



GOOD MICROBIOLOGICAL PRACTICE (GMP) AND CONTAINMENT

Work with micro-organisms involves the use of both good microbiological practice and containment. Often these terms are misused and misunderstood. The simplest way of describing them is as follows:

- Good microbiological practice is used to keep the micro-organisms being handled in the "test tube" without any other organisms getting in and contaminating the work.
- Containment is also used to keep the micro-organisms being handled in the "test tube" but the main objective is to ensure that if any did get out of the "test tube" (either by accident or when the work is finished) they are unlikely to present a danger to laboratory workers and they do not get out of the laboratory.

The principles of good microbiological practice and containment are set out below.

GOOD MICROBIOLOGICAL PRACTICE

The use of aseptic techniques and other good microbiological practices achieves two very important objectives. These are:

- The prevention of contamination of the laboratory by the organisms being handled, and
- The prevention of contamination of the work with organisms from the environment.

The first is of prime importance as regards working safely whereas the second is a key consideration in relation to the quality of the research. The incentive to apply these principles should therefore be high.

The principles of good microbiological practice should be applied to all types of work involving micro-organisms (including genetic modification work) irrespective of containment level. In addition aseptic technique is also invaluable for preventing contamination of tissue cultures.

Aseptic technique is based on creating a special (clean) micro-environment in which to grow and keep the micro-organism of interest and prevent all contact with the outside world. This micro-environment is usually some sort of culture or holding vessel such as a flask, bottle (bijou, McCartney, universal etc) or petri dish and the organisms can either be on a solid agar medium or be suspended in a broth, diluent or other fluid medium.

The principles of aseptic technique are:

1. Prior to use all components of the system (the inside of the vessel, the medium and any objects used in the manipulative processes) must be sterile; and



2. In the inoculation, incubation and processing steps particular care must be taken to avoid cross-contamination. This involves:

- Keeping the vessel closed except for the minimum time required to introduce or remove materials;
- Working with a bunsen burner and flaming the opening of the vessel (passing it quickly through the bunsen flame) whenever tops are removed. The upwards current of hot air created by the bunsen prevents contaminated air or particles dropping into the culture vessel when the lid is open.
- Using manipulation techniques that minimise any possibility of cross contamination eg: opening lids with the little finger so that tops are not put down on the benches; and
- Ensuring that all of the objects that may come into contact with the culture , such as loops and pipette tips, are sterile before use, are not contaminated by casual contact with the bench, fingers or the outside of the bottle etc during handling and are decontaminated or disposed of in a safe manner immediately after use.

In addition to aseptic technique good microbiological practice encompasses a wide range of other working methods that minimise the bi-directional cross-contamination of work and workplace. These include for example:

1. Using manipulation techniques that minimise the possibility of producing aerosols:

- Mix by gentle rolling and swirling rather than vigorous shaking (to avoid frothing);
- Pipette by putting the tip into a liquid or onto a surface prior to gently ejecting the pipette contents (to avoid bubbling and splashing);
- Have vessels in very close proximity when transferring liquids between them (to avoid falling drops splashing);
- Use loops only after they have cooled down after flaming (to avoid sizzling);
- Do not over-fill centrifuge pots (to avoid leakage into centrifuge); and
- Always carry and store cultures etc (bottles and plates) in racks or other containers (to avoid accidental dropping and smashing).

2. Keep the laboratory clean and tidy:

- Only have on the bench those items necessary for the task in progress (to avoid unnecessary clutter which would increase the likelihood of
- things getting knocked over and also to minimise the problems of cleaning up in the event of a spill);
- Plan and lay out work so that everything needed for an experiment is ready to hand (this should allow the worker to sit at the bench and work comfortably);



- At the end of each experiment tidy and clean the bench and always wash hands. In the event of spillage etc always clean it up immediately and wash hands;
- Avoid putting anything on the floor (to avoid tripping hazards and minimise the problems of cleaning up in the event of a spill);
- Regularly clean out water baths (to minimise microbial contamination in the water);
- Regularly clean down open shelving, benching, window-sills etc and items on them (to prevent build up of dust and debris, store infrequently used items in cupboards and drawer);
- Regularly clean floors (to prevent build up of dust and debris, particularly in areas that are difficult to get to);
- Regularly sort through items stored in fridges and freezers, on shelves and benches etc and throw away unwanted items (to prevent clutter); and
- Keep sinks clean (hand wash basins and taps should be cleaned daily).

3. Designating areas in the laboratory for storage of items at different stages in use cycles, and where appropriate, using visual systems (e.g. autoclave tape) for indicating status :

- For example: clean/clean awaiting sterilisation/clean and sterile ready for use/used not decontaminated/used being decontaminated/used and ready for wash up (these types of systems allow for compartmentalisation of work activities into clean and dirty areas);
- Everyone in laboratory to be aware of system to ensure no mix ups occur; and
- System should be logical and easy to follow in working practices (otherwise it won't work).

An important aspect of good microbiological practice that often gets overlooked by the non-specialist is that experienced microbiologists handle all micro-organisms and cultures as if they are pathogenic (even if they are working with Hazard Group 1 organisms) by routine use of aseptic techniques and other good microbiological practices. Whilst intending to grow a particular (non-pathogenic) organism, the possibility of unintentionally culturing a (pathogenic) contaminant should always be acknowledged.

Furthermore whilst it is unlikely that organisms in Risk Group 1 will cause disease, many have the potential to cause opportunistic infections and pathogenic potential may well be altered under laboratory growth conditions

Purity checks should be incorporated into experimental protocols and undertaken at various stages of experiments as a matter of routine. This involves:



- Taking a loopful of fluid from the vessel and plating (or streaking) it out for single colonies onto a non-selective solid nutrient medium;
- Incubating at a suitable temperature (usually 30°C as this will allow growth of contaminants originating from both the general environment and human sources); and
- Examining the plates following incubation for evidence of any contamination (as indicated by colony types).

An indication of the purity of a liquid culture can also be obtained by microscopic examination. The advantage of this method is that the result is instant. A loopful of the culture is placed on a microscope slide and this is then either examined wet (by phase contrast microscopy) or fixed and Gram stained. Contaminating organisms should be clearly visible.

Purity checks are particularly useful in evaluating competence in good microbiological practice. Workers with poor aseptic techniques will suffer frequent contamination problems whereas skilled microbiologists will only occasionally have problems. It is important to recognise that poor practices resulting in cultures being contaminated probably also result in contamination from the work being spread in the laboratory.

There are three commonly preferred excuses for not needing to apply rigorous standards of good microbiological practice:

"I only work with E. coli and this multiplies so quickly that it outgrows all other organisms so I don't have to worry about cultures being contaminated"

"I use antibiotic-containing media so I don't have to worry about cultures being contaminated since only my strain will grow because it has antibiotic resistance markers"

"I wear gloves to protect myself because you always get things on your hands, it also means you don't have to worry about washing your hands"

These beliefs are totally misguided, reflect sloppy laboratory practices and attitude, indicate poor safety standards and compromise the quality of the research.

CONTAINMENT

Work with micro-organisms (using good microbiological practice) is undertaken in containment laboratories. There are 4 different levels of containment and the level of containment under which a particular micro-organism should be handled is indicated by the corresponding hazard group of the organism.

The principles of containment are applied in both the basic design and facilities in the laboratory and the working practices of all the people in the laboratory. The purpose of containment is not only to prevent the micro-organisms getting out of the laboratory but also to ensure that the workers are safe in the laboratory. The latter is achieved by blocking infection routes.

The working practices that are fundamental to containment, and the reason for these, are described below. The additional constraints that apply for



Containment Level 3 are not included here because there is relatively little work undertaken at this level in the University. Specialised training in safe working practices is required for all workers in Level 3 laboratories.

1. Restrict access - only let those people into the laboratory who have good reason for entry:

- Keep the laboratory door closed (the sign restricting access is then clearly visible and people are less likely to wander in); and
- Limit access to the laboratory to laboratory staff and other authorised persons to minimise the number of people likely to come into contact with (and spread) any contamination.

2. Wear protective clothing - any contamination of clothing should be left in the laboratory and not taken to other areas in the building or home at the end of the day:

- Laboratory coats or gowns, which ideally should be side or back fastening, must be worn in the laboratory and removed when leaving the laboratory suite; and
- Laboratory coats should be kept or stored separately in the laboratory suite so that any contamination is not transferred to personal belongings by close contact in locker etc.

3. Block routes of infection by the consistent application of simple precautions:

- Ingestion route - never ever put anything in the mouth:
 - Eating, chewing, drinking, smoking, storing of food and applying cosmetics in the laboratory are all banned.
 - Mouth pipetting, licking labels, chewing pens and finger nails, biting to cut or tear things instead of using scissors, holding things between the teeth, licking fingers or spitting to wet things, etc all must not take place in the laboratory.
 - Hands must be disinfected or washed immediately when contamination is suspected, after handling infective materials and also before leaving the laboratory (contamination on hands commonly gets transferred to mouth by everyday activities).
- Percutaneous route - avoid likelihood of puncture wounds and always keep breaks in skin covered:
 - Avoid using sharps wherever possible. If this is not feasible then handling procedures should be designed to minimise the likelihood of puncture wounds. Wherever possible glass items (including glass pipettes) should be replaced with plastic alternatives.
 - Used sharps should be placed directly into a sharps bin.
 - Unless safe means have been introduced, needles should not be resheathed. Sharps bins should not be overfilled, used sharps



- protruding from bins are very dangerous for those who have to handle them.
- The term sharp should be taken to refer to any item that is sharp and not be restricted to needles and scalpels. Commonly used items that could easily cause damage to the skin include all glass items (including microscope slides and cover slips), ampoules, pointed nose forceps, dissection instruments, scissors, wire loops that are not closed circles and gauze grids used in electron microscopy work. This list is not exhaustive and all items should be assessed for sharp edges. Cracked and chipped glassware should always be discarded immediately.
 - All workers in the laboratory should cover cuts and abrasions with waterproof dressings.
 - To prevent workers from spreading contamination that can be picked up from various sources by all staff in the laboratory, good basic hygiene practices, including regular handwashing, must be practised at all times; at the end of each working session (or day) benches and equipment should be routinely cleaned and disinfected.
 - Eye protection (goggles or safety glasses) and a plastic overall should be worn if splashing is likely to occur.
 - Wearing of gloves gives additional protection if the micro-organism being handled infects via the percutaneous route. If gloves are worn for this reason then it is recommended that two pairs of disposable gloves be worn when handling samples (minor damage to thin gloves often goes undetected until skin contamination is noticed). If during use the outer glove become punctured or grossly contaminated it should be removed and disposed of. If the inner glove is also damaged or contaminated it should also be disposed of and hands should be washed and clean gloves put on.
 - On completion of work gloves should be removed and discarded, and hands should be washed. Disposable gloves should not be re-used as contamination is likely to be transferred when these are put back on.
 - Inhalation route - care must be taken to minimise the production of aerosols:
 - Good microbiological practice must be used to prevent aerosols being produced
 - For manipulations such as vigorous shaking or mixing and ultrasonic disruption etc, a microbiological safety cabinet or equipment which is designed to contain the aerosol must be used.
 - A microbiological safety cabinet should be used for procedures involving a micro-organism that is infectious via the respiratory tract.



- Microbiological safety cabinets only protect against airborne hazards. Good microbiological practice should always be used when working in a cabinet as no protection is afforded against skin contamination (infections may therefore result by percutaneous and ingestion routes as described above).

4. Organise workstations so there is sufficient room to work safely:

- There should be enough benchspace to ensure the workstation is not cluttered and working practices are not compromised due to lack of space.

5. Use disinfection procedures to prevent spread of any contamination:

- Effective disinfectants must be available for routine disinfection and immediate use in the event of spillage;
- Bench tops should be routinely disinfected after use;
- All surfaces should be disinfected immediately following any spillage;
- All surfaces should be disinfected before any maintenance staff are permitted to work in the area; and
- All specimen containers, glassware and used equipment should be immersed in a suitable disinfectant before cleaning or disposal. Used laboratory glassware and other materials awaiting sterilisation must be stored in a safe manner. Pipettes, if placed in disinfectant, must be totally immersed.

6. Use waste disposal procedures that ensure that all contaminated materials are disposed of safely:

- All waste materials must be made safe by autoclaving or disinfection before disposal; and
- Material for autoclaving must be transported to the autoclave in robust containers without spillage.

7. All accidents should be reported so that appropriate action can be taken to minimise the likelihood of illness developing (and minimise the risk of passing this on to family, friends and others outside the laboratory):

- All accidents and incidents must be immediately reported to and recorded by the person responsible for the work;
- A full accident record should be prepared and forwarded to the Health and Safety Department. In the event of potential exposure the Occupational Health Service should be informed immediately;
- In the event of an accident resulting in a wound, it should be encouraged to bleed and the area washed with soap and water but without scrubbing. The wound should be covered with a waterproof



dressing. Any contaminated skin, conjunctivae or mucous membranes should be washed immediately;

- Particular care should be taken to ensure that others in the laboratory do not help with the clear up of accidental spillage (especially where there has been an accident that involves broken glass) if they are not aware of the potential risks and trained in safe working practices; and
- Where an accident results in a release of contaminated material the clear up procedures must be carefully assessed for risk and safe working practices adopted.

8. Staff must be trained and proficient in safe working practices and techniques for the safety of themselves and other persons in the laboratory:

- Workers must be able to recognise how exposure can occur and how it can be prevented.

It is important to emphasise that gloves and microbiological safety cabinets are used as additional control measures for some Containment Level 2 work depending on the route of infection of the micro-organism being handled. They do not need to be used for all Hazard Group 2 pathogens. The use of gloves and cabinets for work protection (such as tissue culture work) should not be confused with their use for worker protection although in some instances cabinets will have the dual function of providing both work and worker protection.