectious waste is primarily responsible for its safe handling and disposal.
- This responsibility extends to the final disposal, even if other parties are involved.
- The generator should inspect all operations to ensure safe final disposal.
- Contingency plans should be developed to handle accidents.
- The generator must follow all regulations and any record keeping that must be observed.
- Principal investigators are reminded that their responsibility is from the cradle to the grave.

3.7.2 Clean-up of Microbiological Spills

This is covered in Section 5 of AS 2243.3. Because of its importance it is set out here in full. Accidents involving spills of living microorganisms and toxic chemicals defy systematic categorisation and prediction. If a material can be spilled, at some time it will be, so as to cause the greatest distribution in the most crowded or poorly ventilated area of a facility (Murphy's Law). Planning for such an emergency involves teaching staff the proper response to accidents, provision of suitable equipment for Clean-up and having available sources of information to help a trained Clean-up group select the correct method for the circumstances.

a. Spills Outside Biological Safety Cabinets

Spills outside biological safety cabinets are complex events. They may be within laboratories where a limited number of persons work, or they may be in corridors used by a considerable number of persons. This is the reason why considerable effort must be made to minimise spill events by containing material being moved by staff between service rooms and laboratories. Spills can involve amounts of material ranging from a millilitre or less up to several hundred millilitres or more. The amount spilled, the physical characteristics of the material and how the spill occurred are important factors in determining the area of involvement. Each spill is made up of three fractions,

- The bulk that stays in an irregular puddle.
- A portion separated from the bulk in large separated splashes and rivulets.
- A small portion separated from both the above by becoming airborne as particles of various sizes. The larger particles separate out quite rapidly, but very small particles have low separation rates and can remain airborne for some time. These smallest particles can be moved away from the spill site by the ventilation system. It is generally agreed that a waiting time of 30 minutes should be observed before attempting to clean up a complex spill. This allows large particles to settle and smaller ones to be removed by the ventilation system. In an air conditioned building, recirculated air could theoretically spread aerosols around the building, but this will occur whether clean up occurs immediately or after a delay. Consideration should be given to whether the air conditioning system should be switched off. It is therefore recommended, following usual practice, to wait at least 30 minutes before clean-up is

commenced. High security laboratories, of biosafety levels 3 and 4, are provided with a ducted and filtered exhaust air ventilation system and air is not recirculated to any other area.

b. Staff Training for Spill Control

All new staff should learn the basic rules of spill control and accident response. The materials necessary to contain and clear up a spill, together with specialized protective clothing should be kept in easily transportable containers in an accessible location.

c. Procedure Following a Spill

Immediate action in the event of a biohazard spill outside a Biological Safety Cabinet,

1. Stop breathing and leave the room immediately.
2. Close the door and place "DO NOT ENTER" AND "BIOHAZARD" signs.
3. Remove, and place in a biohazard bag, laboratory coat and any other garment suspected of being contaminated.
4. If shoes are suspected of contamination, remove and place in a separate biohazard bag.
5. Wash hands and face and put on a clean laboratory coat.
6. Warn others of the spill and to keep out of the area.
7. Notify area supervisor or have someone do it for you.
8. Stay out of the spillage area for at least ***30 minutes.***
9. A full body shower is recommended.

Notes:
a) Items 3 and 6 can be often done simultaneously.
b) It is not correct procedure to go looking for help before removing contaminated clothing, as spill material may be tracked around the building.
c) Spills in certain areas require special consideration, e.g. in cold rooms.

Consult the biosafety officer before commencing clean up to ensure that correct procedures are followed, according to the circumstances, including use of appropriate protective clothing and respiratory protection. The proper emergency response for an accidental spillage of biohazardous material in the laboratory will depend upon the hazard of the material and the volume. A minimally hazardous material that is spilled without generating significant aerosol may be cleaned up with paper towel soaked with an effective decontaminating agent. A spill of a large volume of infectious material with the generation of aerosols will require Clean-up personnel wearing protective clothing and respiratory protection. With M. tuberculosis, for example, the risk of exposure from the spill of a small quantity might be many times that of a much larger spill of *E.coli.* Therefore, if the agent is known, the recommended procedure and protective equipment should be used. Waiting approximately 30 minutes for the aerosols to settle before Clean-up of a large spill is essential. A spill kit or the best utensils available should be placed in containers for decontamination and safe handling by others. Following Clean-up, personnel should

wash or shower. Other types of spills that may generate hazardous aerosols include; spills within centrifuges and the release of biohazardous materials within refrigerators, incubators, or shaker baths. The same principles discussed apply. The area should be left immediately, protective equipment worn, the spill should be cleaned up, and the area should be disinfected. The personnel should then wash or shower. As with biological spills, the proper emergency response to a chemical or mixed chemical/biological release will depend upon the hazard of the chemical and biological agents, the volume, and the location of the incident. The spill should be confined to a small area while avoiding the airborne release to as little as possible. The spill should be neutralized or flushed with water and followed with a Clean-up or mopping up, with careful disposal of the residue. If the spilled material is highly volatile and non-infectious, it should be allowed to evaporate and be exhausted by the ventilation system. **Note:** Do not use hypochlorite disinfectant on a mixed biological/radioactive spill containing radioiodine.

3.7.3 General Procedures for Clean-up

1. Wait a minimum of 30 minutes before entry.
2. Clean-up personnel should wear gowns, rubber boots and rubber gloves. Respiratory protection may be required.
3. The team should consist of three people, one to stand back and observe and direct the other two to contaminated areas.
4. Before commencing observe the spill area to determine the area of contamination.
5. Hypochlorite (5000 mg/L chlorine) or iodophor (8000 mg/L iodine) disinfectants are appropriate. Pour the solution carefully around the outside of the spill and allow to flow into the spill. Lay paper towels wetted with disinfectant over the spill.
6. Wait 20-30 minutes to allow disinfectant to act.
7. Transfer all materials from the spill area to a metal pan for removal. Note, do not autoclave hypochlorite solutions.
8. Wash and mop the adjacent as well as the spill area with fresh disinfectant solution.
9. The decontamination team should wash their boots and gloves before removing them and leaving the area. Any other protective clothing should be autoclaved.

Note: Commercially available spill kits may be used for treatment of simple spills. Check the suitability of the disinfectant in these. Such kits may not be suitable for treatment of complex spills where materials is widely spread on the laboratory floor and furniture. **Monitoring of Sterilization Methods** The need for monitoring of sterilisation cycles by visual indicators, biological indicators and calibration with thermocouples is also discussed in AS 2243.3. Some additional notes on these are as follows.

a. Colour Change Tape

Tapes are available for dry heat, moist heat and ethylene oxide cycles. They are not interchangeable.The main disadvantage is that these tapes are qualitative, not quantitative. They indicate exposure to a cycle but not that articles are in fact sterile.

b. Physical indicators

Browne's tubes and Thermalog indicators are claimed to provide evidence of time/temperature parameters for moist heat cycles.Although better than autoclave- type tapes, they should not be regarded as absolute indicators of sterility.

c. Biologcial indicators

B. stearothermophilus, with available count of 105 per strip, are used to indicate that in moist heat cycles, a temperature of at least 116^{o}C has been achieved for sufficient time to inactivate the microorganisms. *B. pumilus,* usually 105 per strip are used for radiation sterilization cycles. These are inactivated at about 1.5-2.0 kGy (0.15-0.20 Mrad) whereas the usual radiation dose is 25 kGy (2.5 Mrad). Extremely variable performances of biological indicators have been observed in Australia and overseas. For this reason, while their use is recommended, they should not be regarded as providing evidence that sterility of the exposed articles has been achieved.