Principles of Cancer Cell Culture

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Abstract

The basics of cell culture are now relatively common, though it was not always so. The pioneers of cell culture would envy our simple access to manufactured plastics, media and equipment for such studies. The prerequisites for cell culture are a well lit and suitably ventilated laboratory with a laminar flow hood (Class II), CO₂ incubator, benchtop centrifuge, microscope, plasticware (flasks and plates) and a supply of media with or without serum supplements. Not only can all of this be ordered easily over the internet, but large numbers of well-characterised cell lines are available from libraries maintained to a very high standard allowing the researcher to commence experiments rapidly and economically. Attention to safety and disposal is important, and maintenance of equipment remains essential. This chapter should enable researchers with little prior knowledge to set up a suitable laboratory to do basic cell culture, but there is still no substitute for experience within an existing well-run laboratory.

Key words: Cell culture, Serum, Medium, Buffer, Adherence, Equipment, Plastics

1. Introduction

Cell culture is an important tool for biomedical scientists and is widely practiced within academic, hospital and industry laboratories. It permits everything from the discovery of new molecules and their function, to high throughput testing of potential drugs – and not just for cancer. Culture of normal cells is now relatively routine and it is possible to obtain non-neoplastic cells (e.g. inflammatory cells, endothelial cells) from tumours for study. Culture of neoplastic cells directly from human tumours is routine, though multiple methods exist. However, most experiments are done with cell lines, grown from tumours and often passaged many times in media containing serum and other additives to promote their growth.
The history of cell culture, reviewed in the previous edition of this book by Langdon (1), goes back 125 years to a publication by William Roux who successfully cultured chick embryo tissue in saline for several days. The first human experiments were also performed in the nineteenth century when Ljunggren (1898) showed that human skin could survive in ascitic fluid. Ross Harrison (1907) is regarded as the father of tissue culture, as he introduced tissue from frog embryos into frog lymph clots and showed that not only did the tissue survive, but nerve fibres grew out from the cells. The lymph was quickly replaced by plasma, and then by more systematic studies to identify factors required for cell growth in culture. The first cancer cells were cultured by Losee and Ebeling (1914) and the first continuous rodent cell line was produced by William Earle in 1943 at the National Cancer Research Institute. In 1951, George Gay produced the first human continuous cell line from a cancer patient, Helen Lane, and HeLa cells are still used very extensively. The next two decades saw a huge expansion in the number of studies which defined the media we still use today. Serum free media started with Ham’s fully defined medium in 1965, and in the 1970s, serum-free media were optimised by the addition of hormones and growth factors. There are now thousands of cell lines available, and for some, many stable variants have also been produced.

Some common definitions are listed in Table 1 (1). “Cell culture” refers to the maintenance in vitro of disaggregated cells, while “organ culture” refers to a culture of a non-disaggregated tissue. The term “tissue culture” encompasses both terms. The initial culture is known as a “primary culture” and undergoes multiple “sub-cultures” or “passages” to produce a “cell line”. Cell lines may be continuous (capable of unlimited growth) or finite, characterised by senescence after a limited number of population doublings. Continuous cell lines are “transformed” – this seems to equate to their acquisition of telomerase activity in most instances (see Chapter 1) and may occur during or at the time of generation of a cell line. There are now artificial methods by which cells can be “immortalised” (synonymous with transformation),
for example, hTERT transfection, and these methods may be applicable to non-neoplastic cells.

Cell lines may exist as adherent cell cultures, grown on a “substrate” such as plastic or glass, or they may grow “in suspension” This tends to go with their cell of origin, so most lymphocyte cell lines will grow readily in suspension, while the reverse is true of carcinoma cell lines. The coating of plastic or glass with proteins or other molecules can promote adherence and change the phenotype of cells in culture, even after many passages. All grow in a cell culture “medium”– plural media – which can be made up from its constituents, but is more commonly bought from a commercial manufacturer.

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Cell culture</td>
<td>Maintenance of dissociated cells in vitro</td>
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<tr>
<td>Tissue culture</td>
<td>Maintenance of tissue explants in vitro</td>
</tr>
<tr>
<td>Primary culture</td>
<td>The initial culture of cells dissociated directly from tumour or obtained from the blood or a malignant effusion</td>
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<tr>
<td>Cell line</td>
<td>Cells sub-cultured beyond the initial primary culture</td>
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<tr>
<td>Finite cell line</td>
<td>A cell line with a limited life span, one that undergoes senescence after a defined number of doublings</td>
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<tr>
<td>Continuous cell line</td>
<td>A culture which is apparently capable of an unlimited number of population doublings; often referred to as immortal cell culture</td>
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<tr>
<td>Clone</td>
<td>The cells derived from a single cell of origin</td>
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<td>Immortalization</td>
<td>Enabling cells to extend their life in culture indefinitely</td>
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<tr>
<td>Lag phase of growth</td>
<td>Initial slow growth phase which occurs when cells are sub-cultured</td>
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<tr>
<td>Log phase of growth</td>
<td>Most rapid phase of exponential cell growth</td>
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<tr>
<td>Plateau phase of growth</td>
<td>Slowing of cell growth when cells become confluent</td>
</tr>
<tr>
<td>Population doubling time</td>
<td>Time taken for cell number to double</td>
</tr>
<tr>
<td>Cell bank</td>
<td>Repository of cancer cell lines and materials derived from them</td>
</tr>
<tr>
<td>Tissue bank</td>
<td>Repository of tissue samples from patients</td>
</tr>
<tr>
<td>Substrate</td>
<td>The matrix on which an adherent cell culture is grown</td>
</tr>
<tr>
<td>Passage</td>
<td>Subculture of cells from one container to another</td>
</tr>
<tr>
<td>Confluent</td>
<td>Situation where cells completely cover the substrate</td>
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</table>
The requirements of cell culture were defined during the last century, and have not changed, despite our increasing knowledge of the molecular biology of the cell on which these requirements are based. Nutrients are provided, as well as oxygen and a means for the removal of carbon dioxide. Most cells require a substrate and this is now nearly always plastic, though growth of cells on glass coverslips is still a useful method.

A number of common media are listed in Appendix 1, and most are based on a basal saline medium to which various components are added. To grow, cells require amino acids, vitamins, metal ions, trace elements, and an energy source, usually provided as glucose. Other additives include buffer (HEPES is particularly common) and phenol red to indicate the acidity of the culture. Eagle’s basal medium (BME) was modified by adjusting the amino acid concentration to produce Eagle’s minimum essential medium (MEM). This was further modified by Dulbecco who quadrupled the amino acid and vitamin concentration to produce Dulbecco’s minimum essential medium (DMEM). Iscove’s modification of DMEM is designed to support haematopoietic cells and contains even more amino acids and vitamins, as well as selenium, pyruvate and HEPES buffer.

Morgan et al. (2) used a medium that supported an even wider range of cells and is now known as Medium 199. Less complex versions were produced, including one by the Connaught Medical Research Laboratory (CMRL 1066) which was one of the first to examine serum-free growth of cell lines to examine the factors in serum required for the growth of certain cell types. Ham’s nutrient mixtures F10 and F12 were developed for the growth of Chinese Hamster Ovary (CHO) cells with or without serum supplementation. However, a 50:50 mixture of Ham’s F12 and DMEM is now widely used for serum-free cell culture as this combines the use of trace elements and vitamins in the F12 with the nutrients present in DMEM. Ham’s group went on to design a series of media (MCDB) for a series of individual cell lines.

A basic medium described by McCoy et al. in 1959 (3) has also been widely used and was the basis for one developed by Moore et al. (4, 5) at Roswell Park Research Institute – RPMI1640 is perhaps the most widely used cell culture medium in use today.

Serum is often added to the media described above and in Appendix 1 to replace many requirements which are missing, even from the most sophisticated media. These include proteins, lipids
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and carbohydrates as well as growth factors and attachment factors. The concentration of components present is often variable and the practice of using newborn or fetal bovine sera has become common. Concentrations of 5–20% are normally used, depending on the cell type. There is batch to batch variation in the content of factors present and it is often best to stick with one supplier found to work well with a particular cell line. Serum can be divided into aliquots and frozen at −20°C following heat treatment for 30 min at 56°C (if necessary) to remove complement components.

6. Serum-Free Media

The inherent variability in the composition of sera can lead to variation in results obtained using media which contain sera. The uncertainty serum supplementation introduces to cell culture has lead many researchers to explore the use of serum-free media. Two different approaches have been taken – one, espoused by Sato and colleagues (6, 7), was to add specific supplements, while the other route, taken by Ham’s group (8, 9), was to increase the concentration of various components of existing basal media. The addition of insulin, transferrin, selenite, hydrocortisone and cytokines has become common, with varying degrees of success. The addition of albumin to improve the protein content of media has proven useful, and components to promote adhesion signalling such as fibronectin are often useful. Other additives include prostaglandins, hormones and triiodothyronine (10–13). One useful tip for transferring cells from serum-containing media to serum-free media is to gradually reduce the serum content, as this allows cells to adapt gradually to changing conditions (14–16).

7. Obtaining Media

Media can be obtained commercially or made up from constituents. It is important to choose appropriately for the cell line and experimental design – shelf life varies from 9–12 months for made up media, to 2–3 years for powdered media that can be made up with sterile water. A good compromise is often to buy bottled 10× concentrated media with an extended shelf life (12–24 months) that is diluted with sterile water before use.

7.1. Substrate Specificity

Cell culture plates come in many different sizes and compositions. The majority in use allow adherence and are made of polystyrene, but in some circumstances it can be useful to use polypropylene which prevents most cells adhering to the plastic.
Plastics designed to promote adherence have a charged surface resulting from chemical treatment or irradiation. If cell adhesion is desired, it is often possible to improve this by coating plates with fibronectin or collagen. Several commercial preparations are available, but pre-coating plates with serum or conditioned medium can also be effective. Three-dimensional growth within collagen or more complex mixtures such as Matrigel can be used, and dual cell cultures either with direct contact or separation by a filter can allow the study of interactions under defined experimental conditions. The choice of well size or culture flask is dictated largely by the number of cells required for a particular assay and the time for which they will be in culture before passage. Cell culture has been performed successfully in 384-well and even in 1,536-well plates. At the other end of the scale are large systems with enormous surface areas capable of producing many millions of cells in a single passage.

7.2. Environment

Most cell culture is performed in incubators at 37°C with a tolerance of one degree from this. Above 40°C, cells will usually die rapidly. Culture media are usually designed with buffering capacity, but require an atmosphere of 5% CO₂ to ensure optimal performance and are kept at pH 7.2–7.4. Acidification of the media can be seen by changes in the colour of phenol red, if present. This is yellow at pH 6.5, orange at pH 7.0, red at pH 7.4 and purple at pH 7.8, providing an instant readout to the investigator of the pH of cultures. The use of 5% CO₂ in air means that cells are exposed to much higher partial pressures of oxygen than would be the case in tissues. Hypoxic chambers are available to control this, if necessary. Humidity is also critical, particularly in microplates, where drying artefact is to be avoided. Finally, sterility is all-important! These requirements dictate the necessity for a considerable outlay in terms of equipment to support cell culture – if any one of these facets is ignored, the result will usually be a collection of failed experiments.

8. Cell Lines

8.1. Cancer Cell Line Collections

There are thousands of cell lines available, and most can be accessed from culture collections. Table 2 lists a number of culture collections and companies that provide verified cell lines for research use. There are often constraints on the use of such lines imposed by the originating laboratory or the collection. In general these regulate to what extent cell lines can be used for purposes other than research in the institution purchasing the cell line. In addition to academic not-for-profit culture collections, a number of companies will now supply cell lines. These are sometimes
more appropriate for commercial use or where the purchaser has little control over their use.

Cell culture collections will characterise cell lines new to them using techniques such as DNA fingerprinting and expression analysis to ensure that the identity of the cell line can be verified. They will then grow the cell line for a few passages to provide a Master Cell Bank, stored in liquid nitrogen, which will in turn provide material to Working Cell Banks from which researchers will be sent aliquots for their work. The samples stored in the Working Cell Bank will be authenticated and certified free of Mycoplasma and other common contaminating organisms.

Table 2
Major cell line banks

<table>
<thead>
<tr>
<th>Bank</th>
<th>Website</th>
<th>Location</th>
<th>Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>American Type Culture Collection (ATCC)</td>
<td><a href="http://www.atcc.org">www.atcc.org</a></td>
<td>USA</td>
<td>Contains 950 cancer cell lines, and expanding collection of hTERT immortalized cell lines from human tissue</td>
</tr>
<tr>
<td>European collection of animal cell culture (ECACC)</td>
<td><a href="http://www.hpacultures.org.uk">www.hpacultures.org.uk</a></td>
<td>UK</td>
<td>Contains 1,100 cell lines originating from over 45 different species</td>
</tr>
<tr>
<td>Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (German Collection of Microorganisms and Cell Cultures)</td>
<td><a href="http://www.dsmz.de">www.dsmz.de</a></td>
<td>Germany</td>
<td>Contains 652 human and animal cell lines, and a separate collection of 586 human leukaemia–lymphoma cell lines</td>
</tr>
<tr>
<td>Banca Biologica e Cell Factory (Interlab cell line collection)</td>
<td><a href="http://www.iclc.it">www.iclc.it</a></td>
<td>Italy</td>
<td>Lists 268 cell lines.</td>
</tr>
<tr>
<td>HyperCLDB</td>
<td>//bioinformatics.istge.it/hypercldb</td>
<td>Italy</td>
<td>Hyperlink database from the Interlab project, allowing rapid searches</td>
</tr>
<tr>
<td>Japanese Collection of Research Bioresources (JCRB)</td>
<td>cellbank.nibio.go.jp</td>
<td>Japan</td>
<td>Contains more than 1,000 cell lines.</td>
</tr>
<tr>
<td>Asterand</td>
<td><a href="http://www.asterand.com">www.asterand.com</a></td>
<td>USA</td>
<td>Commercial supplier of Human Primary Cells/Cell lines, including breast, prostate and haematopoietic cell lines</td>
</tr>
<tr>
<td>RIKEN gene bank</td>
<td><a href="http://www.brc.riken.go.jp">www.brc.riken.go.jp</a></td>
<td>Japan</td>
<td>Contains over 1,000 human and animal cell lines.</td>
</tr>
</tbody>
</table>
More recently, many large academic hospitals have developed tissue banks, repositories of samples from patients, often but not always including frozen tissue samples stored in liquid nitrogen from which viable cells may be obtained.

8.2. General Growth Characteristics

When a new cell line is obtained, it is important for the laboratory to document its growth characteristics, including morphology, adhesion and serum requirements. Most laboratories passage new lines to produce their own master and working cell line banks. Storage of carefully labelled aliquots of early passage cells in liquid nitrogen is standard within most experienced cell culture laboratories, as with time and many passages, any cell line can lose or gain characteristics. Changes in genotype are common and direct comparison of results between laboratories is sometimes difficult as a result.

8.3. Develop or Use Existing Lines?

With such a wealth of cell lines available, it might be thought unnecessary to propagate new cell lines from primary cell cultures, but this is still valuable. As an example, it has recently become clear that some non-small cell lung cancers have activating mutations of the EGFR gene (17). However, only a fraction of these mutations are available within cell lines and established cultures from lung cancers with unusual mutations would be very valuable to researchers.

Although few laboratories now develop their own cell lines, many produce variants of existing lines, for instance by exposure of cell lines to drugs at increasing concentrations to produce variants with enhanced resistance. This allows the study of resistance mechanisms. Technical methods now exist to allow stable transfection of cells so that they overexpress genes of interest (18), and this is even provided as a service by some companies. Some variants, for instance cell lines expressing luciferase (19) or green fluorescent protein (GFP) (20) provide a simple means of assessing their viability, and such markers can be linked to genes of interest to provide simple markers of the level of gene expression under different conditions (19).

9. Primary Cell Culture

Several chapters within this book deal with primary cell cultures. Cell lines have many advantages, but over time show genetic and phenotypic drift as a result of their adaptation to cell culture. There is much to be said for their reproducibility and ease of use, but there is increasing realisation that overdependence upon cell lines has some drawbacks. Many of these can be overcome by the use of primary cell cultures.
Cells can be extracted from tumours in the same ways used to produce cell lines – the options are essentially mechanical or enzymatic dissociation of cells, followed by culture in media with the characteristics required by the experiment. Further selection of dissociated cells by selective culture media, antibody-labelled magnetic beads, or density centrifugation can be helpful to provide a more homogeneous population of cells for study.

10. Basic Laboratory Design and Equipment

Cell culture requires a clean and well-designed laboratory with sufficient equipment to allow safe, sterile working. The design should be as ergonomic as possible – with areas designated for the handling of new material and cultures free of contaminants. Completion of work on clean material in an area should precede work on potentially contaminated material, and there is something to be said for having separate incubators for potentially contaminated cultures and for clean cultures. Shared facilities are most at risk and need firm management to ensure that all users respect the rules which are there to protect them and their work. The guidance issued for Category 2 laboratories based on the Advisory Committee on Dangerous Pathogens dates from 1985, but makes useful points on lighting, heating, work surfaces, flooring, hand washing and air pressure (negative to corridors) that should be considered carefully.

The key equipment needs are listed and discussed below, but there are many small items that may not be immediately obvious to those starting up such facilities. Perhaps the most underrated requirement is for storage space. While most consumables can be ordered “just in time”, the sudden lack of media, plasticware, or liquid nitrogen can render several months’ work worthless within a few days. Stock control is a necessary concern for any cell culture laboratory and investment in this aspect of working practices will pay dividends later.

10.1. Laminar Flow Cabinets

Laminar flow cabinets are essential to allow cultures to be passaged safely and reliably. The Class II or Class 100 safety cabinet is ideal, as this provides operator and sample protection by drawing air into a grill at the front of the hood, and recirculating the air through a filter to provide sterile laminar airflow onto the specimen. Laminar flow cabinets contain high efficiency particulate air (HEPA) filters which need to be cleaned or replaced on a regular basis. While extraction to the outer atmosphere is preferred by most, cabinets with extraction to the room via a second or third HEPA filter work well. Cabinets extracting
to the outer atmosphere may need a pump on the roof of the building to maintain a negative air pressure within any ducting required.

Laminar flow cabinets obviously require laminar air flow, yet this is often interrupted by equipment placed in the hood, rendering the cabinet a lot less effective. The aim should be to have as little in the cabinet as possible when working – a set of drawers on wheels beside the cabinet can prevent the need to have too much inside it. Environmental testing with agar plates exposed to the air within the hood can be useful, and if cells which may have a higher level of contamination (e.g. with known pathogens) are to be used, then it is wise to consider a higher level of containment and to seek advice from a microbiologist. Finally, all laminar flow cabinets require regular servicing – however costly and unnecessary this may seem.

10.2. Centrifuges

Cells do not require high speed centrifugation, and for short periods up to 40 min, refrigeration is also unnecessary. Most cell culture laboratories have multiple centrifuges of benchtop type with sealed buckets to prevent aerosol contamination. It is useful to purchase a type that has the ability to take multiple sizes of tubes, and some will even spin microplates. Tubes should not be over-filled and the centrifuge balanced carefully to avoid strain on the rotor as well as aerosol production. Think about where centrifuges are sited to avoid overheating, and to allow easy access without the need to juggle precious samples. Spills should not be ignored, but cleaned up immediately according to the manufacturer’s instructions. A clear area of bench beside the centrifuge helps enormously, and we have always sited our centrifuges close to the laminar flow hoods to minimise the need to carry samples half-way round the laboratory. Again, servicing is essential to ensure that the centrifuge is performing within its expected limits – the engineer will check that rotors are not cracked, that there is no corrosion, and that electric motor brushes (if present) are replaced when necessary.

10.3. Incubators

Cell cultures require a carefully regulated environment in which to grow. This usually means that one of the larger purchases within the cell culture lab is likely to be at least two CO₂ incubators with temperature (animal cultures are usually performed at 37°C) and humidity control. Some more expensive versions have copper coating or HEPA filters to provide microbiological control, but placing copper filings in the water tray or adding a treatment fluid to the tray will also help. Why two? Well, they do break down, need regular (weekly) cleaning and frequent servicing!

10.4. Microscopes

It is usually necessary to look at cells within flasks or microplates, and this is virtually impossible with a standard upright microscope.
Even an inexpensive inverted microscope will prove a useful addition to the laboratory. It should have a series of lenses from \( \times2 \) to \( \times200 \) magnification and phase contrast as well as direct light facilities. Fluorescence can be very useful and should be considered, though it adds significantly to the cost.

10.5. Counting Cells

The ability to count cells is essential – in the past this was usually performed using a haemocytometer and microscope, but recently a number of simple instruments have come onto the market which provide greater accuracy and are of similar cost to an average upright microscope. Most use a CCD camera system and a manufactured disposable slide chamber or cassette which can be filled in the laminar flow hood. An alternative is a flow based cell counter, though these tend to be more expensive.

10.6. Storage and Other Facilities

The average cell culture laboratory requires several refrigerators, \(-20^\circ\text{C} \) and \(-80^\circ\text{C} \) freezers, and liquid nitrogen storage. Inventory control is essential to keep these under control. The plasticware required will fill a large number of drawers, cupboards, all of which should be carefully labelled with their contents. Pipettes should be of good quality and regularly serviced – automated pipettes are often more accurate, even in the hands of an experienced operator, and should be considered. It is worthwhile having a strict policy to determine which pipette can be used in which hoods to further control contamination.

10.7. Work Surfaces and Flooring

Benches, walls and flooring should be easy to clean. If an older laboratory cannot be redeveloped, then disposable or easily wiped surfaces fixed to existing benches should be considered. Walls should be capable of withstanding a variety of chemicals including cleaning fluids, and floors should be resistant to cracking if liquid nitrogen is in use. Continuous flooring with a coved skirting board will reduce dust, and windows should be sealed unless required as fire exits. Benches abut walls and need similar consideration – many are now offered with a lip to prevent fluid spills from finding a way between wall and bench. Safe working heights for standing or sitting should be considered, and we recommend having at least two electrical sockets fitted with on/off indicator lights every metre or so. Some under-bench sockets and high wall mounted sockets may be required for fridges and freezers.

11. Quality Control

All cell culture laboratories should be run according to Good Laboratory Practice Guidelines. A number of organisations provide accreditation of clinical laboratories, and although
most cell culture is probably done within research laboratories, similar principles should be applied. The major issues are infection and contamination such that the results obtained are inaccurate.

**11.1. Infection and Microbial Contamination**

Cell cultures may be produced within endogenous (mainly viral) infections, but most infections are acquired.

**12. Bacterial or Fungal**

Bacterial or fungal infections of cell cultures are usually obvious – the phenol red, if present, will turn yellow as the infection uses up available nutrients and acidifies the medium, and under the inverted microscope the cells will be replaced by hyphae, yeast, or colonies of bacteria. However, it is common practice to add antibiotics to cultures and this can mask low level infection for a considerable time.

**13. Mycoplasma**

The most common and most missed infection in cell culture laboratories is probably *Mycoplasma*, so much so that we have devoted an entire chapter to it in this volume. Several species are involved and their effects are insidious. The indicators that there might be a problem include reduced growth rate, morphological changes, chromosomal aberration, and altered metabolism. There are several ways of testing for *Mycoplasma* – and many manufacturers provide kits. It is possible to treat *Mycoplasma* infection with antibiotics, but avoidance is the best policy. Most laboratories simply dispose of infected cultures and start again.

**14. Viruses**

Viral infection is also insidious – some cultures contain viruses in any case, either integrated into their genome, or as endogenous non-lethal infections. In many cases, these are not regarded as infections, and viral transformation of cells is a time-honoured method of producing continuous cell lines. Bovine serum may contain viruses – particularly bovine viral diarrhoea virus (BVDV), though manufacturers are aware of this and test for it.
15. Antibiotics

The commonest antibiotics used in cell culture are penicillin and streptomycin. In some circumstances, particularly primary cell culture methods, this may be necessary and the addition of antifungal agents may also be desirable. However, in many cases, these agents are not necessary and leaving them out has the advantage that they cannot interfere with experimental results, particularly if potential drugs are being tested. Equally overuse of antibiotics leads to the selection of resistant organisms in cell cultures, just as it does in the population at large.

15.1. Contamination of Cell Lines

Many cell lines look the same under the microscope and confusion of one vial with another or mislabelling is all too easy. Working with one cell line rather than several at a time will help, but may not be feasible. Good practice will help, and it is generally best to use authenticated cell lines obtained from a tissue culture collection.

16. Safety

16.1. Risk Assessment

The performance of risk assessment is a legal requirement in many countries. The purpose is to identify and mitigate risks to individuals. Cell lines can be classified as low risk (e.g. non-human/non-primate continuous cell lines and those with finite lifespans), medium risk (poorly characterised mammalian cell lines) and high risk (primary cell cultures, human cell lines, or those with experimental or endogenous infections). Category 2 containment is generally sufficient, but category 3 is required for cell lines containing HIV and some other viral pathogens.

16.2. Maintenance

The correct maintenance of the infrastructure and equipment within a laboratory is essential. This should include the equipment listed above and should be performed at regular intervals.

16.3. Disinfection and Waste Disposal

Disinfection of culture waste minimises the risk of harm to individuals and is necessary before equipment is serviced.

Hypochlorites are good general purpose disinfectants, active against bacteria and viruses, but can corrode metal surfaces – for example in centrifuges. They need to be made up daily before use, and commercial product instructions should be followed.

Alcohol is effective against all except non-enveloped viruses and can be used on most surfaces. Aldehydes such as formalin or glutaraldehyde are used for fumigation and are irritants. They can be used on metals. Phenolic disinfectants are not active against viruses, and are of limited use.
Waste disposal may be handled by a central contractor, but it is important to know how they do this – regular disposal is necessary. Tissue culture waste and smaller used plastics such as pipette tips should be decontaminated overnight in hypochlorite and then incinerated. Larger items can be bagged for incineration with care taken to ensure that any edges cannot penetrate the bag. Sharps boxes are useful for pipette tips and needles.

References

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