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The Cell Culture Laboratory

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1.1 Introduction

Cell culture dates back to the early twentieth century (Table 1.1) by which time some progress had already been made in cryopreservation, the long-term storage of mammalian cells in a viable state.

The laboratory process of cell culture allows cells to be manipulated and investigated for a number of applications, including:

- studies of cell function, for example metabolism;
- testing of the effects of chemical compounds on specific cell types;
- cell engineering to generate artificial tissues;
- large-scale synthesis of biologicals such as therapeutic proteins and viruses.

The pioneering work of Ross Harrison in 1907 [1] demonstrated that culturing tissue *in vitro* (in glass) not only kept cells alive, but enabled them to grow as they would *in vivo* (in life). However, the early development of cell culture technology was hindered by issues of microbial contamination. The growth rate of animal cells is relatively slow compared with that of bacteria. Whereas bacteria can double every 30 minutes or so, animal cells require around 24 hours. This makes animal cell cultures vulnerable to contamination, as a small number of bacteria soon outgrow a larger population of animal cells. However, tissue culture became established as a routine laboratory method by the 1950s with the advent of defined culture media devised by Eagle and others. The discovery of antibiotics

Animal Cell Culture: Essential Methods, First Edition. Edited by John M. Davis.

Table 1.1 The early years of cell and tissue culture.

Late nineteenth century – Methods established for the cryopreservation of semen for the selective breeding of livestock for the farming industry
1907 – Ross Harrison [1] published experiments showing frog embryo nerve fibre growth *in vitro*
1912 – Alexis Carrel [2] cultured connective tissue cells for extended periods and showed heart muscle tissue contractility over 2–3 months
1948 – Katherine Sanford *et al.* [3] were the first to clone – from L-cells
1952 – George Gey *et al.* [4] established HeLa from a cervical carcinoma – the first human cell line
1954 – Abercrombie and Heaysman [5] observed contact inhibition between fibroblasts – the beginnings of quantitative cell culture experimentation
1955 – Harry Eagle [6] and, later, others developed defined media and described attachment factors and feeder layers
1961 – Hayflick and Moorhead [7] described the finite lifespan of normal human diploid cells
1962 – Buonassisi *et al.* [8] published methods for maintaining differentiated cells (of tumour origin)
1968 – David Yaffe [9] studied the differentiation of normal myoblasts

by Fleming was of course another major milestone that facilitated prolonged cell culture by reducing contamination issues.

In the 1940s and 1950s major epidemics of (among others) polio, malaria, typhus, dengue and yellow fever stimulated efforts to develop effective vaccines. It was shown in 1949 that poliovirus could be grown in cultures of human cells, and this became one of the first commercial 'large-scale' vaccine products of cultured mammalian cells. By the 1970s methods were being developed for the growth of specialized cell types in chemically defined media. Gordon Sato and his colleagues [10] published a series of papers on the requirements of different cell types for attachment factors such as high molecular weight glycoproteins, and hormones such as the insulin-like growth factors. These early formulations and mixtures of supplements still form the basis of many basal and serum-free media used today (see Chapters 4 and 5).

Recombinant DNA technology (also known as genetic engineering) was developed in the 1970s and it soon became apparent that large complex proteins of therapeutic value could be produced from animal cells. Another milestone came in 1975 with the production of hybridomas by Köhler and Milstein [11]. These cell lines, formed by the fusion of a normal antibody-producing cell with an immortal cancer (myeloma) cell, are each capable of the continuous production of antibody molecules with (in the modern embodiment of the technology) a single, unique amino acid sequence. By 2007, the centenary year of tissue culture, such monoclonal antibodies were being commercially produced in multi-kilogram quantities.

Large-scale culture applications have led to the manufacture of automated equipment, and today's high-end cell culture robots are able to harvest, determine cell viability and perform all liquid handling. The Cellmate^{TM}, for example, is a fully automated system for T-flasks and roller bottles [12] that was first produced for Celltech's manufacturing operations, and which has since been used in vaccine production. The latest version includes software to support validation if it is used in processes requiring compliance

with regulations such as 21CFR Part 11 (see Chapter 11, Section 11.3.5). Also on the market are automatic cell culture devices that handle the smaller volumes used by high-throughput laboratories. This recognizes the growing importance of cell-based assays, particularly in the pharmaceutical industry. The Cello™ is an automated system for the culture of adherent and non-adherent mammalian cells in 6-, 24-, 96- and 384-well plates for the selection of optimal clones and cell lines. It automates operations from seeding through expansion and subculturing, and thereby decreases the time required for cell line development.

In the biomedical field cultured cells are already used routinely for a variety of applications, for example Genzyme's Epicel® (cultured epidermal keratinocyte autografts) for burns patients and Carticel® (cultured autologous chondrocytes) for cartilage repair, as well as at *in vitro* fertilization (IVF) clinics where the zygote is cultured – usually for a few days – prior to implantation in the mother's uterus. Stem cell research is another cell culture application that holds huge promise for the future, especially now specific cell programming is possible. Although much stem cell research used to depend on the use of embryonic stem cells (obtained from early-stage embryos,) scientists can, at least in some cases, now change differentiated somatic cells into stem cells (iPS – induced pluripotent stem cells) using genetically engineered viruses, mRNA or purified proteins, thus avoiding the ethical issues surrounding the use of embryos as a source of cells. These iPS cells are similar to classic embryonic stem cells in many of their molecular and functional features, and are capable of differentiating into various cell types, such as beating cardiac muscle cells, neurons and pancreatic cells [13]. Stem cells can potentially be used to replace or repair damaged cells, and promise to drastically change the treatment of conditions such as cancer, Alzheimer's and Parkinson's diseases, and even paralysis.

1.2 Methods and approaches

1.2.1 Cell culture laboratory design

When planning a new cell culture facility, it is important to clearly identify the type of work anticipated within the laboratory, as much will depend on the nature and scale of the culture to be performed. For any design, the access doors need to be large enough for the passage of any major equipment (an obvious point, but sadly one often overlooked). Even routine small-scale work, such as much of the tissue culture undertaken in healthcare, biotechnology and academia, has varied needs that require careful consideration in the planning stage.

Certain types of laboratory involved in highly specific work – such as IVF laboratories, environments dedicated to the production of biopharmaceuticals under Good Manufacturing Practice (GMP) conditions, or work with Hazard Group 4 pathogens (biological agents that are likely to cause severe human disease and pose a serious hazard to laboratory workers, are likely to spread to the community and for which there is usually no effective prophylaxis or treatment available) [14] – are not dealt with here. They require expert help for laboratory design because of the need to comply with stringent legislation and/or the highly significant associated health and safety risks.

Some of the questions that need to be answered before commencing the design of a laboratory are set out below. This list of questions is by no means exhaustive.

1.2.1.1 Health and safety implications

- What is the highest Hazard Group of material to be handled [15]?
- Will all work need to be carried out at the related containment level [14]? If not, what facilities are required at what level? (The lower the containment level, the less onerous and expensive it is to build, equip, run and work in the laboratory.)
- Will genetically modified organisms (GMOs) be used? (Note that, in the UK, any work with (or storage of) GMOs falls under the Genetically Modified Organisms (Contained Use) Regulations 2000. These require that the laboratory is registered with the Health and Safety Executive for the performance of GMO work, and that various other safeguards are put in place, *before* any GMOs enter the laboratory. Many other countries also have regulations/legal requirements covering work with GMOs.)

These are extremely important considerations as the relevant health and safety legislation may require (or recommend) features that need to be incorporated into the laboratory design from the start (e.g. specific air handling, the need for a changing lobby, accessibility of autoclaves), as well as constraining the specifications of the equipment required within the laboratory.

1.2.1.2 The scale of the work

- How many and of what size are the largest vessels that will be used within the facility?
- Will they require support vessels? (For example, a large fermenter would also require media preparation and storage vessels as well as smaller fermenters in which to grow up the cell inoculum.)
- For both of the above, will they be fixed or mobile? Is special handling equipment required? How will they be cleaned and/or sterilized?
- How are any spillages to be dealt with?

Thought needs to be given to the safe preparation, handling, inactivation and disposal of cells and media, as well as the cleaning and sterilization (or disposal) of *all* the equipment used. Disposal and discard areas need to be clearly identified, and of sufficient size to cope with the amount of waste generated by the laboratory. Good practice dictates that they are kept tidy and cleaned regularly.

Medium- to large-scale manufacturing facilities may have very specialized requirements, for example systems for handling large volumes of media, such as lifting devices or under-floor kill tanks. Even laboratories using smaller-scale benchtop cultures, for example 10-l glass fermenters or wave-type bioreactors (see Chapter 10, Section 10.2.2.5), might find that trolleys and lipped benches help with risk management, especially if multiple units are to be in use at any one time.

1.2.1.3 Segregation requirements

Consideration needs to be given to the physical segregation of work:

- Are separate rooms needed for (for example) primary cell culture or the quarantine of incoming cells?
- Do several smaller rooms give more flexibility than one large one?

Often full segregation is not possible and in this situation the full implications of this need to be understood. Ideally, work place practices can then be implemented to reduce the risk of cross-contamination, for example the use of dedicated cabinets and incubators for specific cell types, with associated records of what was handled when, and by whom. Valuable stock cultures should be duplicated in incubators with independent services (electricity, CO_2) to avoid their complete loss. Use of an uninterruptible power supply is worth consideration in this respect. Thought must also be given to the flow of work within the room – try to keep dirty areas, such as those used for waste disposal, near the door, with critical clean areas containing items such as the microbiological safety cabinet(s) and incubator(s) as far from the entrance as possible (see below).

1.2.1.4 General considerations

One feature that is common to all cell culture laboratories is the need to prevent the contamination of the cell cultures with adventitious agents from external sources, that is operators and the environment. For this reason, the microbiological safety cabinet (MSC) is a central component to all tissue culture laboratories. It provides protection to the operator and, in the case of Class II cabinets, also offers the culture some protection against any microbes that might be present in the environment. As discussed in Section 1.2.3.1, the positioning of MSCs is extremely important – not only to ensure that the correct airflow is maintained, but also to provide an ergonomic environment for the user – and will have a significant impact on the design of the culture laboratory. For more demanding applications, such as in the production of biological medicines, MSCs will be situated in a dedicated culture suite supplied with sterile-filtered air. The air in such rooms is generally kept under slight positive pressure with regard to neighbouring areas, to ensure that non-sterile air is not drawn in [16].

In order to minimize further the risk of contamination to cultures, the passage of people through the cell culture laboratory should always be minimized, especially past the cleanest areas – the MSCs and incubators. Therefore, as mentioned above, these critical work areas should be positioned away from the entrance. Sticky mats can be placed on the floor immediately inside the laboratory to remove loose dust and grime from shoes. These mats should be changed regularly to prevent them becoming a source of contamination themselves. Disposable overshoes or shoes dedicated for use in the laboratory provide another option.

To reduce dust build-up within the laboratory, there should be as few horizontal surfaces as possible, commensurate with the work to be undertaken and along with any allowance for future developments (see Section 1.2.1.5). All surfaces must be cleaned regularly. In order to facilitate cleaning, all plumbing, cabling etc. on entering the laboratory should be boxed in, with any access points through walls, floors or ceilings being well sealed. Flooring should be flat and even, as seamless as possible and joined smoothly to the walls. Sufficient storage needs to be at hand for work surfaces to be kept

clutter-free. Ideally, under-bench storage cupboards and drawers would be moveable to facilitate cleaning, rearrangement and removal. Even if the room is not designed to be fully fumigable (using formaldehyde or vaporized hydrogen peroxide – see Chapter 2, Section 2.2.4.1), it should at least lend itself to thorough cleaning with a biocidal agent (see Chapter 2, Section 2.2.4.2).

Once workers have entered, there should be sufficient space to change into clean laboratory coats, with adequate provision for storage of these coats and safety glasses when not in use. Hand wash sinks with soap and alcohol rub should be nearby to allow thorough hand cleaning on entry and exit. Eye wash stations are best positioned by the doors where they are easily accessible. Users require sufficient room for drawers or moveable trolleys of consumables to be at hand, and to have easy access to basic equipment such as the incubator, microscope and centrifuge.

Having entered, the need to exit/re-enter should be reduced by having all necessary small items of equipment within the laboratory. Ideally any equipment that does not need to be operated under sterile conditions (e.g. analytical flow cytometer, fluorescence microscope) should be housed in a separate but nearby room. Adequate stocks of unopened media and frozen reagents for use in the short term should be stored within a laboratory refrigerator or freezer, but larger quantities of unopened supplies should be housed elsewhere, preferably in dedicated clean storage.

1.2.1.5 Future needs

Often, requirements change within the lifetime of the laboratory due to fast-moving technology and changes in scientific focus. Therefore, it is worth considering what the requirements may be in the future, as designing in flexibility for upcoming work may be cost-effective in the long run. What seems routine now may well be superseded with time, and innovative technology and instrumentation may need to be brought in. Although one cannot predict exactly what these changes may be, it is worth ensuring that sufficient power, utility and computer network connection points are installed at the outset. Leaving plenty of room for workers, and free bench space, not only allows safe and full access to equipment when needed but can give scope for some rearrangement at a later date.

1.2.2 Services

The service requirements of tissue culture laboratories are very similar to those of other laboratories, with the additional need for gas supplies, for example carbon dioxide for incubators, oxygen and nitrogen for fermenters, and mixed carbon dioxide/air for the manual gassing of culture vessels.

The laboratory should have some form of continuous environmental control (heating/air conditioning) to ensure that there is little variation in room temperature during different times of the day or year. This is important as many pieces of equipment will not be able to operate as required (e.g. an incubator may not be able to maintain constant temperature, and could potentially malfunction) if the ambient temperature varies significantly. Many pieces of instrumentation also require a steady ambient temperature in order to give reproducible results.

Numerous power sockets are essential in the laboratory, both above and below bench level. Although most laboratory equipment will require only normal single-phase electricity (230 v, 15 A in the UK), it should be noted that certain items such as large centrifuges may require a single-phase electricity supply that will deliver a higher amperage (e.g. 30 A), and some items such as large fermenters and autoclaves may require a three-phase supply.

IT network points and communication ports may be necessary to network many of the pieces of computer-driven instrumentation that may be used in the laboratory. This can help to reduce the amount of paperwork moving in and out of the laboratory by giving workers access to the computer network from within the laboratory.

1.2.2.1 Water

High-purity water is essential in any cell culture laboratory, not only for preparing media and solutions but also for glassware washing. A source of ultra-pure or RO (reverse osmosis) water is therefore required. RO is a process that typically removes 98% of water contaminants. Tap-water fed to an RO unit should first be passed through a conventional water softener cartridge to protect the RO membrane. RO water can then be fed directly to a second-stage ultra-purification system, comprising a series of cartridges through which the water is filtered for ion exchange and the removal of organic contaminants, and finally through a microporous filter to exclude any particulate matter, including microorganisms. Water purity is monitored by measuring resistivity, which should reach about 18 megohm/cm. Most water purification units, supplied by companies such as Millipore (Milli-Q system), have semi-automatic cleansing cycles requiring minimum effort to maintain. Water should be collected and autoclaved or filtered immediately prior to use for sterile applications.

If in doubt about the quality of the water from a purification system, a simple *Limulus* amoebocyte lysate (LAL) assay should be performed to check endotoxin levels. If the water is found to be a source of endotoxin and the problem cannot be solved, then non-pyrogenic water for injection (WFI) can be purchased and used for preparing media and other critical solutions. Most laboratories now buy ready-made media and supplements for cell culture use.

Further details on the requirements for, and purification of, water for laboratory use can be found in reference 17.

1.2.2.2 Pressurized gases

Ideally, gas cylinders should be kept outside the main cell culture laboratory and the gas piped through, to maintain cleanliness in the sterile handling area. CO_2 cylinders should be secured to a rack and the gas fed via a pressure-reduction valve on the cylinder head, through pressure-rated tubing to the incubator intake ports. It is critical to maintain an uninterrupted gas supply, and it is advisable to have two cylinders connected to the CO_2 supply system via an automatic changeover unit that will switch to the second cylinder when pressure in the first drops below a certain level. (Note that some automatic changeover units require an electricity supply, and in the event of an interruption to the

electricity these may malfunction or even cut off the gas supply. Thus a purely mechanical changeover unit may be preferable.) An in-line 0.2-μm filter is a useful precaution against dirt or microorganisms in the supply system, and some incubators will require the use of a further pressure-reduction valve to bring the CO_2 pressure down to that for which the incubator was designed.

In order to check the integrity of pressurized gas connections, a wash-bottle filled with soap solution (household washing-up liquid is ideal) for squirting around connections makes a cheap and effective leak detector. In particular, newly connected cylinders should be tested in this way; dirt on either face of the connection can cause significant leakage.

It is the responsibility of the laboratory manager, not the gas supplier, to ensure that gas systems, storage and operating practice on site comply with any relevant laws and regulations.

1.2.2.3 Liquid nitrogen

Liquid nitrogen (LN) is required for the long-term storage of cell stocks (see Chapter 6). All vessels containing LN must be kept and used in well-ventilated areas, and certainly *not* coldrooms or basements. LN refrigerators containing cell stocks (see Section 1.2.3.6) need to be kept in a secure location fairly close to the culture laboratory, but preferably not in the laboratory itself. If keeping such vessels in the laboratory is unavoidable, a risk analysis must be carried out first, taking into account the maximum amount of LN that could be released in the event of a spillage or vessel failure (and the volume of nitrogen gas this would become), the volume of the laboratory, and any other relevant factors such as air exchange rates of the ventilation system and the presence in the room of an oxygen depletion monitor (a good precaution in any event). Larger-volume stocks of LN for keeping the cell stores topped up are best stored in a secure enclosure on the outside of the building, and must be easily accessible for filling by a delivery vehicle. As these vessels will usually be pressurized, they must be designed specifically for the storage of LN, and maintained on a regular basis. For further details, and general advice on the handling of LN, see Chapter 4, Section 4.2.8.1.

1.2.3 Equipment

1.2.3.1 Microbiological safety cabinet

The most important piece of equipment within a cell culture laboratory is the MSC. Although cell culture can be performed on the open bench with the help of a Bunsen burner [16], laboratory techniques may produce aerosols that can contain hazards such as infectious agents which can be inhaled by laboratory workers. MSCs are used as primary barriers during the handling of materials that may contain or generate hazardous particles or aerosols, in order to prevent exposure of laboratory personnel and contamination of the general environment. Some MSCs are designed to also provide a clean work environment to protect cell cultures or sterile apparatus/solutions

All MSCs purchased in the UK must comply with British Standard BS EN 12469:2000, and the USA [18] and other countries have similar specifications. There are three classes

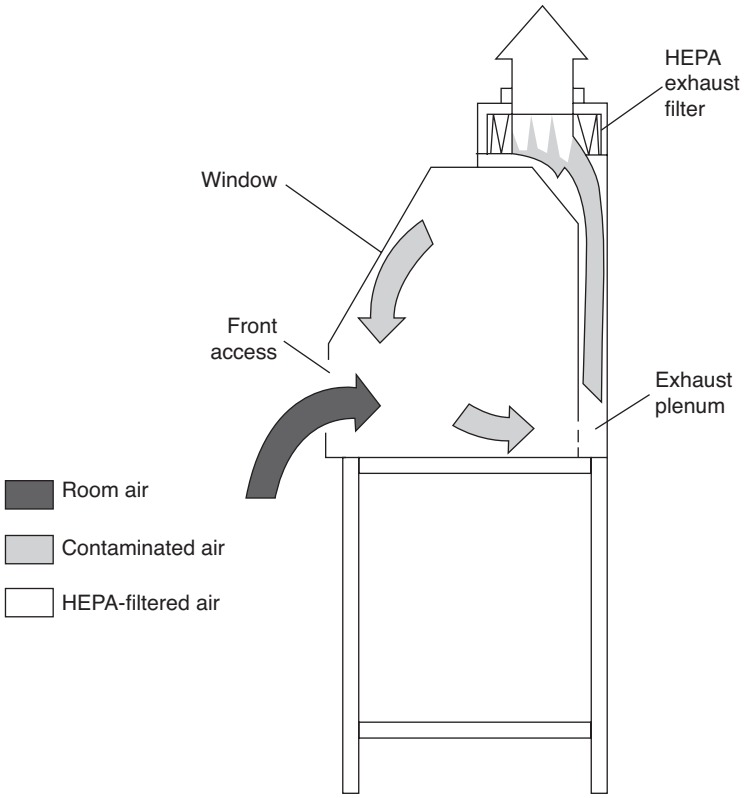


Figure 1.1 A diagrammatic cross-section of a Class I microbiological safety cabinet, showing airflow and HEPA filtration.

of microbiological safety cabinet available. They are used in different contexts, but all are suitable for cell culture work.

- **Class I** (see Figure 1.1) – *gives operator protection only*. These cabinets work by drawing air into the cabinet away from the worker and then the exhaust air passes through a HEPA (high efficiency particulate air) filter to remove particulate matter before it is discharged outside the building, thus protecting the environment.
- **Class II** (see Figure 1.2) – *both the work and the operator are protected*. This cabinet functions as above, but air drawn in from the laboratory via the front access area immediately flows downwards through holes or slots in the front of the work surface into the base of the cabinet before being HEPA filtered and recirculated, so providing an in-flowing curtain of air that offers protection for the operator. Similarly, some of the recirculated filtered air flowing downwards within the hood is also draw through the same holes or slots in the front of the work surface, thus providing a curtain of air that protects the work from particles entering through the front access. Generally this is the cabinet of choice in a cell culture laboratory, allowing handling of all but the most hazardous cell lines.

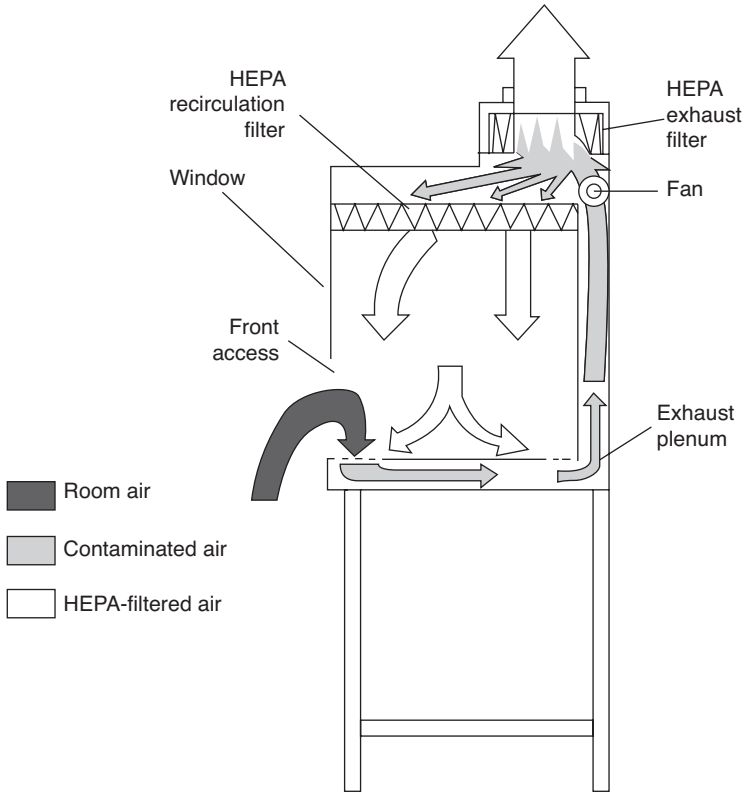


Figure 1.2 A diagrammatic cross-section of a Class II microbiological safety cabinet, showing airflow and HEPA filtration.

- **Class III** (see Figure 1.3) – *These hoods provide protection to the operator and the work*, for those working with Hazard Group 4 organisms. The hoods are totally enclosed, with access to the interior via glove ports, or an air lock. They are onerous to use, and only employed for the handling of extremely dangerous pathogens.

NOTE THAT THERE ARE OTHER TYPES OF AIRFLOW CABINETS THAT ARE NOT MICROBIOLOGICAL SAFETY CABINETS. Unidirectional airflow cabinets (UDAFs) – formerly known as laminar flow cabinets – come in horizontal and vertical versions. They protect the work area, **but not the operator**, by blowing HEPA-filtered air over the work surface. Thus THESE CABINETS MUST NOT BE USED FOR HANDLING CELL CULTURES. Horizontal UDAFs (where airflow is directed at the operator) can be used for the assembly of sterile apparatus, and vertical UDAFs may be used for the filtration of non-hazardous solutions, and the filling of such solutions into containers.

Extreme care needs to be given to the siting of MSCs. Not only is the space available important, but the presence of any obstructing features such as walls, windows and pillars that may disturb air flow will influence the positioning of the cabinet. MSCs need to be

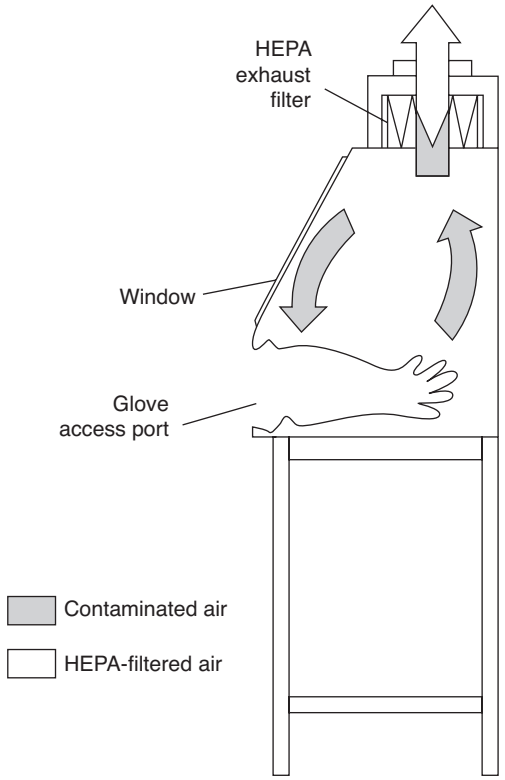


Figure 1.3 A diagrammatic cross-section of a Class III microbiological safety cabinet, showing airflow and HEPA filtration. Fresh air is drawn into the cabinet through a HEPA filter (not shown).

positioned to minimize traffic past them, and away from features and equipment that may disturb airflow in the vicinity. Examples can be seen in Figures 1.4 and 1.5.

There are many options available for configuration of Class II MSCs – the exhaust can be ducted to the exterior of the building or recirculated to the laboratory depending on the organisms to be used, the type of work to be undertaken and the procedures to be employed within the laboratory. (Note that it is essential that if cabinets are to be fumigated independently of the laboratory (see Chapter 2, *Protocol 2.6*) then they MUST exhaust outside the building.) Different manufacturers can supply varying widths and heights of cabinet; some even have adjustable base heights. Reputable suppliers will be happy to make site visits and advise on the best locations and hood types for your specific laboratory and applications.

Some of the considerations when choosing an MSC include the following.

- Are you likely to want to use volatile hazardous substances in the MSC (e.g. toxic chemicals, radioisotopes) where the hazard is non-particulate? If so, consult reference 18 for the different possible subtypes of Class II cabinet. Such cabinets must always be securely ducted to exhaust outside the building.

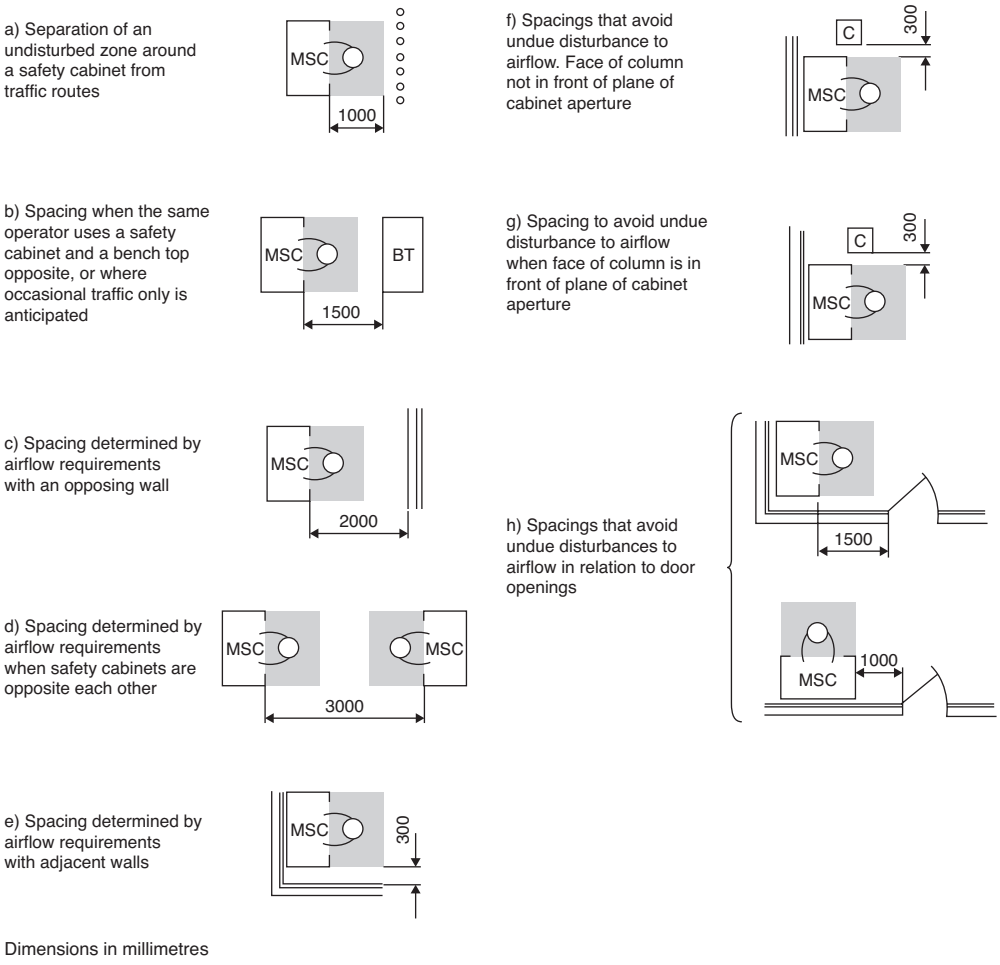
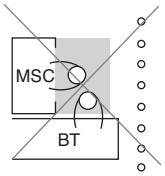
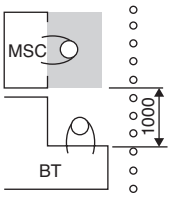


Figure 1.4 Recommendations for minimum distances for avoiding disturbance to the safety cabinet and its operator. For key, see page 14. Reproduced with permission from British Standard 5726:2005.

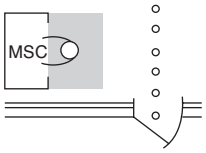
- Does the cabinet you are thinking of purchasing have airflow alarms? For the safety of the user, audible and visible alarms should be present to indicate when the airflow rates fall below specification.
- Do you require electrical sockets within the hood?
- Is the type of work surface in the cabinet suitable for your purposes? Thought should be given to any issues that may affect your work. The work surface can be made in several (lighter) sections or one large piece, but ease of cleaning is important.
- Most cabinets have the optional extra of ultraviolet (UV) lighting for decontamination. Would you find this useful? UV radiation is directional, and thus for it to be effective the cabinet must be totally empty. UV lamps are active microbicidally for a relatively short part of their working life. Efficacy must therefore be monitored regularly.



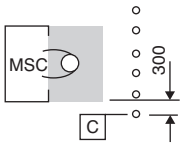
a) bench at right angles to a safety cabinet may keep traffic away from the undisturbed zone but work at the bench will cause disturbances in the air flow



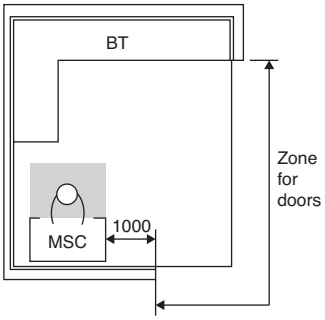
b) A projecting bench will help to keep traffic clear of the undisturbed zone and the work at the bench will have little effect on air flow if sufficient distance is allowed between the cabinet and the projecting bench



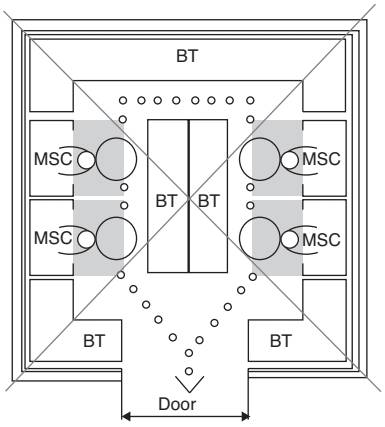
c) Projecting walls and the positioning of doors can be effective in defining traffic routes



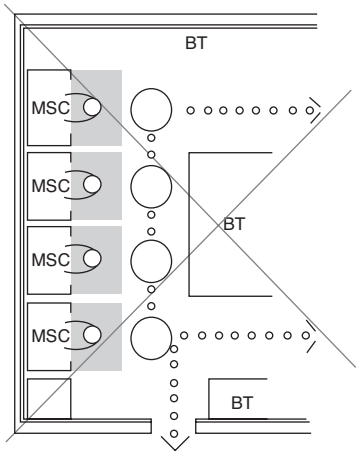
d) Columns can assist in the definition of traffic routes



e) In a small laboratory, the safety cabinet should be clear of personnel entering through the doors



f) Danger of too much air movement in front of safety cabinets should be alleviated by allowing more space between the apertures of the safety cabinets and the bench tops

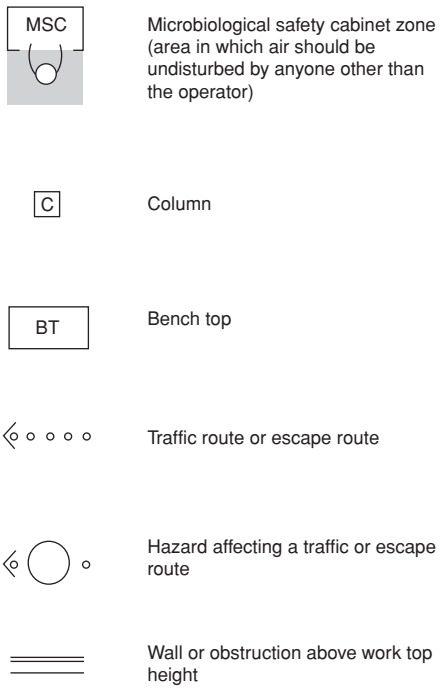


g) Danger of too much movement in front of safety cabinets should be avoided by allowing more space between the apertures of the safety cabinets and the bench tops

Dimensions in millimetres

Figure 1.5 Recommendations for minimum distances for avoiding disturbances to other personnel. For key, see page 14. Reproduced with permission from British Standard 5726:2005.

Key to figures 1.4 and 1.5



NOTE In Figures 1.4 and 1.5, siting arrangements which should be avoided are overlaid with a cross.

- Are gas taps required within the hood?
- Are all internal surfaces accessible to enable thorough cleaning?
- Do you have additional requirements? For specialist laboratories, various pieces of equipment including microscopes and heated bases can be built in.

All MSCs must be regularly serviced and tested to ensure their continued safe performance. Twelve-monthly would be the minimum frequency for cabinets used in low-risk work, but if handling Hazard Group 2 or higher organisms 6-monthly or even more frequent testing may be appropriate. This must be decided on a case-by-case basis, with regard to the relevant risk assessment.

1.2.3.2 Incubators

Incubators for cell culture must maintain an environment which is optimal for cell growth. This requires that three parameters be measured, controlled and reliably maintained: temperature, humidity and carbon dioxide concentration. Generally for mammalian cell culture the incubator is set to 37 °C (or to be safe, 36.8 °C or 36.9 °C, as cells are more

tolerant to a low incubation temperature than a high one) with a relative humidity level of 95% and with CO_2 concentration matched to the media in use (usually 5% – see Chapter 4, Section 4.1.2.1 *i Basal Medium*).

i. Temperature Different types of incubator maintain their internal temperature in different ways. Careful thought should be given to your particular situation and requirements.

- Direct heat allows increased chamber size for a given footprint, while reducing the overall size and weight of the incubator.
- Air-jacketed incubators are lighter, and quicker to reach their temperature set-point than water-jacketed ones, but conversely lose heat faster in the event of a fan or electricity supply failure.
- Water-jacketed incubators are by nature much heavier, and require filling before use and draining before moving. While they take longer to reach their temperature set-point they can maintain it longer in the event of a power failure.

ii. Carbon dioxide concentration CO_2 can be monitored and controlled in various ways. Older-style incubators use a continuous flow of both CO_2 and air, which is mixed. This method can be wasteful of CO_2. Most modern incubators have an internal sensor that monitors the CO_2 in the chamber, and opens a valve to draw pure CO_2 in should the level drop below the set threshold. Infrared sensors are the most sensitive and accurate; they can respond extremely quickly and are unaffected by changes in humidity and temperature. Sensors may need to be removed from the incubator to allow thorough cleaning or fumigation; if in any doubt, check with the manufacturer.

iii. Humidity The atmosphere within the incubator is normally humidified by means of a tray of water placed in the bottom of the incubator, with a fan ensuring even distribution of humidity around the chamber. De-ionized or RO water should be used, with a regular and frequent preventative maintenance schedule in place to ensure the tray is emptied, thoroughly cleaned and refilled, as it can potentially be a source of bacterial and fungal contamination. It is therefore important that the tray can be easily accessed. Non-volatile cytostatic reagents such as thimerosal or a low concentration (1%) of a disinfectant detergent such as Roccal can be included.

- Alarms should be fitted to the incubator to alert the user if the chamber conditions fall outside pre-set limits with regard to any of the above. Some models are also able to keep error logs identifying such issues, which can aid the user should there be an intermittent problem.
- The incubator chamber should be designed in such a way as to minimize the risk of contamination. Ideally, there will be a smooth inner casing and shelves should be easily removable to allow cleaning and disinfection. Many manufacturers offer incubators with copper interiors and shelving, or automatic decontamination routines which can reduce the risk of contamination.

- An additional factor to consider is the culture capacity required by the laboratory – can the incubators be stacked on top of each other should the need arise at a later stage for more? The interior layout can also be varied with full or half shelves, multiple gas-tight inner doors, or roller rig adaptors. Most incubators also offer the option of left- or right-opening doors.

1.2.3.3 Centrifuges

A low speed benchtop centrifuge capable of generating at least 200 *g* is needed for pelleting cells during various culture operations. A variety of sizes and models are available, usually with assorted accessories such as interchangeable rotors (swing-out or fixed angle), different size buckets with compatible inserts to accommodate numerous tube types, and sealable lids for use in more hazardous applications where containment is essential. The requirement of different laboratories will vary, but most would need adapters for standard 50-, 30- and 15-ml tubes and perhaps holders for 96-well plates. Refrigerated versions of these centrifuges are also available.

Ideally, the centrifuge should be sited on its own table, bench or stand to prevent any vibration affecting nearby equipment. A small high-speed microfuge is also useful for centrifuging small tubes (<2 ml capacity). Laboratories handling large quantities of cells or cell-derived products may also require a large refrigerated floor-standing centrifuge, but the availability of an appropriately rated electricity supply should be checked before purchase (see Section 1.2.2).

Many modern centrifuges have the capacity for several programs (with varying speeds/forces and times) to be stored, allowing users to quickly select commonly used parameters. With all centrifuges it is worth checking that the whole of the bowl is easily accessible, and the rotor can be easily removed to allow thorough cleaning.

1.2.3.4 Microscopes

A good inverted microscope is essential in any tissue culture laboratory to allow visualization of cultures in flasks and plates, and also for cell counting. Several objective lenses are required such as 4×, 10×, 20× and perhaps 40×, and phase contrast is a desirable feature to improve images. The siting of the microscope is important. Although it should be located in a convenient position not too far from the MSC and incubator(s), it must also be positioned away from sources of vibration. For operator comfort, it should be located at a convenient height with adequate space below the microscope to accommodate the operator's knees; a height-adjustable laboratory chair should also be supplied.

Microscopy is addressed in detail in Chapter 3.

1.2.3.5 Refrigerators and freezers

For storage of media and additives at least one refrigerator is needed. Ideally, unopened media should be kept separated from that in use – preferably in a cold room or refrigerator nearby. Once opened, each bottle of medium should be labelled with the user's name and date of opening, as well as the cell line it is to be used with, to ensure both that the medium is used within 1 month and also that it is not mixed between users and cell lines (in order

to prevent cross-contamination of cultures). Refrigerators should not be glass fronted, as many media types are light-sensitive and may be degraded by constant exposure to UV.

Freezers at both −20 °C and −80 °C should be available for storage of media components that are unstable at higher temperatures. If possible, aliquots should be stored separately from unopened stocks, but must be clearly labelled with contents, date and lot numbers. Cells frozen under suitable conditions (see Chapter 6) may be stored short term (for a few days) at −80 °C but will gradually lose viability thereafter. Liquid CO_2 back-up can also be added to low-temperature freezers, to maintain the temperature in the event of a power failure.

If possible, data logging and 24-hour monitoring of all freezers containing stock reagents and cultures is desirable to prevent expensive loss in the case of equipment failure. If this is not available then regular monitoring of equipment should be performed.

1.2.3.6 Liquid nitrogen refrigerators for cell storage

Cell stocks for long-term storage are usually kept in vials in either the liquid or vapour phase of LN (see Chapter 6). A wide range of Dewar vessel-type storage refrigerators are available from companies such as Taylor–Wharton. These range from small vessels with a capacity of around 90 1-ml vials, through the popular under-bench-sized vessels holding up to 4000 vials, to huge vessels that might be found in a cell repository and can hold over 33 000 vials.

There are two main methods of storage within these vessels. Vials can either be attached to aluminium canes, which are then placed within canisters in the vessel, or alternatively the vessel can contain tesselating towers of drawers, each of which contains many small compartments each designed to accept a single vial. Small vessels only tend to use the former system, and large vessels the latter, but intermediate-sized vessels can be purchased in either format. The drawer-type vessels tend to use more nitrogen, due to the larger opening required to access the drawers. Note also that LN vessels can hold cells either in the liquid or the vapour phase, the latter being preferable in order to minimize the risk of cross-contamination (see Chapter 6, Section 6.2.4).

As the cell stocks may be a laboratory's most valuable asset, it is important that the LN refrigerators never be allowed to run anywhere near dry. To ensure this, they must be filled on a regular and frequent rota (see Chapter 4, *Protocol 4.2*). For added protection, low level alarms can be fitted to most vessels, and larger vessels can be equipped with an auto-fill system that will add LN from an attached storage vessel if the level drops below a certain point. In addition, important cell banks should be split between vessels and locations whenever possible, in order to avoid total loss in the event of a vessel failure or a fire. For a detailed discussion of cell freezing and banking, see Chapter 6.

1.2.3.7 Miscellaneous equipment

Numerous other smaller pieces of equipment are desirable; some of these are listed below with brief descriptions of their use and key attributes.

- Water bath/dry incubator: for thawing and warming of reagents prior to use. Owing to the risk of contamination the water bath needs to be easy to empty and clean on

a regular basis. Use of a cytostatic reagent (but not azide) in the water is advised. It should be noted that heat dispersal is less efficient in an incubator than in a water bath, but an incubator has the benefit of reducing the risk of contamination from water and associated aerosols. As it may be necessary to use these pieces of equipment at different temperatures (e.g. 37 °C to warm media, but 56 °C to inactivate complement) a working thermometer should always be present, and the current set temperature marked clearly and prominently on the outside of the equipment.

- Aspirator: to remove spent supernatants from culture vessels and plates. The aspirator jar should contain an anti-foam and small amount of detergent such as Decon (Decon Laboratories Ltd). A small volume of Chloros (sodium hypochlorite) solution should be drawn through the line between handling different cell lines and at the end of the working session.
- Automatic hand-held, battery- or mains-operated pipette aid: for use when pipetting with disposable volumetric pipettes (1–100 ml).
- Single- and multi-channel micropipettes covering volumes 1–20 μl, 20–200 μl, 100–1000 μl and 1–5 ml, preferably autoclavable. These are used for accurate pipetting of small volumes and reducing the manual handling involved in using 96-well plate formats. They are available in manual or rechargeable electronic form.
- Laboratory vortex mixer: for cell resuspension, particularly of cell pellets prone to clumping, for example following trypsinization. (Note that it may not be appropriate to use this method of resuspension with fragile cells.)
- Label printer: useful for clear and accurate identification of information on aliquots and tubes of frozen cells.
- Trolleys: may be useful for the transportation of large volumes of media and waste.
- Cell freezing device: providing controlled rate freezing for the long-term storage of cells (see Chapter 6).
- Weighing balance, magnetic stirrers: for preparation of media and reagents.
- pH meter and osmometer: to check pH and osmolarity of media.
- Automated cell culture instrumentation: for example cell counters, or spent medium analysers to assess culture growth.

1.2.4 Culture plasticware and associated small consumable items

Laboratories differ in their preference for the use of glass or plasticware for cell culture and related operations. However, whenever possible the use of single-use disposable plasticware is highly recommended over the alternative method of recycling and reusing glassware. Glassware has a propensity for adsorbing substances such as alkaline detergent onto its surface. If glassware is to be used it needs to be of a high-quality borosilicate and supported by the use of good washing facilities. There is no doubt that plastic containers are less prone to leaching trace elements that may be deleterious to cells.

1.2.4.1 Tissue culture plasticware

There are a number of companies that can offer the standard items of sterile cultureware required for tissue culture at comparable cost and ISO9001-certified quality. Each manufacturer treats their plastics in a slightly different manner, and some cell lines may adhere better to one than another. Comparative testing of samples from a number of suppliers can therefore be a worthwhile investment of time if dealing with demanding cultures. When assessing different products, some factors to be considered are handling benefits of different shapes/formats such as uniform footprint for ease of stacking – bad handling can itself be a source of contamination; alphanumeric identification – some products offer clearer labelling, for example individual alphanumerical codes for well identification; or other features such as (for multi-well plates) non-reversible lids with condensation rings to reduce the risk of contamination in multi-well plates, or culture flasks with built-in filters in the caps to aid gas exchange. In addition, certain products may be guaranteed to be non-pyrogenic and/or DNAse free, characteristics that may be essential for certain applications; these contaminants are known to have deleterious effects on certain cell cultures, assays and cell-derived protein products. It is much better to avoid possible issues from the beginning by scrupulous control of starting materials.

The basic procedures of cell culture that require plasticware fall into three categories.

i. Cell growth The most common vessels used for growing cells are 'tissue culture' treated for maximization of cell growth, be it in anchorage-dependent (adherent) or independent (suspension) mode. They are available in many formats depending on the scale of culture required including tubes, flat-bottomed flasks, spinner and Erlenmyer flasks, Petri and multiwell dishes, flasks, roller bottles (Chapter 10, Section 10.2.1.1) or stacked-plate vessels (Chapter 10, Section 10.2.1.2). Some manufacturers also offer non-tissue culture-treated versions, for use in applications where unwanted cell attachment is a problem.

Although uncommon, poor or uneven cell growth can occasionally be attributed to product quality issues – either poor design or moulding – and could be batch specific. Other possible causes of poor cell growth are discussed in Chapter 4, Section 4.3.

ii. Liquid handling and filtration This equipment, includes volumetric pipettes, Pasteur pipettes, centrifuge tubes, autoclavable micro-pipette tips, and filters for sterilizing tissue culture solutions.

The pore size of sterilizing filters should normally be 0.2 μm, or even 0.1 μm in order to exclude mycoplasma as well as other micro-organisms. Some filter membranes, for example those made of polysulphone, have low protein-binding properties and are essential where the protein concentration of the filtered solution is critical, particularly if the molecule is highly charged as are some of the polypeptide growth factors. Very small volumes of valuable reagents should be filtered through units where the dead space (hold-up volume) is minimal. It is cost-effective, therefore, to have some of these more expensive filters reserved for special purposes. Larger volumes can be filter sterilized using either pre-assembled, disposable units which attach to a vacuum line or, more economically, a washable, autoclavable, filter housing unit for repeated use (see Chapter 2, Section 2.2.5).

iii. Storage These include sample tubes, Eppendorf tubes, cryotubes and larger-volume screw-capped bottles or bags.

1.2.4.2 Miscellaneous small items

As in any other laboratory, items of general use, such as a calculator and test tube racks, are required and these items should be readily available, in sufficient quantity for the workload, and stored in the cell culture laboratory. This limits the transfer of materials in and out of the culture facility and thus helps to reduce contamination issues. If reusable pipettes are used, it is convenient to sterilize them in pipette cans, which should be marked as dedicated for use in one room, and once opened kept for exclusive use by an individual worker. Listed below are some of the small items most cell culture laboratories will require, but these lists are in no way intended to be comprehensive.

General items that may be required include:

- autoclave tape or bags
- pipette cans
- sharps- and waste-disposal units
- cryomarker pens
- tube racks
- cages or boxes for washing up
- personal protective equipment, for example gloves, safety glasses
- disinfectant spray bottles
- timers
- calculators.

Specialized items that may be required include:

- haemocytometer and/or other cell counting equipment (see Chapter 4, Section 4.2.6.3)
- cell dissociation grinders and sieves
- cell scrapers
- cloning rings (see Chapter 8)
- well membrane/cover slip inserts
- forceps
- scalpels and/or scissors
- microcarrier beads.

1.2.5 Washing reusable tissue culture equipment

All tissue culture equipment which can be washed, sterilized, and reused should go through the same general process, as described in *Protocol 1.1*. This can either be performed manually or by a tissue culture-dedicated automatic washing machine with both acid rinse and distilled water rinse facilities.

Over time a film of cell debris, protein and other materials can develop, and such glassware can become a source of endotoxin contamination. Therefore, periodic stripping by washing in sodium hydroxide (NaOH) is recommended. Recoating of certain vessels like spinner flasks with a silicone reagent to prevent cell attachment will be necessary. All reusable glassware will have to be sterilized after washing and drying. This topic is covered in Chapter 2.

PROTOCOL 1.1 Washing and sterilization of reusable labware

Equipment and reagents

- Ultra-pure water
- Chloros (hypochlorite) solution for soaking
- Phosphate-free detergent (e.g. Micro (International Products Corp.) for manual washing; low-foam for machine, as recommended by manufacturer)
- AnalaR (or similar high purity) HCl for manual washing; formic or acetic acid for machines
- Rigid plastic soak tanks, cylinders, and beakers (for small items and instruments)

Method

1 Soak items either in Chloros solution (except metals), or directly in detergent.
2 Wash with detergent (by soaking or machine).
3 Perform tap water rinses (continuous or sequential).
4 Rinse with acid (except for metals).
5 Repeat tap water rinses as in step 3 above.
6 Rinse with distilled/reverse osmosis/ultra-pure water (two or more times).
7 Dry with hot air.
8 Store temporarily, capped or covered, for example on preparation bench.
9 Prepare for sterilization (see Chapter 2).
10 Sterilize by autoclaving or in dry oven.
11 Store for use in dedicated area.

1.2.5.1 Pipette washing

Unless a pipette-washing facility is available in the automatic washing machine, in which case the supplier's instructions should be followed, this should be carried out using *Protocol 1.2.*

PROTOCOL 1.2 Washing glass volumetric pipettes

Equipment and Reagents

- Forceps
- Compressed air supply, or water jet
- Soaking cylinders and associated plastic pipette carriers
- Detergent suitable for use with cell culture equipment such as 7X or Decon 90
- Syphon-operated pipette washer
- Concentrated HCl
- Ultra-pure (tissue culture-grade) water (see Section 1.2.2.1)
- Drying oven

Method

1 Unplug the pipettes using forceps or a high pressure air or water jet applied to the opposite (tip) end.

2 Place them, tip upwards, in a plastic pipette carrier.

3 Insert the carrier into an outer cylinder containing detergent solution and leave overnight to soak.

4 Transfer the carrier to an automatic, water syphon-operated pipette washer[a].

5 After 3–4 h, add about 50 ml of concentrated acid (HCl or acetic) at the filling stage of the cycle and turn off the tap when full.

6 Leave for about 30 min to remove residual traces of detergent.

7 Run the tap water rinse cycle again for at least 1 h.

8 Immerse the pipettes in their carrier in two or three changes of ultra-pure water, in a clean plastic cylinder kept for the purpose.

9 Drain for a few minutes and transfer the carrier to a drying oven.

Note

[a] Pipette washers operate on tap water, the flow rate of which must be within certain limits, set empirically. The container fills and empties repeatedly, by a syphon mechanism.

Problems can frequently occur if equipment is inadequately cleaned. Such problems are largely avoided by attention to the following details.

- Handle all glassware with care, especially pipettes, and discard any broken or chipped items immediately using appropriate procedures.
- Rinse all items before soaking, remove labels and fully immerse.
- Soaking should be carried out as soon as possible after use, as many tissue culture solutions contain protein, which, if left for any prolonged period, can result in microbial growth and difficult-to-remove dried-on protein.
- Separate ready-filled soak tanks should be available near sinks for both easily broken delicate glassware and more robust vessels.
- The solutions in tanks need to be changed regularly. A convenient-to-use tablet form of sterilizing agent is always helpful as it speeds the preparation of soaking solutions.
- If washing is to be performed manually, further soak items overnight in detergent followed by an acid rinse to neutralize alkaline detergent residue.
- Ensure adequate rinsing to remove any residues of cleaning materials that can be toxic to cells.

1.2.6 General care and maintenance of the tissue culture laboratory

The proper care of equipment and attention to tidiness and cleanliness are especially important in a tissue culture laboratory. Consequently, it is useful to have a checklist of tasks that must be completed on a daily, weekly or monthly basis. The following lists can be used as a guide but should be modified and extended to suit the individual laboratory.

Daily checklist:

- Where appropriate, check room air handling pressure differential(s) before entry.
- Record incubator, refrigerator and freezer temperatures.
- Check incubator temperature and CO_2 are at set points.
- Check CO_2 cylinder pressures.
- Check conductivity of purified water.

Protocols for laboratory start-up and shutdown can be found in Chapter 4, *Protocols 4.1* and *4.2*.

Weekly checklist:

- Wash floor and bench work surfaces (if not done daily).
- Clean underneath MSC work surface.
- Change water in water baths.

- Change water in humidifier trays in incubators.
- Replenish stocks of routine reagents and plasticware. (Do not allow cardboard boxes to enter the sterile work area).
- Empty aspirator jars as necessary (if not done daily).
- Top up LN in cell freezers (or twice weekly).
- Change floor sticky mat (or more often if required).
- Change used laboratory coats for clean ones.

Monthly checklist:

- Cleanse and sterilize reverse osmosis unit.
- Check water ultra-purification cartridges.
- Check whether equipment services or safety tests are approaching.
- Defrost freezers (as necessary).
- Calibrate instruments and monitors – if not done more frequently or on a regular basis before use.
- Strip down and clean/sterilize the insides of incubators
- Perform environmental monitoring if required, and if not performed more frequently – see *Protocol 1.3*.

PROTOCOL 1.3 Environmental monitoring

Equipment and reagents

- 37 °C incubator – separate to culture facility
- Tryptone Soya Agar (TSA) settle plates (90 mm irradiated, Cherwell Labs)
- Sabouraud Agar (SDA) settle plates (90 mm irradiated, Cherwell Labs)

Method

1 Remove from outer packaging 2 × TSA and 2 × SDA plates and label each plate with details of the area to be monitored, for example MSC or bench position, and date.

2 Open one of each plate with agar exposed to the testing environment and with lid face down. The second plate is a negative control for the same area and remains closed.

(*continued*)

3 Leave for 4 h, then close.

4 Incubate (closed) with agar uppermost at 37 °C for 2 days.

5 Record number and type of colonies on the settle plates. Incubate further at room temperature for four more days and record.

6 Establish background colony count levels for specific areas and refer to troubleshooting section if above upper limit and decontamination is required. Typical acceptable counts for a general cell culture laboratory are MSC <5, bench <20, incubator <10.

7 More extensive testing, possibly including the use of swabs and contact plates, may be required in specialized areas, for example aseptic dispensing rooms.

If the laboratory is a multi-user facility, then one individual should be assigned the tasks of overseeing the running and maintenance of the laboratory and the training of staff, and ensuring that all users abide by an agreed code. Examples of good practice include:

- Clearing away promptly and effectively after finishing sterile work, i.e. vacating the MSC and cleaning up any spills, followed by swabbing down the work surface with alcohol and drawing disinfectant through the aspirator line.
- Update hood log records – details of use, for example time, date, operator, cell type handled, cleaning procedure adopted.
- Dealing promptly and appropriately with waste and any used glassware to be soaked.
- Labelling opened sterile equipment and cell line-specific solutions, for reuse only by the same named individual.
- Maintaining good communal records, for example of shared cell or reagent stocks.
- Checking incubated cells regularly (daily) for condition and early signs of contamination.
- Checking incubators regularly and discarding surplus or contaminated stock cultures according to approved safety procedures (e.g. by inactivation, autoclaving or soaking).

Further guidance on good practice in the cell culture laboratory can be found in reference 20.

1.3 Troubleshooting

In a well designed laboratory, where routine monitoring is kept up to date and best practice followed (as outlined above in Section 1.2.6 and in other chapters of this book) few problems should arise.

The major issues likely to be encountered are poor cell growth, or contamination; these are covered in greater detail in Chapters 4 and 9 respectively. There are three main routes whereby culture conditions and performance can be compromised:

- **Operator error** – poor aseptic technique
  These are normally sporadic events and easily identified and rectified by careful disposal and additional cleaning.
- **Quality issues** – introduction of inherent problems via raw materials (including cell lines) or consumables
  Examination of batch records can often identify the causative material – highlighting the necessity for good documentation. If the source of the problem is not easily identified, switch to alternative batches, and/or refer to the supplier if appropriate, to identify the cause via a process of elimination. Identity testing should be implemented, if relevant.
- **External source** – equipment failure
  This can be separated from the above two points by the global nature of the problems encountered, for example if the incubator is faulty all the cultures within it will be affected. Extra equipment checks can be implemented immediately to help pin-point the cause of a problem more accurately prior to calling a specialized engineer. For example, in the case of poor cell growth, check the incubator. Assuming the displays are showing the expected readings, check the actual parameter values by another means, e.g. place a thermometer inside so that you can take a reading without opening the door, and use a CO_2 monitoring device to check the accuracy of the displayed reading. Recalibrate the incubator's sensors as required.

1.3.1 Microbial contamination

This particular type of contamination can be subdivided into two basic categories: those easy to detect, usually by eye or by microscopic observation of the effects on the culture (such as a change in pH and/or turbidity) caused by bacteria, mould and yeast; and those more difficult to detect such as viruses, mycoplasma or cross-contamination by other cell cultures. For further details see Chapter 9 and reference 19.

Microbial contamination can result in persistent or recurrent problems for the whole laboratory, which are much easier to prevent than to cure. The length of time during which a culture contaminant escapes detection will be an important determinant of the extent of the damage it creates in a laboratory or on a research project. The maintenance of high standards is therefore fundamental, and best practice should be actively encouraged [20].

Each occurrence of microbial contamination should be recorded and investigated as far as possible: no incident should be simply ignored once the clean-up is complete, as this is only storing up problems for the future. With frequent checks and good record keeping contamination events can be dealt with far more effectively or avoided entirely, minimizing disruption to the laboratory.

In general, there are three factors to assess and act upon immediately: extent, containment and clean-up. These are crucial to the correct handling of the situation and are

immediate considerations. At a later time it is important to try to identify the source of the contamination. This may not be so easy to pin down – but especially in the case of recurrent problems, this becomes increasingly important.

1.3.1.1 Extent

As soon as possible, all users should be alerted to the fact that there is microbial contamination in the laboratory. All cultures must be checked in a thorough manner, with any found to be contaminated being removed and disposed of appropriately as soon as possible. It is important to establish how widespread the problem is – this will help in the other steps of the investigation.

1.3.1.2 Containment

Often the contamination is restricted to one flask or plate – an isolated incident for one user. In this case it may be possible to trace the movements of that culture and operator so that any equipment involved can be selectively cleaned and decontaminated, causing minimal disruption but ensuring there is no further reservoir of contamination. This process can be aided when MSC logs are in use and work is segregated by type within incubators. If it is suspected that poor technique or practice is involved, it may be necessary to give the individual involved further training and/or supervision.

Sometimes the number of users and cultures involved indicate a larger problem, and a full shut down and decontamination of the whole laboratory is advisable.

1.3.1.3 Cleaning up

Once the spread of the contamination has been established, a course of action can be identified and undertaken.

i. Limited spread → selective cleaning

- Identify, remove, sterilize and dispose of any contaminated cultures.
- Trace the movement of users and cultures.
- Identify MSCs, incubators and other equipment used.
- Dispose of all opened consumables used within affected MSCs.
- Thoroughly clean and decontaminate the MSCs: including all internal and external surfaces (fumigate if possible – see Chapter 2, *Protocol 2.6*).
- Empty the affected incubator, remove water tray. Thoroughly clean and decontaminate the incubator and shelves; run a decontamination programme if the incubator has one.
- Clean and decontaminate any other equipment affected.

- Decontaminate all work surfaces.
- Discard opened media used by the operator(s) involved.
- Send operators' lab coats for laundering.

ii. Wide spread problem → full shut down

- Sterilize and dispose of all growing cultures.
- Dispose of all opened consumables within the laboratory.
- Discard all open media, and reagent aliquots.
- Clean and decontaminate all equipment.
- Thoroughly clean and decontaminate all MSCs: including all internal and external surfaces (fumigate if possible – see Chapter 2, *Protocol 2.6*).
- Thoroughly clean and decontaminate all incubators (including shelves); run a decontamination programme if the incubators have them.
- Decontaminate all work surfaces.
- Thoroughly clean and decontaminate the laboratory; both high and low level including the floor.
- Send all lab coats for laundering.
- If possible, fumigate the laboratory (see Chapter 2, *Protocol 2.5*).

1.3.1.4 Identifying the source

The source of the contamination should be identified – if possible. This will enable remedial action to be taken, and help to prevent a repeat.

There are four main potential sources of contamination to consider:

- consumables
- raw materials (cells, media or media component)
- operator error
- external sources.

i. Consumables Has the sterility of a consumable been compromised?

This may be local (e.g. pipette wrappings damaged when opening a box), or more widespread, for example a batch of tips failing to be irradiated by the manufacturer. The identity of the contaminated consumables can often only be confirmed if batch numbers are recorded.

ii. Raw materials

- *Media*
  Has a medium or medium component been contaminated?
  Again this may be a local issue (such as contamination upon aliquotting, or while preparing a bottle of medium), or could be due to a manufacturing failure. Samples of a new batch of medium can be incubated prior to use to confirm sterility.
  With good record-keeping it should be possible to see who has used which aliquots, or batch numbers, to see if there are any patterns which reflect the spread of contamination.
- *Cells*
  Cells are a potential source of contamination, for example primary cells or incoming cell lines. Once again where possible cell lines should only be obtained from reputable suppliers (preferably culture collections such as the American Type Culture Collection (ATCC) or the European Collection of Cell Cultures (ECACC) – see Chapter 9 for contact details) and cells quarantined on receipt. Once cell growth is established, tests (e.g. for sterility and the absence of mycoplasma – see Chapter 9) can be performed to ensure acceptability for further use and storage.

1.3.1.5 Operator error

Is the contamination due to one operator?

While occasional sporadic contamination can occur for any operator, it may become evident that some users are more prone to this than others. This reinforces the need for operator training, especially for those new to cell culture, and also when new techniques are being employed. Training can be 'in house', an experienced operator looking over the shoulder to spot any obvious errors and offer advice, or could be provided by an external agency (such as the courses offered by ECACC – see Chapter 9 for contact details).

1.3.1.6 External sources

Sometimes the source of the contamination comes from outside the laboratory, and may be beyond the control of the users. One issue for cell culture labs is nearby building work – it is well known for spreading fungal spores far and wide. There may be shared areas that can cause problems (nearby bacterial fermenters etc.), or common utilities that may fail – such as an autoclave or air handling unit.

1.3.2 Quality control testing

The best strategy for reducing contamination is to be proactive by routinely monitoring supplies, media and solutions, work areas and, most importantly, cell cultures for contaminants before they are used in critical applications and experiments. Unfortunately, there are no easy solutions: no single microbiological medium can detect all types of biological contaminants, and practical testing methods often miss low levels of contaminants.

The process of detection is made even more difficult by the use of antibiotics in culture media. However, it is recommended that laboratories, as a minimum, incorporate QC tests for sterility, mycoplasma, and cell culture identity into their monitoring process. Each of these is described in detail in Chapter 9.

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