Good microbiological practice (GMP)

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1. Scope

Good microbiological practices (e.g. aseptic technique) when handling micro-organisms have developed to prevent contamination of the work with other micro-organisms. They also supplement the containment facilities, procedures and processes to limit the spread of microbial contamination and help prevent exposure of people and the environment to the micro-organisms that are being deliberately manipulated either by accident or once work has finished.

This document summarises the principles of GMP to be adopted when working with micro-organisms and it should be read in conjunction with the guidance on containment. The information contained in this document has been adapted from sector guidance and information from the Society for General Microbiology

2. Good microbiological practice

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

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

The first is an important principal of safe working 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 GMP should be applied to all types of work involving micro-

organisms, including genetic modification work, irrespective of containment level and is a specific requirement of the Genetically Modified Organisms (Contained Use) Regulations. In addition, aseptic technique also prevents contamination of tissue cultures.

Aseptic technique is based on creating a 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
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 e.g.: opening lids with the little finger so that tops are not put down on the benches
    - 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 GMP encompasses a wide range of other working methods that minimise the bi-directional cross- contamination of work and workplace. These include for example:

- 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 then splashing)
- Use loops only after they have cooled down following flaming (to avoid sizzling)
- Do not over-fill centrifuge pots (to avoid leakage into the centrifuge)
- Always carry and store cultures etc. (bottles and plates) in racks or other containers (to avoid accidental dropping and smashing).

- Keeping 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)
  - 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 the laboratory to be aware of the system to ensure no mix ups occur

    - Any system should be logical and easy to follow in working practices (otherwise it will not be followed and will not work).

An important aspect of GMP 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 is always acknowledged. Furthermore whilst it is unlikely that organisms in Hazard 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 loop-full 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^{o}C as this will allow growth of contaminants originating from both the general environment and human sources)

- 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 loop-full 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 GMP. 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.

3. Bibliography

1. Health and Safety Office. Biological and Genetic Modification Safety Policy. *Health and Safety Office.* [Online] http://www.bris.ac.uk/safety/biosafety/#policy.