Planning and design of a cell and tissue culture laboratory

1. INTRODUCTION

A major feature that distinguishes tissue culture laboratories from most other types of laboratory is the requirement to maintain the replication of a biological culture under sterile conditions (monoseptic), particularly ensuring the absence of bacteria and fungi. The facilities therefore must reflect this unusual need in the nature of equipment and laboratory design. The ideal way to plan a tissue culture laboratory is to start from scratch with a blank piece of paper. Realistically, however, this is rarely possible as budgetary constraints usually impose the need to convert existing facilities rather than build from new. This does not necessarily mean that adapted facilities will be inferior, in operation and/or safety terms, to a purpose-designed laboratory, provided that certain guidelines are followed. With this concept in mind, this chapter will both outline the basic requirements for providing a safe and efficient working environment, and suggest principles for the design of a cell culture suite.

2. PLANNING A CELL CULTURE LABORATORY

2.1 General principles of cell culture laboratory planning

Designated working areas are a prerequisite to providing a smooth running cell culture laboratory. By isolating each activity, contamination risks to both the operator and materials are reduced to a minimum. Examples of key activities, each of which ideally should have their own dedicated work area, are:

- preparation of sterile equipment and media;
- reception and culture of newly established or incoming cells;
- culture of cells which have received in-house testing for microbial contamination;
- banking of cells requiring special conditions to prevent contamination (e.g. cells used in the production of diagnostic and therapeutic agents);
- cryopreservation and cell storage.

Laboratories should be laid out so as to be in sympathy with the natural flow of activity in a normal working day, while at the same time minimizing the possibility of contamination of cells and of

workers by extraneous organisms (bacteria, fungi, mycoplasmas and viruses). It is important that waste and contaminated material should not be allowed to accumulate, as accidents are potentially more hazardous with larger volumes of waste which will be more awkward for laboratory staff to handle. Appropriate operation is more easily achieved by providing separate working areas designated for specific functions. A theoretical representation of these separate areas and the movement of material between them is illustrated in Fig. 1. An example plan for a tissue culture laboratory is shown in Fig. 2. Before finalizing the structure of these work areas it is essential to confirm that the proposed design is compliant with local or national regulations. This will avoid wasted time, energy and resources. When preparing designs for an international sponsor it may also be necessary to consider foreign and international regulatory requirements for quality and safety.

2.2 Influence of the type of work and management policy

Whilst all well designed cell culture laboratories will have many basic features of design and operation in common, the specific features of each laboratory will depend on many factors. These include not only the obvious constraints of existing facilities and resources, but also the remit for the laboratory's work, and management policy.

The laboratory may be required to carry out very specific research or analyses with clearly delineated hazards. Alternatively the work may involve general procedures (e.g. diagnostic work) incorporating a wider range of less well defined hazards. Thus a general risk assessment of proposed work may be required.

It is important to remember that, as a matter of management policy, quality and safety go hand-in-hand and should not be dealt with in isolation, particularly at the laboratory design and planning stages. Quality and safety are significant features of local laboratory management and national regulation. Quality policy is determined by the end use of the research, production or service work. This may be directed by peer review of published data, approval by national and international regulatory bodies or acceptability to the customer or sponsor.

These driving forces will identify the appropriate standards of reproducibility, reliability and traceability in the work. Local and national legislation and guidelines which are well developed in Europe, the USA (US Department of Health and Human Services) and

Japan (Ministry of Health and Welfare) should be the primary source of reference. However, the laboratory designer or planner should also bear in mind the possible consequences of quality and safety guidelines in other countries, which the laboratory management may wish to adopt to satisfy the requirements of outside sponsors or collaborators.

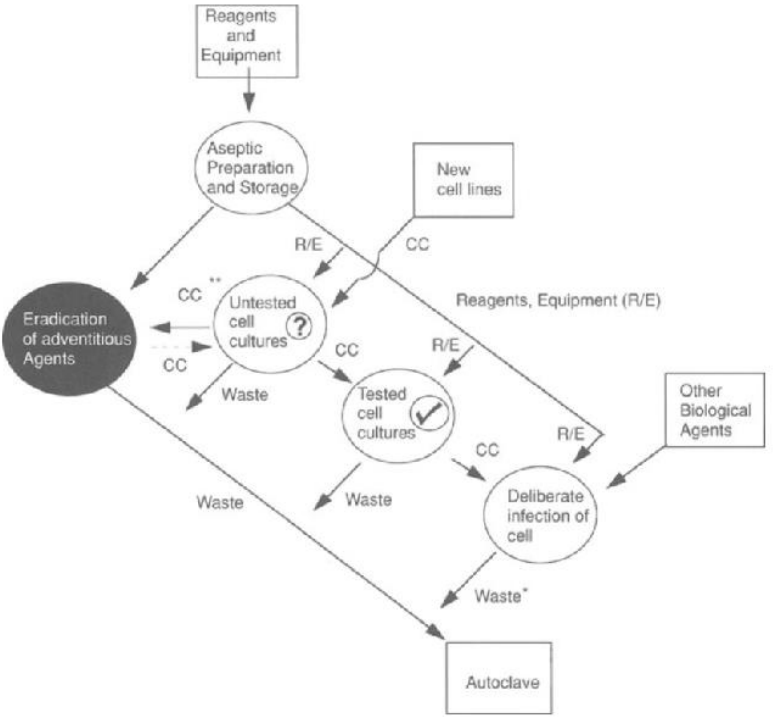

Fig. 1 Separation of work activities and one-way flow of materials in a tissue culture laboratory. Cultures should be carefully screened after eradication. *RiE,* sterile reagents and equipment; CC, cell cultures; **, eradication should be considered as a last resort; ?, quality control incomplete; Waste, tissue culturecontaminated materials which have been disinfected; '/, quality control procedures complete; *, for high-grade pathogens special handling and disposal procedures may be required. (Figure contributed by Glyn Stacey, NIBSC.)

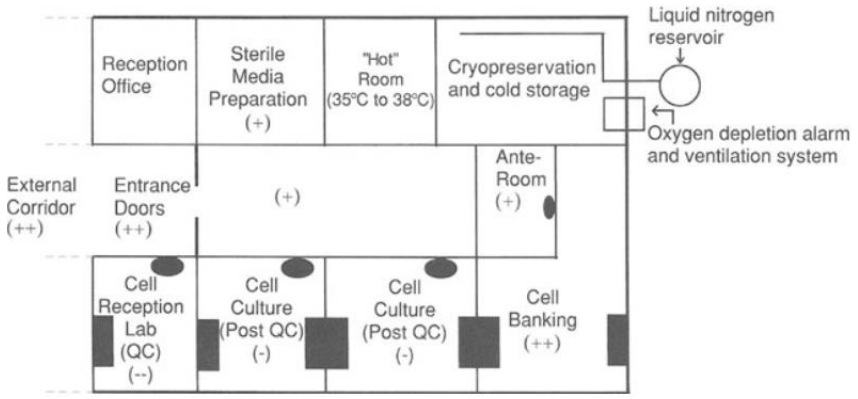

Fig. 2. Schematic layout of a tissue culture laboratory. This plan illustrates one possible arrangement for a tissue culture laboratory which provides a 'through flow' from low to high contamination risk, i.e. cell banking to cell reception. Each room environment is independently controlled to provide a positive-pressure (+) or negative-pressure (-) air flow to minimize this risk. Sterile air is supplied to the positive-pressure rooms via HEPA-filtered systems, and negative pressure can be maintained by venting Class II cabinets (filled oblongs) to atmosphere. The balance in the cell banking room is achieved by an over-supply of the HEPA-filtered air, which provides a sterile working environment outside the class II cabinet(s). The cell reception laboratory receives all incoming calls either from primary sources or as established cell lines. Cells undergo all quality control tests while in this area, only passing to the main cell culture laboratories on satisfactory completion of these tests, e.g. screening for bacteria and mycoplasma. Routine culture work is conducted in the cell culture laboratory or laboratories, which ideally should handle only one cell line at a time. However, if the rooms contain two or more class II cabinets, more than one cell line can be cultured provided each is designated to a specific cabinet. Cell banks are prepared in a positive-pressure, sterile-air room which, if required, can be operated to Good Laboratory Practice or Good Manufacturing Practice standards. Designated staff enter this room only after removing their normal laboratory coats in the ante-room, and putting on special clean-room clothing. Each culture room is shown with two Class II cabinets and a wash-hand basin (filled small oval) adjacent to the entrance door. Note that the cabinets are located away from the entrance to minimize interference from air movements during entry into the room. The nitrogen storage room is adjacent to an external wall, through which an insulated line supplies nitrogen from a reservoir to the storage vessels.

2.3. Regulatory considerations for laboratory construction

Many of the national guidelines are geared towards the protection of the laboratory worker rather than the protection of the work, but the main criteria are common in both instances. These include:

- Adequate space - at least 20m^2 of floor space and 3m^2 of bench space per person (50% of which should not be covered by equipment).
- Adequate ventilation - there should be at least six complete room air changes per hour (ideally 10 per hour if mechanical ventilation is used).
- If mechanical ventilation is used, it should be input at high level and extracted at low level to help prevent contamination from microorganisms of the operator's personal flora.
- Standard laboratories should be maintained at negative pressure in relation to corridors or atmosphere in order to help contain microbiological aerosols resulting from incidents or accidents. For more information on controlled ('sterile') environments.
- Adequate lighting and heating in accordance with national guidelines and other guidance.
- Floors should be impervious to water and also acid- and solventresistant. Floors should be coved to the wall to limit the collection of dust and for ease of cleaning. In liquid nitrogen-storage areas the flooring should also be resistant to cracking when cooled rapidly following a nitrogen spillage.
- Bench surfaces should be fully sealed, impervious to water, and resistant to strong acids, alkalis, solvents and disinfectants. These can also

be coved for ease of cleaning. Wooden surfaces are, in general, not acceptable.

- Benches should be at an adequate height for laboratory staff depending upon whether they normally sit or stand at their work.
- Hand-washing facilities must be provided either at or as close as possible to the exit. Taps must be operable without the use of the hands (i.e. foot or elbow controls).

The regulations which apply to working conditions for the manufacture of diagnostic and therapeutic products have important implications for cell culture operations related to particular products. These regulations and systems for inspection and validation are established in Europe.

3 DESIGN AND OPERATION

3.1. Laboratory surfaces

Strict and frequent cleaning routines are essential to ensure removal, and prevent build-up, of environmental contamination due to spills, splashes, aerosols and ingress of organisms from the external environment. The construction of the laboratories must incorporate design features and materials which will facilitate cleaning and hence safe operation. Thus all work surfaces must be smooth, continuous, waterproof and resistant to a wide range of chemicals. If an existing laboratory is to be converted, any wooden surfaces will have to be either removed or sealed. Similarly, flooring should not have any gaps or cracks that can trap dirt or microorganisms. There must be a continuum where the floors and walls meet (i.e. coved). When a new laboratory is constructed, the walls and ceiling will often be covered in the same material as that used on the floor, to provide a totally sealed shell. The types and volumes of waste materials should be considered so that appropriate collection and disposal procedures can be put in place. Separate areas should be designated for work with infected cell lines. Those cultures releasing viable higher grade pathogens, e.g. human immunodeficiency virus (HIV), human Tlymphotropic virus and haemorrhagic fever virus, require special precautions for containment.

3.2. Air flow

By controlling the air flow between rooms the risk of cross-contamination to materials and workers in adjacent laboratories can be controlled. The choice of positive- or negative-pressure operations will depend on the function of the room. In general, it is advisable that cell culture facilities should operate at negative pressure and preparation of sterile materials should be performed in positive-pressure environments (Fig. 2). To prevent the spread of infectious agents that may be present in incoming cell cultures, a separate reception (quarantine) laboratory is necessary. Here cultures can be screened for the presence of microbial contaminants such as mycoplasmas before they are handled in other areas. This room must be maintained at a higher negative pressure than the surrounding rooms. It is advisable to install pressure manometers outside all areas that are maintained at pressures adjusted from ambient, and to monitor pressure differences regularly to ensure that any deviation from the acceptable range can be dealt with swiftly. An essential part of this work is staff training to ensure that each person involved in

routine monitoring is aware of correct recording procedures and is able to instigate appropriate action when pressure tolerances are exceeded.

It is desirable to be able to control the ventilation of each room individually, usually by the use of dampers in the ventilation ducting. This also enables each room to be isolated when it has to be fumigated. As part of these routines, annual fumigation is also desirable to eliminate organisms in inaccessible places. However, fumigation should not be used as a substitute for regular laboratory cleaning: thorough and regular surface cleaning is an essential preventative measure against a build-up of environmental contaminants. Exhaust air from one room must not be able to enter another via common ducting. In rooms where cell culture operations are to be performed the supply air should be sterile. This can be achieved by drawing air in through a supply duct fitted with a highefficiency particulate air (HEPA) filter.

3.3. Safety cabinets

Cell cultures should be handled in a Class II microbiological safety cabinet. In the majority of cases where no known adventitious agent is present, level 2 containment facilities, including Class II microbiological safety cabinets, are appropriate. Laminar flow cabinets should not be used for manipulation of cell cultures or indeed for preparation of cell culture media where there is a toxic or allergenic hazard. In circumstances where cell lines are known to produce European Category 3 biological agents, then Class III safety cabinets will be used under containment level 3 conditions. In the USA the equivalent containment level for HIV-l, for example, would be Biosafety Level 3 (BSL3) practices in a BSL3 facility for production of virus. However, for research procedures a BSL2 can be used although practices must still operate at BSL3. A full explanation and references for these categories and for safety cabinets are given in Chapter 4 and Appendix A. Before deciding on a particular make of cabinet, ensure that it passes the current national standard. In addition it is advisable to check that the cabinet has passed these tests at an independent, authorized testing laboratory. Copies of the test certificates should be obtained, preferably from the testing laboratory.

At the time of installation each cabinet must be tested for both filter integrity and operator protection (KI test) and this should be witnessed under expected operational conditions by a member of

staff. Subsequent tests should be undertaken in accordance with current recommendations, but this should be not less than once a year. The location of safety cabinets is crucial to their efficiency of operation in the laboratory. They should be sited in a position where there is a minimum of 'human traffic', to avoid creating air movement in front of the cabinet. A location as far away from the entrance or exit to the room is best. There are recommended minimum clearance distances from walls, benches, laboratory equipment and other cabinets, which the installing company should also take into account.
Class II cabinets can operate by recirculating the exhaust air into the room, which promotes containment. However, ducting this air to atmosphere has certain advantages. When exhaust air is vented into the room it may be deflected downwards by adjacent walls, which can in turn increase the air turbulence at the front of the cabinet. By ducting exhaust air directly to atmosphere, this problem is avoided. An obvious advantage from the point of view of safety is that the continual removal of air from the room will produce a negative air pressure, which will prevent potentially contaminated air from leaking into adjacent rooms. Each laboratory should ideally have only one safety cabinet to minimize the risk of cross contamination between operations. Only one cell culture should be handled at a time in each cabinet. To ensure that carry-over of any adventitious agents to subsequent culture work does not occur, each operation should be followed by total internal surface decontamination and 15min waiting time before proceeding to the next operation. These precautions will minimize the risks of cross-contamination and culture switching due to mislabelling.

3.4. Incubators

Humidified CO2 incubators are very popular for cell culture since they avoid the necessity to purge each vessel individually with gas. However, such incubators represent an open culture system which represents a direct route for exposure of laboratory workers to agents within cell cultures. In addition the humidified atmosphere will promote the growth and rapid spread of bacteria and fungi from a single contaminated culture to all others in the incubator. Thus there is a risk of spread of contamination between cultures during incubation, and a risk to laboratory workers when opening an incubator. This risk can be largely eliminated either by strict use of specially manufactured gas-permeable closures, which are more

expensive, or by the use of dry incubators. In the dry incubation system, gas-tight culture vessels such as culture flasks are pre-gassed with a carbon dioxide/air mixture through an in-line filter (0.2 mm pore size). Open-culture vessels such as petri dishes and 24- and 96-well plates can be sealed in air-tight containers fitted with gas taps which are then used to purge cultures with the gas mixture. Operating a dry incubator/ closed vessel system is clearly beneficial for laboratory safety and quality assurance in cell culture procedures. Furthermore this system improves the economy of laboratory procedures due to the lower cost of dry incubators and savings on pressurized gases and special gas-permeable closures.

3.5. Services

A high-quality water purification system or some other reliable supply of sterile pure water is essential if cell culture medium is to be produced in the laboratory. A combination of reverse osmosis and organic filtration will provide the level of purity required. Addition of a 0.2 μm filter in the supply line will help to maintain a sterile supply of water, and the purification units and storage tanks should be sanitized regularly with chlorine tablets or as recommended by the manufacturer. Tap water and sink drainage facilities should be excluded from the tissue culture area as they are important sources of contaminating bacteria and fungi from the environment. Where sink or sluice drainage is required then a sterilizable drain trap should be fitted. It is useful to connect as many services as possible on-line in the tissue culture laboratory by piping in from an external supply area. This avoids any unnecessary manual handling within the laboratory area (a significant safety issue where pressurized gases are concerned) and further reduces the chance of entry of microorganisms. Additional controls such as in-line filters for gas supplies are also advisable.

Two areas often overlooked in the planning of a tissue culture laboratory are those for sterilization and preparation of glassware, and for storage of cryopreserved material. Both will require good ventilation, the former because of the high heat output from autoclaves and drying ovens, and the latter to maintain safe oxygen levels. An often-neglected risk for laboratory staff is nitrogen evaporation from liquid nitrogen refrigerators. Loss of consciousness due to displacement of oxygen by nitrogen gas can occur without

warning and an oxygen monitor should always be fitted as a precaution for staff working in the room. All staff likely to enter such areas must be instructed on the function of the oxygen meter and receive strict instructions on the evacuation and notification procedure when the low-oxygen alarm is triggered. If the room is sited by an outside wall, an external liquid nitrogen reservoir can supply nitrogen to the storage vessels through an insulated pipe. This will facilitate nitrogen deliveries and improve safety by reducing the need for staff to handle liquid nitrogen. It is worth calculating the future space required for cryopreserved material, i.e. the number of ampoules, to assess the total number of storage vessels that will be needed as, in the authors' experience, very few researchers will wish to discard stored material. To avoid a sudden loss of valuable material due to a vacuum failure, it is advisable to fit nitrogen-level alarms to all vessels. To provide 24-hour surveillance these can be linked to a telemetric monitoring system which will automatically dial pre-set numbers for staff on call. If large storage vessels are to be used, the ergonomics of working with such vessels must be considered carefully to avoid awkward and dangerous manipulation of storage racks.

The way in which cryopreserved cells are organized and contained is also an important consideration, and a recent report that hepatitis B virus can be transmitted between samples during storage in the liquid phase of nitrogen storage has highlighted this issue. Thus storage in the vapour phase of liquid nitrogen refrigerators is recommended and, in the UK, is a legal requirement for infections material. Secondary containment of frozen ampoules and/or storage tanks dedicated for different uses are additional precautions which can be considered.

4. CELL CULTURE ACTIVITIES REQUIRING SPECIAL CONTROL AND MONITORING

When performing cell culture procedures for which absence of contamination must be guaranteed (e.g. generation of cell products or viable cells for pharmaceutical use) a separate and specially prepared room is required. Unless the cells require the European Level 3 containment facilities, the room must be operated at a pressure positive to surrounding areas, to ensure that external contaminants are not able to enter. However, it is important to remember that the use of positive pressure is specific to cell lines intended for pharmaceutical use and should not be adopted for general tissue culture. The use of specialist flooring or entrance mats which attract

and retain dust (e.g. Dycem, Bristol, UK) will also prevent movement of contaminated particulate material between the laboratory and the outside environment.
Operations in the room should follow strict guidelines which must include:
• designated and specially trained staff;
• dedicated clean-room clothing to be worn;
• fully documented procedures including cleaning and disinfection;
• dedication of the work area to one cell line at a time.
Routine testing of air quality (i.e. particle counts and determination of airborne viable bacteria) may be useful, as well as a process 'integrity test'. The latter involves process simulation using bacterial culture medium in place of cells and their growth medium. The processed bacterial medium is then incubated to demonstrate the aseptic integrity of the process. While such tests are of limited importance for the safety of the laboratory worker, they are critical to the safety of the cell culture products which may ultimately be used for patient therapy. For such products, cell culture facilities, procedures, practice and raw materials will come under the scrutiny of regulatory agencies responsible for establishing standards and approving manufacturing facilities for pharmaceutical products.

5. WORKING PRACTICES

Maintenance of a smooth and safe throughput of work requires not only good laboratory design, but also the establishment of, and strict adherence to, working procedures controlled by authorized protocols and safety procedures. In particular these should cover decontamination, disinfection and waste disposal. Safe working practices must be promoted by developing protocols that take into account the current local and national safety regulations, such as those guidelines provided by the UK Health and Safety Executive. These protocols should be reviewed annually to allow for changes both in procedures used and in the safety regulations.
In the European Union, risk assessment of microorganisms should be carried out in accordance with EC directives.

6 ESTABLISHMENT OF LABORATORY RULES AND PROCEDURES

The following is a list of recommended procedures which can be used as a guide to efficient, and consequently safe, operation of an animal cell culture laboratory.

• Provide a specific training programme for all staff which is officially documented and regularly updated and recorded. This should include aspects of waste disposal, decontamination and laboratory cleaning. There should also be procedures in place to exclude unauthorized staff from specialized laboratory areas.
• Exclude staff who are likely to be infectious (especially in the case of respiratory infections) from sterile media preparation and cell culture areas of the laboratory.
• Subject all cell culture reagents, whether bought from commercial suppliers or prepared in the laboratory, to appropriate, documented and
recorded quality control procedures. Screen cell culture media by dilution in bacteriological growth media followed by incubation at 25 and 37°C to enhance the detection of low-level or fastidious contaminants. In addition set up and enforce a reagent surveillance procedure to ensure that old or contaminated reagents are discarded.
• If different cell culture procedures cannot be isolated physically, arrange the work schedule for the day so that operations involving the lowest risk of contamination are performed first, i.e. preparation of sterile reagents prior to handling cell cultures.
• Establish procedures that will isolate work at different levels of risk of infection (i.e. sterile, quality controlled cell cultures, untested cell cultures and infected cell cultures) by use of separate reagents, equipment, protective clothing and staff in each area. Particularly strict controls should be placed on movement from areas of relatively high risk of contamination to cleaner areas.
• Aseptic technique is an important element in protecting the operator as well as the work. Address any factors which may lead to lax aseptic technique (e.g. routine use of antibiotics) in training programmes for staff new to tissue culture. Emphasize general tidiness and hygiene procedures in staff training.
• Instigate routine checks on cells in culture, i.e. daily observation for the presence of microbial contamination, and testing for mycoplasmas on each occasion that a cell line is recovered from cryopreservation.
• Arrange regular rosters for the cleaning and maintenance of laboratories and equipment. These should be signed and dated by the staff performing the duties.
• Ensure that key equipment is routinely monitored and serviced, e.g. Class IT cabinets, autoclaves, laboratory pressure manometers and

centrifuges. In some cases this may involve statutory safety tests, e.g. pressurized containers for liquid gases.

• Set up an air quality monitoring regime for critical aseptic operations in specialized rooms and Class IT safety cabinets, e.g. particle counts, agar bacteriological 'settle' plates.

• Provide a clear, unambiguous and documented system for containment of different types of waste with suitable containers for the disposal of paper, plastic, glass and liquid waste. These should then be disposed of either as clinical waste (i.e. autoclaved and/or incinerated) or domestic waste for public disposal systems. A strict and documented regime for waste collection and autoclaving is essential for safe operation of a laboratory.

• Ensure that all aspects of cell culture work (staff training, media preparation, cell culture, culture storage, decontamination and waste disposal) are adequately documented and included in staff training schemes.

7. MAINTENANCE OF SAFETY STANDARDS

Once a safe working environment in the tissue culture laboratory has been established, it is essential to ensure that procedures are in place to maintain safe practices. It is useful to allocate each laboratory a named supervisor from the technical staff in that area who will check that routine safety practices and equipment are in use. The responsibilities of such staff should be clear, unambiguous and documented. An example currently used at the Centre for Applied Microbiology and Research is shown in Box 1.

Overall assessment can be achieved by initiating safety audits on a regular basis carried out by staff not working day-to-day in the tissue culture laboratory. It is important that any necessary actions from audits are followed up, and it is therefore useful to record the audit. An example of an audit record form which incorporates both safety and quality. Such initiatives, in combination with effective staff training programmes, will ensure that the laboratory (designed with the safety of workers and the environment in mind) will meet with the requirements addressed in the original design.

Box 1. An example of the responsibilities allocated to laboratory supervisors for maintenance of safety and quality standards within the tissue culture laboratory

RESPONSIBILITIES OF THE LABORATORY SUPERVISOR

The list below describes the duties of the laboratory supervisor, the key aspects of which are the maintenance of documentation, ensuring the smooth and safe operation of procedures in the laboratory,
and the notification to line management of any poor quality or unsafe working practice.

1. Check on a regular basis (i.e. monthly) that current copies of relevant risk assessment forms, laboratory procedures and standard operating procedures are available in the laboratory and that old protocols and equipment record sheets are archived.
2. Check that all staff are familiar with the location and contents of risk assessment forms, laboratory procedures and standard operating procedures.
3. Ensure that current emergency procedures are prominently displayed and that all staff are familiar with fire regulations and procedures.
4. Notify the line manager of breaches of good laboratory practice, quality systems and local code of safety practice.
5. Ensure good housekeeping in the laboratory, i.e. that the laboratory is kept clean and tidy and necessary consumables are always available, and that correct disinfection, fumigation and waste disposal procedures are adhered to.
6. Keep a record of all chemical reagents and microorganisms used within the laboratory, discarding out-of-date reagents and stock chemicals where appropriate.
7. Provide the Biological Safety Officer with any information concerning the activities of the laboratory as required.
8. Ensure that all laboratory equipment is maintained in a safe and operational condition. All obsolete equipment must be removed to storage or disposed. This includes maintenance of equipment logs, record sheets, service/maintenance records, calibration check records and lists of equipment requiring calibration completed within the appropriate quality system (e.g. ISO 9001).
9. Discuss laboratory repairs to fabric or fittings with the line manager and liaise with contractors to implement these.