Guidance on Good Cell Culture Practice

A Report of the Second ECVAM Task Force on Good Cell Culture Practice

Sandra Coecke,[1] Michael Balls,[2] Gerard Bowe,[1] John Davis,[3] Gerhard Gstraunthaler,[4] Thomas Hartung,[1] Robert Hay,[5] Otto-Wilhelm Merten,[6] Anna Price,[1] Leonard Schechtman,[7] Glyn Stacey[8] and William Stokes[9]

[1]ECVAM, Institute for Health & Consumer Protection, European Commission Joint Research Centre, Ispra (VA), Italy; [2]FRAME, Nottingham, UK; [3]Research and Development Department, Bio-Products Laboratory, Elstree, Herts., UK; [4]Department of Physiology, Innsbruck Medical University, 6010 Innsbruck, Austria; [5]ATCC, Manassas, VA, USA; [6]Généthon, Evry, France; [7]National Center for Toxicological Research, Food and Drug Administration, Rockville, MD, USA; [8]Division of Cell Biology and UK Stem Cell Bank, National Institute for Biological Standards and Control, Blanche Lane, Potters Bar, Herts., UK; [9]National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods Environmental Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, USA

Scope of the Report

The maintenance of high standards is fundamental to all good scientific practice, and is essential for maximising the reproducibility, reliability, credibility, acceptance and proper application of any results produced. The aim of this Guidance on Good Cell Culture Practice (GCCP) is to promote the maintenance of these standards and to reduce uncertainty in the development and application of animal and human cell and tissue culture procedures and products, by encouraging greater international harmonisation, rationalisation and standardisation of laboratory practices, quality control systems, safety procedures, recording and reporting, and compliance with laws, regulations and ethical principles.

The scope of the document has deliberately been broadly defined, to include systems based on cells and tissues obtained from humans and animals, and issues related to the characterisation and maintenance of essential characteristics, as well as quality assurance, recording and reporting, safety, education and training, and ethics.

Background

The first ECVAM Task Force on GCCP was established in the autumn of 1999, in response to proposals made at a workshop on the standardisation of cell culture procedures (1), held during the 3rd World Congress on Alternatives and Animal Use in the Life Sciences, Bologna, Italy, in 1999 (2). The proposal that guidelines should be developed to define minimum standards in cell and tissue culture, to be called Good Cell Culture Practice (GCCP), led to the publication of outline guidance on GCCP in 2002 (3). The principles of GCCP are analogous to the OECD Principles of Good Laboratory Practice (GLP), which cannot normally be fully implemented in basic research, including *in vitro* studies (4).

In October 2003, a new task force was convened in Ispra, Italy, with a broader range of expertise in cell and tissue culture, in order to produce a more-detailed GCCP guidance document which could be of practical use in the laboratory.

This Guidance is required to serve the rapidly expanding use of *in vitro* systems: in basic research, to meet regulatory requirements for chemicals and products of various kinds; in the manufacture of various products; in medical diagnostics; and in therapeutic applications such as tissue engineering, and cell and gene therapy.

Further significant developments are certain to result from, *inter alia*: the use of *in vitro* systems for high throughput screening in pharmacology and toxicology; the human genome project; the emerging fields of genomics, proteomics and metabonomics; and the use of biomarkers of disease, susceptibility, exposure and effect.

This Guidance is intended to support best practice in all aspects of the use of cells and tissues *in vitro*, and to complement, but not to replace, any existing guidance, guidelines or regulations.

Address for correspondence: Sandra Coecke, European Centre for the Validation of Alternative Methods (ECVAM), Institute for Health and Consumer Protection, European Commission Joint Research Centre, Via Fermi 1, 21020 Ispra (VA), Italy.
E-mail*: sandra.coecke@jrc.it*

The Principles of GCCP

Based on review by a broad range of experts and organisations, the aim of this Guidance is to foster consensus among all concerned with the use of cell and tissue culture systems, in order to:

— establish and maintain best cell and tissue culture practice;
— promote effective quality control systems;
— facilitate education and training;
— assist journal editors and editorial boards;
— assist research funding bodies; and
— facilitate the interpretation and application of conclusions based on *in vitro* work.

This GCCP Guidance is based upon the following six operational principles.

1. Establishment and maintenance of a sufficient understanding of the *in vitro* system and of the relevant factors which could affect it.

2. Assurance of the quality of all materials and methods, and of their use and application, in order to maintain the integrity, validity, and reproducibility of any work conducted.

3. Documentation of the information necessary to track the materials and methods used, to permit the repetition of the work, and to enable the target audience to understand and evaluate the work.

4. Establishment and maintenance of adequate measures to protect individuals and the environment from any potential hazards.

5. Compliance with relevant laws and regulations, and with ethical principles.

6. Provision of relevant and adequate education and training for all personnel, to promote high quality work and safety.

The Application of GCCP

GCCP sets the minimum standards for any work involving cell and tissue cultures. However, its detailed implementation depends on the nature of the work involved. Whilst this guidance is considered a minimum standard for the preparation and maintenance of cell cultures, deviations from its specific elements may be necessary under certain conditions, in which case they should be justified.

Research and development

This guidance is important for research work, to avoid poor reproducibility of data and the invalidation of results from cell culture processes. Research involving the derivation of new cell lines should always include detailed records of the derivation process and of the reagents and materials used, as the cells may go on to be used for purposes not anticipated at the time, and in some cases, this may include clinical use. Particular care should be taken where cells and tissues are to be used as a reference point or reference material, especially for data interpretation related to the equivalent cells and tissues *in vivo*.

Critical testing procedures

In diagnostics, toxicology and pharmacology, specific regulations are in place in order to protect human health and the environment, such as European Pharmacopoeial requirements and EU and OECD test guidelines. This Guidance has been written to ensure that the appropriate standards can be maintained when cells or tissues are used in meeting these regulations and requirements.

Manufacture of products and therapeutic preparations of cells and tissues

A number of specific regulations and requirements relate to the nature and quality of cells and tissues used in the manufacture of products, including vaccines, monoclonal antibodies, hormones, and cells and tissues for therapeutic use, as well as to the preparation of the final products. The application of GCCP must be consistent with these regulations and requirements, and provides guidance generally not covered in requirements for specific applications.

Principle 1: Establishment and maintenance of a sufficient understanding of the *in vitro* system and of the relevant factors which could affect it

The essential elements for assuring reliable and accurate work when using cell and tissue-based systems, are:

— authenticity, including identity of the system, for example, provenance and confirmation of genotypic and/or phenotypic characteristics;
— purity, for example, freedom from biological contamination; and
— stability and functional integrity of the system in relation to its intended use.

The standardisation of *in vitro* systems begins with the original animal or human donor and the cells or tissues derived, and also embraces their subsequent manipulation, maintenance and preservation.

Standardisation is a difficult task, since cells and tissues are prone to change in culture, and inevitably are subjected to physical and/or chemical insults during their isolation, culture, use and storage. However, by establishing a framework of procedures for factors that can be controlled, variation and other adverse effects on reproducibility and reliability can be minimised. The availability of well-characterised and quality-controlled stocks of cells and tissues, and of media and other critical reagents, further reduces variability.

Various classifications have been published, which define different types of *in vitro* cell and tissue systems (see Figure 1 and, for example, reference 5). Three broad categories will be considered in this Guidance:

— isolated organs or tissues;
— primary and early passage cultures; and
— cell lines (including finite, continuous and stem cell lines).

1.1 Cells and Tissues

Isolated organs or tissues

Isolated organs and tissues, taken for direct use from animal or human donors, are used for a wide variety of *in vitro* applications. These systems are difficult to standardise, because they often have complex environmental and nutritional needs, and because of variation between donors.

Tissues or organ fragments can be used, often perfused with physiological buffers, in a variety of devices. Such *in vitro* systems, including isolated skin and eye models, are very popular for toxicological applications, due to their similarity with the *in vivo* situation. It is important to be able to study an adequate number of replicates in such experiments, and one approach is to use slice technology. Ultra-thin slices of tissues such as liver, lung, kidney or brain, can be used to provide a preparation retaining some of the structural and functional features of the original organ. Inevitably, however, such features tend to be rapidly lost.

Methods involving the isolation and reaggregation of cells from organs such as the skin, brain and liver, can lead to the reconstruction of three-dimensional structures, again with some of the structural and functional properties of the original organ or tissue.

Cells from blood and other body fluids are readily prepared as homogeneous preparations, which are very useful for *in vitro* studies. Preparations such as umbilical cord blood and bone-marrow offer rich sources of stem cells, and could become the basis of an expanding range of other systems.

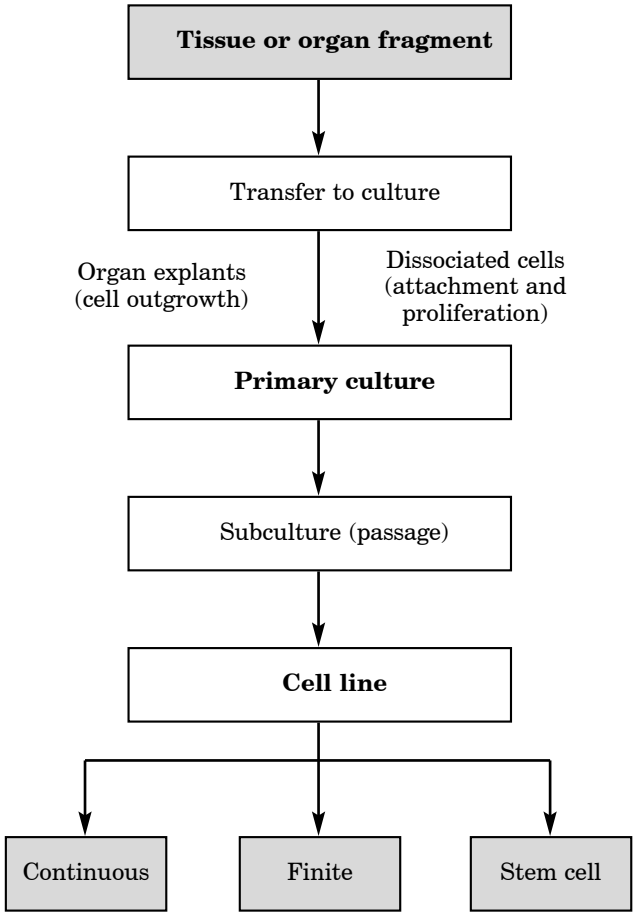
Primary cultures and early passage cultures

The initial *in vitro* culture of harvested cells and tissues taken directly from animals and humans is called *primary culture*. In many cases, such cultures also exhibit key characteristics similar to those seen *in vivo,* so they are widely used for basic research and for a number of *in vitro* applications.

Although cells in some primary cultures can proliferate and can be subcultured (as *early passage cultures*), they generally have a limited life-span and are known to change their differentiated characteristics with time in culture. They commonly require complex nutrient media, supplemented with animal serum and other non-defined or ill-defined components, although serum-free medium formulations are becoming increasingly available. Primary cultures often represent heterogeneous cell populations, and are difficult to standardise and to reproduce, because of uncontrollable variations between preparations.

Primary cultures have traditionally been maintained either in suspension or, more commonly, as

Figure 1: Relationships between the main types of *in vitro* systems



monolayers on glass or plastic surfaces. However, methods employing extracellular matrix components, and innovative techniques such as the co-culture of different cell types and three-dimensional culture, now offer much greater potential for maintaining differentiated structure and function.

Cell lines

Cell lines comprise cells that are able to multiply for extended periods *in vitro* and can therefore be maintained by serial subculture. They can be subdivided into finite cell lines, continuous cell lines and stem cell lines.

Finite cell lines
Finite cell lines are cultures of cells that possess the ability to be subcultured numerous times, but which eventually cease replication and enter a state of senescence, in which cell division has stopped, but the cells remain viable and may also retain some functional activity.

Finite cell lines have a useful life-span *in vitro*, and can be maintained as well-characterised and quality-controlled cell banks. However, changes occur as they approach senescence, so they should not be used above defined population doubling limits, which can be established by experimental investigation.

Numerous finite cell lines have been established. Many of them are human diploid fibroblast cell lines, which are genetically stable and remain diploid for many passages, but which generally reach senescence after 60–70 population doublings.

Continuous cell lines
Certain cell lines show an apparent ability to be subcultured indefinitely, and are known as continuous cell lines. They do not show the senescence experienced with finite cell lines. Continuous cell lines are typically derived from tumours or normal embryonic tissues.

While many continuous cell lines have proved to be stable over long-term passage *in vitro*, they may undergo substantial and irreversible changes. It is therefore important to avoid subjecting cell lines to variable culture and passage conditions, and to establish cryopreserved stocks of early passage cells.

Some continuous cell lines can be a heterogeneous mixture of phenotypes (for example, human promyelocytic HL-60 leukaemia cells, RD, SH5Y-SY). Other cell lines may undergo changes to the differentiation state due to certain medium additives (for example, retinoic acid, dimethylsulphoxide) or culture conditions (for example, when adherent cultures, such as Caco-2 or MDCK, are allowed to reach confluency). In such cases, the potential for the selection of certain cell types as a result of sub-optimal *in vitro* maintenance, handling and preservation, is a significant risk for *in vitro* cell-based methods.

Continuous cell lines may arise spontaneously, or can be produced by using a variety of other methodologies, such as:

— exposure of normal cells and tissues to irradiation and/or treatment with chemical mutagens or carcinogens;
— isolation from cultures infected with viruses (for example, Epstein-Barr virus);
— genetic modification of cells by transfection with cloned genes (for example, SV40 large T-antigen, adenovirus E1, telomerase); and
— isolation from transgenic animals.

Stem cell lines
Stem cell lines, such as embryonic and germ cell lines, are types of continuous cell lines that retain the characteristics of stem cells and can produce diverse differentiated cell types. They require great care in their maintenance, handling and preservation, in order to ensure that their stem cell characteristics and capacity for differentiation are retained.

Embryonic stem cell lines are usually established and maintained on embryonic mouse fibroblasts or other feeder cell layers, which are critical to their successful culture. Although serum-free and feeder-free culture methods are currently being developed, the effects of these new developments on the stability and quality of the cultures have yet to be ascertained.

Some continuous cell lines, notably cancer cell lines, are known to contain stem cell or precursor cell populations. For the purposes of this Guidance, these are not included as stem cell lines. The exact nature and significance of the apparent stem cell component in such lines remains to be determined.

Standardisation for specific uses
Standardisation of cell lines used for specialised studies and for production purposes will require attention to specific characteristics, as well as to the fundamental issues which apply to all cell cultures (see GCCP Principle 2). They should be checked, and rechecked at appropriate times, for the expression of critical functions and markers (for example the pathways for biotransformation of xenobiotics, specific cytoskeletal markers, and characteristic morphology and ultrastructure). The number of passages for which they remain usable should be established.

1.2 *In Vitro* Culture Conditions

Cell and tissue culture environments differ in many respects from those found *in vivo*. Key elements of *in vitro* culture conditions include culture media,

supplements and other additives, culture-ware, and incubation conditions.

Basal medium

In vitro work is generally performed in complex nutritive media. Depending on the circumstances, the basal culture medium can be serum-supplemented (as in traditional cell culture methods) or serum-free, but supplemented with additives necessary for obtaining satisfactory cell proliferation and production, or for maintaining a desired differentiation status.

Many slightly different formulations exist under the same general medium names, such as Minimum Essential Medium (MEM), and even subtle changes in the medium formulation can substantially alter the characteristics of certain cells and tissues. In many cases, these variations are deliberate for specific applications. Therefore, the medium to be used should be precisely specified, and it is important to check that new supplies of medium meet the required specifications.

Serum

Serum is essential for the maintenance and/or proliferation of many cell types. It is a complex mixture of a large number of constituents, including low and high molecular weight biomolecules with a variety of physiologically balanced growth promoting and growth inhibiting activities. However, due to its complexity and to batch-to-batch variation, serum introduces unknown variables into a culture system and can interfere with its performance.

Animal serum can be derived from adult, newborn or fetal sources. Bovine sera are most commonly used, and during the last few decades, fetal bovine serum (FBS) has become the standard supplement for cell culture media. It is a cocktail of most of the factors required for cell proliferation and maintenance, and thus is an almost universal growth supplement.

As the composition of serum is highly variable, it is important that, when an existing batch of serum is substantially depleted, a new set of serum batches should be evaluated in parallel with the current in-use batch. A range of growth promotion tests can be used for this purpose, one of the most convenient and most widely used of which is the plating efficiency test (see reference 6).

It may also be useful for individual users to define serum specifications that meet their particular needs, including the maximum acceptable levels of serum components, such as immunoglobulins (which may have inhibitory effects), endotoxins (indicative of bacterial contamination, but which are also powerful cell mitogens), and haemoglobin (indicative of haemolysis during clotting).

Animal sera are a potential source of microbiological contaminants, notably mycoplasma, bovine viruses, and possibly the agent which causes Bovine Spongiform Encephalopathy (BSE). Suppliers use a variety of techniques, including filtration, irradiation and heat-inactivation, to reduce microbial contamination. Nevertheless, it is wise, and for some applications, obligatory, to specify sourcing of serum from countries where there is a low risk of infection, and, in the case of bovine sera, from animals of less than 30 months old.

The use of human serum is restricted to specialised applications, as it carries additional risks, such as the potential presence of human pathogenic viruses. Its use must be subject to the strictest quality controls, including documentation to demonstrate origin and viral safety.

Because of the disadvantages inherent in the use of animal and human sera, there have been many attempts to find alternatives. These have included the use of poorly defined supplements (for example, pituitary extracts, chick embryo extracts, bovine milk fractions, bovine colostrums), and various plant extracts (for example, vegetal serum). In some cases, it is possible to use fully chemically defined media with appropriate hormones and growth factors. A compilation of commercially available serum-free media was published recently, and can be found at http://www.focusonalternatives.org.uk.

Nutritional status

The exhaustion or inactivation of essential nutrients in cell culture media, and rising levels of metabolites, will inhibit cell growth and cell function, and will ultimately cause cell death. Planning an appropriate procedure for medium replenishment (i.e. frequency and volume of medium) and passaging (for example, split ratio) is therefore essential. This should also be considered when using conditioned medium from one culture in an attempt to promote the growth of another.

Antibiotics

It is important to remember that antibiotics are agents that arrest or disrupt fundamental aspects of cell biology, and, while they are effective against prokaryotic cells (i.e. bacteria), they are also capable of causing toxic effects in animal cells. Not surprisingly, antifungal agents, being directed at higher order, eukaryotic micro-organisms, are likely to be more toxic to animal cell cultures. Given these obvious contra-indications, the use of antibiotics in cell and tissue culture should be focused in two areas: a) protection of tissues, organs, primary cultures and cell lines from contamination; and b) the positive selection of recombinant cell clones

based on the expression of antibiotic resistance genes. In addition, it is important to obtain antibiotics from companies that are willing to provide certification for the concentration and purity of the antibiotics they supply.

Where possible, the use of antibiotics should be avoided. It should not become routine in the cell and tissue culture laboratory, and can never be relied on as a substitute for effective aseptic techniques.

Cell culture surface/matrix

The surfaces to be used for cell cultures may need to be pre-washed or pre-treated, for example, to achieve the comprehensive wetting of a complex matrix. Where coating materials are used, the preparation method may lead to toxic conditions (for example, low pH), and washing before cell seeding may be necessary. There may be batch-to-batch variation in coatings of biological origin, so pre-use testing is essential.

1.3 Handling and Maintenance

Care should be taken not to expose the cells or tissues to inappropriate conditions (for example extended periods out of the incubator). Key items of equipment, including incubators, laminar air flow and microbiological safety cabinets, and cryostorage systems, must be set up and used appropriately (see Appendix 1 and Appendix 2).

Aseptic techniques, where appropriate, should be rigorously applied. The routine isolation, handling and maintenance protocols for cells and tissues should be established as Standard Operating Procedures (SOPs).

Temperature

The optimal culture temperature depends on the type of cells involved. Insect cells have a relatively low optimal growth temperature compared to mammalian cells, and their growth characteristics may be altered at higher temperatures, for example, above 28°C. The exposure of mammalian cells to temperatures above 39°C may induce apoptosis, whilst growth below 35°C may slow replication but may also enhance the expression of certain cell proteins. Recombinant cell lines expressing the temperature-sensitive form of SV40 large T-antigen, will replicate at around 33°C, but not at 37°C.

Atmosphere

Oxygen and carbon dioxide are known to be vital for cell growth, and variations in the levels of these gases can have significant effects on cell cultures. High levels of both gases will be toxic, and very low levels will inhibit cell growth and may result in cell death. Oxygen levels may need to be optimised for particular purposes, for example, to promote growth in large-scale cultures in bioreactors. For many cell cultures, the appropriate atmosphere would be 5% v/v carbon dioxide in air, but the optimum carbon dioxide concentration will depend on the medium in use, the cells being cultured, and possibly on other specific considerations.

pH

The optimal physiological pH for mammalian cell cultures is usually considered to be pH 7.2–7.4, and pH 6.0 for insect cells. Variation outside a relatively narrow pH range may have significant effects on cell phenotype, growth and viability.

Cell detachment and subculture

Detachment solutions, such as trypsin/EDTA, can have significant effects on cells, if their use in specific circumstances is not appropriate. Residual detachment solutions can lead to adverse effects, and therefore should be removed after cell dissociation.

Most cell lines are subcultured before they reach confluency. This may be particularly important in some cases, such as where cell differentiation occurs progressively after confluency is reached (for example, Caco-2 cells). The repeated passage of some cell lines after they have reached full confluency, may result in the loss of desired characteristics. For example, the subculture regime can affect the apparent productivity of recombinant cell lines, and the differentiation capacity of Caco-2 cells.

1.4. Cryopreservation

Cells and tissues can be cryopreserved in a stable state for limited or prolonged periods. The cryopreservation process includes freezing, storage and recovery. In the development of a preservation procedure for a new cell culture, the following points relating to the biochemical and morphological nature of the culture system, must be considered:

— original cell or tissue type (i.e. gross morphology or complexity of culture system);
— growth phase (usually, cells should be harvested during exponential growth to increase the proportion of cells with a high nucleus:cytoplasm ratio); and
— status of cells (other biochemical or morphological features, affected by differentiation, adherence, etc., will influence the success of cryopreservation).

There are also a number of key technical elements in the process of cryopreservation that should be considered, including:

— cryoprotectant (select type and concentration to balance the degree of cryoprotection against any toxic effects, for example, 10% v/v DMSO);
— additives to improve cell survival (for example, serum);
— cooling rate (for example, freezing at controlled rate in the presence of the selected cryoprotectant: typically 1°C/minute with 10% v/v DMSO);
— storage conditions (sufficiently low temperature to eliminate biological changes, for example, liquid nitrogen vapour or liquid phase); and
— recovery method (for example, rate of thawing, gradual dilution to minimise osmotic shock, removal of cryoprotectant to avoid any toxic effects).

Storage in the liquid phase of nitrogen provides the lowest, most stable and most convenient storage temperature, but vapour phase storage is generally considered to be safer (see Appendix 1). Electrical storage systems provide a very practical and maintenance-free, low temperature storage solution. However, in a multi-user environment, such systems are prone to the effects of temperature cycling in stored material, and in the absence of liquid nitrogen or carbon dioxide back-up systems, they are at high risk in the event of loss of power supply.

The failure of liquid nitrogen refilling procedures can result in the loss of valuable cells and tissues, so it is vital that there are effective training and monitoring procedures for the filling and maintenance of liquid nitrogen containers. In addition, it is advisable to store aliquots of important stocks at more than one storage site.

1.5 Microbial, Viral and Cellular Cross-contamination

Contamination with bacteria, yeast and other fungi can result in the complete loss of cultures. Undetected contamination with slow growing micro-organisms, or with micro-organisms resistant to antibiotics, can have a significant impact on the quality and/or validity of data obtained from *in vitro* systems. The most common example of such an infection is mycoplasma.

There are various potential sources of viral contamination, including the operator, cell culture reagents of animal origin, and cells or tissues of animal origin. All cell and tissue culture facilities should therefore have appropriate measures for minimising the risk of microbial and viral infections and for their detection.

Viruses can cause lytic infections, thus destroying the host cells, but may also become established as persistent, sub-lethal infections, which are maintained with passage of the host cell line. Many cell lines both carry and express virus sequences without producing infectious virus particles. In a small number of cases, infectious human pathogens are released into the culture medium from lymphoblastoid cell lines (for example, Epstein-Barr virus from the B95-8 cell line, and human T-lymphotrophic virus II from MT4 cells). Animal viruses are expressed by some cell lines (for example, bovine viral diarrhoea virus in certain bovine cell lines). Mammalian genomes contain many retrovirus-like sequences, which, whilst not overtly infectious, may be released in large quantities as retrovirus-like particles in murine myeloma cells, hybridomas and other cell lines (for example, CHO cells and BHK cells). The expression of such virus-like sequences is also observed at the RNA level in many human cancer cell lines and also in primate cell lines.

Cross-contamination of cell lines with other cell lines is a real, but often neglected, problem. Whenever possible, cells should be obtained from certified sources, and appropriate procedures should be applied to minimise the risk of cross-contamination during their storage and use in the laboratory (see Principle 2).

Principle 2: Assurance of the quality of all materials and methods, and of their use and application, in order to maintain the integrity, validity, and reproducibility of any work conducted

The aim of quality assurance is to confirm the consistency, traceability and reproducibility of *in vitro* cell and tissue work. Each laboratory should have designated persons to oversee the quality assurance of:

— the cells and tissues;
— growth media and all other materials;
— the methods, protocols, and SOPs;
— the equipment and its maintenance;
— the recording procedures; and
— the expression of results.

2.1 Cells and Tissues

A laboratory should have specific protocols or SOPs for the receipt of new or incoming cells and tissues, and for the handling, maintenance and storage of all cells and tissues, with regular monitoring for compliance. The following are among the factors to be considered:

— authenticity;
— morphological appearance;
— viability;
— growth rate;
— passage number and/or population doublings;
— functionality;
— differentiation state;
— performance controls specific to the application; and
— contamination and cross-contamination.

2.2 Other Materials and *In Vitro* Culture Conditions

The quality control of media, supplements and additives is both time-consuming and expensive. Since most of these materials are obtained commercially, the supplier should be expected to operate according to standards appropriate to their supply and use, and to provide the relevant quality control documentation (Table 1).

The user laboratory has the responsibility:

— to confirm that all the materials to be used are suitable for their intended purposes;
— to ensure that all materials are appropriately handled, stored and used; and
— to monitor batches of materials with regard to changes or variations which may affect their use (for certain critical reagents, for example, serum, pre-use testing may be necessary).

In the case of critical reagents, the manufacturer cannot be expected to know the user's specific requirements. The user should therefore define a specification to include general details of the reagent, such as quality controls for identity (composition), purity and activity and stability. Where relevant, the specification should include compliance with international standards (such as ISO standards or pharmacopoeial protocols).

All other working materials which come into direct contact with cell and tissue cultures should be regularly monitored, and appropriate procedures should be in place for ensuring; the quality of culture vessels and surface coatings; the cleanliness and sterility of any re-used equipment (for example, glassware); and lack of toxicity (for example, plastic, absence of detergents, and rubber components).

Appropriate procedures are necessary for the purchase, installation, commissioning, correct use, performance monitoring (for example, calibration) and maintenance of the following:

— low temperature storage refrigerators;
— incubators;
— laminar air flow and safety cabinets (see Appendix 2), and other sterile work areas;
— automatic pipettes and pipettors;
— sterilisation ovens and autoclaves; and
— analytical and production equipment.

European Norms and ISO standards can be adopted for these areas, and in some cases, compliance may be a legal requirement (for example, for pressurised gases, such as carbon dioxide/air for cell cultures, where there will be ISO standards for the gases, and safety standards for the cylinders and pressure regulators).

Principle 3: Documentation of the information necessary to track the materials and methods used, to permit the repetition of the work, and to enable the target audience to understand and evaluate the work

In cell and tissue culture, as in any practical science, clear documentation of the systems used and procedures followed is mandatory, in order to permit the traceability, interpretation and repetition of the work. Therefore, accurate records of cell type,

Table 1: Assessment of the quality of reagents used in cell and tissue culture

		Quality assessor	
Reagent	**Parameter**	**Supplier**	**End user**
Serum	Sterility and endotoxin testing	+	
	Physical and biochemical analysis	+	
	Functional testing	+ (general)	+ (specific)
Basal medium, complete medium (e.g. serum-free medium), additives (e.g. non-essential amino acids)	Sterility testing	+	
	Physical and biochemical analysis	+	
	Functional testing	+ (general)	+ (specific)
Detachment solution (e.g. trypsin/EDTA)	Sterility testing	+	
	Physical and biochemical analysis	+	
	Functional testing	+	+
Surface coating for cell attachment	Sterility	+	
	Physical and biochemical analysis	+	
	Functional test	+	+

origin, authentication and characterisation, and of the materials used and the culture techniques performed, are essential.

The documentation should be retrievable, and should include:

— the objective of the work;
— the rationale for the choice of procedures and materials used;
— the materials and equipment used;
— the origin and characterisation of the cells and/or tissues;
— the laboratory records, including results, raw data and quality control records;
— cell and tissue preservation and storage procedures; and
— the protocols and SOPs used, and any deviations from them.

In some circumstances, for example, where compliance with GLP or Good Manufacturing Practice (GMP) is required, there should be formal procedures for the retrieval and review of documentation, and for resolving any questions or disputes that may arise.

3.1 Origins of Cells and Tissues

A minimal set of information is essential when working with cells or tissues of animal or human origin (Table 2).

3.2 Handling, Maintenance and Storage

It is essential that records should be kept on the following:

— culture media (including all supplements and additives) and other solutions and reagents (including details of supplier, batch, storage requirements, expiry date), and methods of preparation (these may be generically specified in SOPs for research and development work, but for specific standards, the traceability of each procedure to ensure the use of appropriate reagents may be required);

— culture substrate (type and supplier of coating material, for example, collagen, fibronectin, laminin, poly-D-lysine, Matrigel®, basal membrane), and recording of the coating procedures, where applicable; and

— procedures for preparation or use of cells or tissues.

The records on handling, maintenance and storage related to culture-ware and equipment should include:

— type and origin of culture-ware (types and suppliers of flasks, Petri dishes, T-flasks, roller bottles, etc.);
— laminar air flow and safety cabinet testing, calibration, maintenance and repair;
— monitoring of humidity (if appropriate), temperature and CO_2 levels in incubators;
— monitoring of refrigerator and freezer temperatures;
— monitoring of liquid nitrogen level and/or temperature in storage containers;
— sterility controls (for example, autoclaving, sterility tests); and
— regular maintenance and calibration of all other critical apparatus (according to manufacturers' manuals).

The level of monitoring and testing may vary, from installation of alarms for research and development work, to continuous monitoring of calibrated monitoring systems for critical work.

With regard to the *in vitro* system, critical information must be recorded, to permit tracing of the history of the biological material, its characteristics, and the treatments, manipulations, measurements and procedures applied to it, including statistical procedures used to analyse the results obtained.

Cell and tissue preservation and storage details should include (but not be limited to) the following (Table 3):

— type of cell or tissue, passage/identity number;
— cryoprotectant used, and its concentration;
— number of cells and volume per cryovial;
— position in storage container;
— viability and plating efficiency after thawing; and
— date and operator.

Any changes in storage location should be formally recorded and, when appropriate, relevant notification should be given (for example, to the owner, safety officer or quality control personnel).

The disposal procedures for culture laboratory waste (used solutions, toxic treatments, biological materials, etc.) must be documented, and compliance with them should be ensured.

3.3 Reporting

Effective communication is an essential part of cell and tissue culture work, so careful attention should be given to the reporting procedures used.

The format of a report will depend on the target audience, for example, in-house personnel, a client or sponsor, a regulatory body, the scientific community, or the general public. The person(s) responsible for the report should be identified.

Table 2: Examples of requirements for documentation concerning the origins of cells and tissues

	Isolated organs and tissues of animal origin (e.g. rat brain tissue)	Primary cultures of animal origin (e.g. rat hepatocytes)	All materials of human origin (e.g. cord blood)	Cell lines (e.g. Balb/c, 3T3)
Ethical and safety issues	+	+	+	Applicable, if human or involving recombinant DNA or pathogens
Species/strain	+	+	+	+
Source	+	+	+	+
Sex	+	+	+	+
Age	+	+	+	+
Number of donors	+	+	If applicable	na
Health status	+	+	+	+
Any special pre-treatment	+	+	+	+
Organ/tissue of origin	+	+	+	+
Cell type(s) isolated	+	+	+	+
Isolation technique	+	+	+	+
Date of isolation	+	+	+	+
Operator	+	+	+	+
Supplier	+	+	+	+
Informed consent	na	na	+	If human, may be applicable
Material transfer agreement	na	na	+	+
Medical history of donor	na	na	+ (if available)	If human, may be applicable (if available)
Pathogen testing	If applicable[a]	If applicable[a]	+[a]	+[a]
Shipping conditions	+	+	+	+
State of material on arrival	+	+	+	+
Cell line identification and authentication	na	na	na	+
Mycoplasma testing	na	na[b]	na[b]	+

[a]*Screening tests for animal colonies or donors of cells and tissue may be appropriate.*

[b]*May be important if material is preserved for longer term use (e.g. as feeder layers for other cultures).*

na = not applicable.

Where appropriate, the report should be formally authorised for its intended purpose.

A high-quality scientific report should cover the objective of the work, the protocols and SOPs used, planning and experimental design, the execution of the study, data collection and analysis and a discussion of the outcome. It should also be made clear that the whole study was established and performed in accordance with any relevant standards, regulations, statutes, guidelines or guidance documents, and safety and quality assurance procedures.

Table 3: Examples of requirements for documentation concerning the handling, maintenance and storage of cells and tissues

	Isolated organs and tissues of animal origin (e.g. rat brain tissue)	Primary cultures of animal origin (e.g. rat hepatocytes)	All materials of human origin (e.g. cord blood)	Cell lines (e.g. Balb/c, 3T3)
Ethical and safety issues	na	na	+	may be applicable, if human or involving recombinant DNA or pathogens
Morphology	+	+	+	+
Histopathology	+	na	If applicable	na
Quarantine[a]	na	+	+	+
Purity of isolation	+	+	+	+
Phenotype	na	+	If applicable	+
State of differentiation	na	+	+	+
Type of culture[b]	+	+	+	+
Culture medium[c]	+	+	+	+
Feeding cycles	+	+	+	+
Growth and survival characteristics[d]	+	+	+	+
Initial passage number	na	na	+	+
Confluency at subculture	na	na	+	+
Subculturing details[e]	na	na	+	+
Induction of differentiation	na	+	+	+
Identification and authentication	+[f]	+[f]	+	+
Ageing[g]	+	+	+	+
Mycoplasma testing	If applicable[h]	If applicable[h]	If applicable[h]	+

[a]isolation from other cultures; [b]type of culture (e.g. monolayer, organotypic, suspension culture); [c]type of culture medium, additives and supplements and volumes used; [d]growth and survival characteristics (e.g. cell survival, time of cell maturation, expression of cell-specific markers, ageing, initial density at plating, doubling time); [e]subculturing details (e.g. date of sub-culture, subculture intervals, split ratios; seeding densities, perfusion rate); [f]cells and tissues should be traceable to a particular animal or set of animals; [g]replication limits, passage number/population doublings for the cells and/or maximum passage number; [h]where there may be a potential risk to other work or where risk assessment of original tissue shows high risk of infection.

na = not applicable.

When submitting a report on cell and tissue culture work, a minimum set of information should be included, which covers the origins of the cells, the characterisation, maintenance and handling of the cells, and the procedures used (see Tables 4 and 5). A statement of compliance with the GCCP principles should also be included.

Principle 4: Establishment and maintenance of adequate measures to protect individuals and the environment from any potential hazards

National and local laws, based on moral and ethical principles, govern safety in the workplace in most

Table 4: Details to be included in papers for publication in journals, using the example of mouse 3T3 cells

	Details	Supplier details
Type of culture	Continuous cell line	na
Cell/tissue type	Fibroblast-like	na
Species	Mouse	na
Origin	Balb/c3 embryo	na
Description	3T3	na
Catalogue/product number	ATCC 407/351C Clone A31 86110401	ATCC, Mannassas, VA, USA
Basic culture medium	Ham's F12	Gibco, Paisley, UK
Serum	10% newborn calf serum (NBCS)	Gibco
Antibiotics	100U/ml penicillin, 100µg/ml streptomycin 0.25µg/ml Fungizone	Gibco
Other additives	4mM glutamine	ICN-flow, Irvine, UK
Complete medium	No further comment	na
Frequency of medium change	At subculture, when used	na
Culture flasks for stock cells	24cm^2 angle-necked tissue culture flasks (163371) or 80cm^2 filter closed flasks (167008)	Nunclon, Roskilde, Denmark, or Scientific Laboratory Supplies, Nottingham, UK
Culture plates for test	96-well tissue culture plates (167008)	Nunclon
Culture well inserts	Not used	na
Surface coating	Not used	na
Subculture frequency	At confluency	na
Subculture split ratio	1:6	na
Detachment solution	0.25% trypsin/EDTA	Cambrex Bio Science, Wokingham, Berks., UK
Usable passage range	25–45	na
Passage number at receipt	30	na
Passage number at use	35–40	na
Maintenance conditions	37°C, 5% CO_2 in air	na
Storage conditions	Stock cells in liquid nitrogen in 40% NBCS/ 20% DMSO	na
Use	3T3-NRU phototoxicity test	na
Relevant Standard Operating Procedures/guidelines	OECD TG 427, EU B.29	na
References	12, 13	na
Further comments	None	na

na = not applicable.

countries. Many countries also issue guidelines on occupational health and laboratory safety, and individual laboratories may also have rules which reflect local circumstances. Thus, the guidance on safety in the cell culture laboratory given here in no respect replaces these laws and regulations, but rather draws attention to certain aspects of them and highlights issues specific to the *in vitro* culture of animal and human cells and tissues. In many countries, each laboratory is required to appoint a biological safety officer, and this individual should be involved in the safety evaluation of any cell culture procedures.

4.1 Risk Assessment

Identifying and evaluating risks, and taking appropriate action to avoid or minimise them, are the foundations on which safety is built. In the work environment, and particularly in the laboratory, where hazards may be complex and their evaluation requires specialist knowledge, risk assessment should be performed in a structured way. Furthermore, the results of such risk assessments should be recorded, not only to confirm that they have been carried out and appropriate action taken,

but also to act as a reference document for individuals performing the tasks assessed. These assessments should be reviewed at regular intervals, to take into account any changes in local practice, national or international regulations, or increases in scientific knowledge.

It is important to pay particular attention to risks which may be specific to, or more significant in, certain groups of workers. For example, where women of reproductive age may carry a (possibly undiagnosed) pregnancy and would be at greater risk from the effects of certain chemicals, such as teratogens or biological agents. Similarly, persons with a diminished immune response (for example, due to medication or to a medical condition) should seek expert medical advice before they are allowed to work in a laboratory where cell and tissue culture is performed.

The safety conditions highlighted below relate not only to the safety of individual cell and tissue culture workers, but also to that of their colleagues, the general public and the environment.

Some of the areas of concern with regard to general laboratory safety, and to which it might be appropriate to apply risk assessment, are shown in Table 6. Hazards of particular concern in the cell or tissue culture laboratory are further discussed in Sections 4.2 and 4.3, below.

Table 5: Details to be included in papers for publication in journals, using an example of primary/early passage human cell culture

	Details	**Supplier details**
Type of culture	Primary cell culture	na
Cell/tissue type	Keratinocyte	na
Species	Human	na
Origin	Foreskin	QMC Hospital Trust, Nottingham, UK
Ethical permission	Required	Ethics Committee, QMC Hospital Trust
Supply to other users	Not permitted	
Transport solution	Phosphate-buffered saline	Gibco, Paisley, Scotland
Basic culture medium	Epi-Life® Medium	Cascade Biologics, Mansfield, Notts., UK
Serum	None	na
Antibiotics	100U/ml penicillin, 100µg/ml streptomycin	Gibco
Other additives	HKGS Kit (5-001 5)	Cascade Biologics
	Calcium chloride	In-house
Complete medium	No further comment	na
Frequency of medium change	Every 2 days and at subculture	na
Culture flasks for establishing cultures	24cm^2 tissue culture flasks (163371)	Nunclon, Roskilde, Denmark, or Scientific Laboratory Supplies, Nottingham, UK
Inserts	Not used	na
Surface coating	Not used	na
Subculture	When 50–80% confluent (not when 100% confluent)	na
Subculture split ratio	1:5 or 1:10	na
Detachment solution	0.25% trypsin/EDTA (R-001-100) with trypsin-neutralising solution (R002-100)	Cambrex Bio Science, Wokingham, Berkshire, UK
Usable passage range	1–4	na
Maintenance conditions	37°C, 5% CO_2 in air	na
Storage conditions	Stock cells in liquid nitrogen, in 90% fetal calf serum/10% DMSO	na
Passage number at use	3	na
Culture plates for use	96-well plates (167008)	Nunclon
Use	3T3-NRU phototoxicity test	na
Relevant Standard Operating Procedures/guidelines	OECD TG 427, EU B.29	na
References	14, 15	na
Further comments	None	na

na = not applicable.

Once a risk assessment has been carried out, all relevant personnel must be made aware of the potential hazards associated with their work, and must be trained in the necessary precautions (typical precautions are shown in Table 7) and designated safety procedures, as well as in the appropriate use of the safety equipment required (including personal protective equipment) and the appropriate handling of spills.

4.2 Hazards Related to Cell and Tissue Culture Work

Hazards can be categorised into three main groups: physical hazards, chemical hazards, and biological hazards. A risk assessment plan should consider all these hazards in relation to the proposed work. As already mentioned, this assessment should not be limited only to the laboratory and laboratory personnel, but should also cover risks to people in the entire facility, people in the external environment, and to the environment itself. This is not only a vital aspect of basic research and testing, but is particularly important when cultured cells and tissues are used for diagnostic purposes or for producing therapeutic products, or when the cells and tissues themselves are used for therapeutic purposes.

Physical hazards

The cell and tissue culture laboratory does not pose any specific physical hazards. However, laboratories and workspaces should always be kept clean and tidy, and free of material stored on the floor or anywhere where it can cause risk to other people. Any equipment or apparatus used should meet national safety guidelines. Equipment such as autoclaves and laminar flow or microbiological safety cabinets should have a programme of maintenance for safe use, usually carried out at a minimum frequency of once a year. The correct operation of equipment should also be regularly checked. Procedures should be in place for ensuring the safest possible use of equipment connected with ultra-violet light, lasers, radioisotopes, liquid nitrogen (see Appendix 1) and pressurised gases.

Chemical hazards

The cell and tissue culture laboratory is not a particularly dangerous place to work with regard to chemical hazards. However, some chemicals have ill-defined or unknown biological effects, so general safety standards should always be maintained to protect workers against these uncertain hazards. Material Safety Data Sheets for all chemicals used in the laboratory should be requested from the suppliers. For any substances which are potentially hazardous to health (for example, mutagens, cryoprotectants, labelling dyes), these data should form the basis of a risk assessment for the use of this chemical, as the level of risk will vary, depending on, for example, the quantities being used and the techniques being employed. This is covered by national legislation in some countries. Approved waste disposal procedures should always be followed.

Materials being tested in *in vitro* toxicity tests represent a particular problem, particularly if the study requires that they be anonymously coded and supplied via an independent, external source. Although the concentrations used in the final test solutions may be very low, the storage of the bulk material and its handling can represent a significant potential risk. It should always be possible to break the code in the event of an accident. Particular care should be taken with certain kinds of materials, such as when women of reproductive age may be exposed to teratogenic test materials during an *in vitro* reproductive toxicity study.

Biological hazards

Many different issues related to potential biological hazards must be considered and, in certain cases, monitored and recorded in the cell and tissue culture laboratory.

Risk assessments should address issues that could arise from the species of origin (i.e. human and primate cells of highest risk, see reference 7), the health status of the donor, the available data from microbiological screening tests, and the culture and storage history (8). Although not usually dangerous to the user, cells and tissues have the potential to permit the replication of viruses potentially pathogenic to humans, and should therefore be routinely treated as if they are a potential health risk (Table 7).

All cells and tissues new to the laboratory should be handled under a strict quarantine procedure, including suitable precautions to prevent the spread of potential contamination, according to the general guidance given in Table 7, with additional controls, as necessary (such as the use of separate dedicated media and equipment, and work by dedicated staff). Horizontal laminar flow cabinets should not be used when handling cells, as such cabinets are designed to protect only the work area and the air flow is directed toward the user.

Where the nature of the work means that there is a significant risk of biological hazard, special precautions must be taken in accordance with national requirements, most of which, where infectious organisms are concerned, are based on the World Health Organisation classification for human pathogens (Appendix 3).

Table 6: Some areas of concern in general laboratory safety to which risk assessment should be applied

Facilities (such as laboratories, offices, storage and sanitation): for example, are they appropriate and adequate for the intended use, well maintained, and properly heated, ventilated and lit?
Security: depending on the work, are special security precautions required, (for example, for restricted access to site/laboratories, and for removal of hazardous material from the site)?
Health and safety of staff: is the health and safety monitoring of staff regularly carried out and documented?
Laboratory equipment: is the equipment used certified as sufficiently safe for its specific and intended purpose?
Infectious/biohazardous materials: are hazard classification, receipt, processing, containment, storage and disposal conducted correctly, with use of the appropriate protective equipment, clothing and other precautions?
Chemicals and radioactive substances: are the receipt, handling, storage and disposal of hazardous materials (for example, radioisotopes, toxic compounds, flammable liquids) conducted according to the correct procedures?
Hazard prevention: are appropriate hazard prevention plans established, are staff regularly trained in these procedures (for example, fire evacuations), and are they applied correctly?
Waste disposal: is a waste management procedure established that ensures prompt and safe removal from the clean cell culture areas, followed by disposal according to approved procedures?

If the cells or tissues originate from a certified source, such as a recognised cell bank, which provides certification of freedom from certain contaminants, this documentation may suffice for risk assessment, provided that the cells have not been exposed to potential sources of contamination since leaving the bank. However, it is recommended that, as a minimum and where advisable, mycoplasma testing should be carried out on all samples received.

Due to the risk that the operators' immune systems may not protect them against, for example, the tumorigenic growth of their own cells which may have been altered via the *in vitro* procedures (for example, by transformation, immortalisation, infection, or genetic modification), most national guidelines make it unacceptable for operators to culture cells or tissues derived from themselves or from other workers in the same laboratory, nor to genetically manipulate such cells or tissues, or treat them with potentially pathogenic organisms.

Many countries have national safety committees, which establish guidelines for work with genetically modified organisms (GMOs) and help and require scientists to classify and perform their work at the appropriate biosafety level. Recombinant cells, (i.e. those produced by genetic engineering or genetic modification [terms used to cover most techniques which artificially alter the genetic make-up of an organism by mixing the nucleic acids of different genes and/or species together]) will generally fall within the requirements of such guidelines. The classification and control of this kind of work differs between countries, and countries may decide to classify work at a higher or lower level when new information on a particular vector/host system becomes available.

Risk assessment is clearly a dynamic process, and has to take into account new developments and the progress of science. It is the responsibility of the scientists involved to keep up to date with developments in this expanding field of activity, and at all times to respect national and international guidelines and requirements.

4.3 Risk to the Environment

Risks to the environment are generally due to poor waste disposal, leading to contamination of water, air or soil, or the escape from containment of hazardous materials. The environment can also be contaminated by release of biological material due to accidents, including transport accidents, and systems should be put in place either to prevent or minimise the potential for such damage. Support from the local biological safety officer should be sought, if available.

Waste disposal

Methods of waste disposal appropriate to the work in hand must be identified during the risk assess-

Table 7: Typical precautions to be used to ensure operator safety when handling cells and tissues

Hands should be washed or disinfected before and after handling cells. An appropriate gown or laboratory coat should be worn, to be put on when entering the laboratory and removed when leaving it.
Personal accessories (for example, rings, watches), which might compromise cell and tissue culture activities, should be removed or covered up to prevent contamination. If appropriate, gloves should be worn, and replaced immediately if torn or punctured or during extended work sessions.
When handling cell and tissue cultures, workers must avoid transferring contamination on the hands from the culture work to unprotected body parts (for example, eyes or mouth), clothing or items in the open laboratory environment.
As far as is reasonably practicable, all cell and tissue work should be performed in a Class II cabinet or other appropriate (micro)biological safety cabinet (see Appendix 2). NB: certain cabinets, such as horizontal flow cabinets, protect the cells and tissues, but not the user or the general environment.
Mouth-pipetting must be strictly prohibited.
All procedures should be undertaken by using methods that minimise the production of aerosols that might spread contamination by micro-organisms or cells.
All disinfectants used should be effective and appropriate for the work.
All work surfaces should be cleaned with an appropriate disinfectant, before and after use.
The use of sharps should be avoided as far as is possible. Any used sharps should be disposed of safely according to approved procedures.
All cultures should be clearly and unambiguously labelled.

ment process. These methods must protect not only the individual tissue culture workers themselves, but also their colleagues, the wider population, and the environment. Work with known pathogens and GMOs must be performed according to the relevant regulations (see above), including methods of waste disposal. Where methods are not specified in these regulations, there is a requirement to assess and justify all proposed methods of waste disposal as part of the risk assessment. Similarly, the appropriate method of disposal of hazardous chemicals must be identified before work with them is undertaken. In line with the above precautionary principle, the following minimum precautions should be taken when disposing of waste from the cell culture laboratory:

— all liquid waste, with the exception of sterile media or solutions, should be either chemically inactivated (by using sodium hypochlorite or another disinfectant) or autoclaved before disposal; and

— all solid waste contaminated with tissue culture liquid and/or cells should either be autoclaved before leaving the laboratory, or should be placed in rigid, leak-proof containers before being transported elsewhere for autoclaving or incineration.

Transport

The transportation of any biological materials, chemicals (including liquid nitrogen) or other materials (for example, dry ice) of potential risk to humans, animals, plants and/or the environment, must comply with national or international regulations (see, for example, http://www.iata.org/whatwedo/dangerous_goods). They should be packed so as to prevent spills in the case of breakage, be correctly labelled (with appropriate hazard symbols), and have the appropriate accompanying documentation (materials safety data sheet, import form, export form, and CITES permit, if applicable). A typical materials safety data sheet for a cell line is shown in Table 8. Where appropriate, the International Air Transport Association (IATA) guidelines should be followed, as they are stringent and are recognised internationally (for regular

Table 8: Typical material safety data sheets for animal cell cultures (Containment Level 1 or 2), but without references to specific national and local legislation[a]

Cultures are not specifically defined as hazardous, but as live cells, they are potential biohazards, and should be treated as if biohazardous.

Emergency Telephone Number:
To be used only in the event of an emergency involving a spill, leak, fire, exposure or accident.

Description:
Either frozen or growing cells shipped in liquid cell culture medium (a mixture of components that may include, but is not limited to: inorganic salts, vitamins, amino acids, carbohydrates and other nutrients dissolved in water).

SECTION I
Hazardous Ingredients:
Frozen cultures may contain 5–10% dimethyl sulphoxide (DMSO).

SECTION II
Physical data:
Pink or red aqueous liquid.

SECTION III
Health hazards:

For Biosafety Level 1 Cell Lines[b]
This cell line is not known to harbour an agent known to cause disease in healthy adult humans. This cell line has NOT been screened for Hepatitis B, human immunodeficiency viruses or other adventitious agents. Handle as a potentially biohazardous material under at least Biosafety Level 1 containment.

For Biosafety Level 2 Cell Lines[b]
This cell line is known to contain an agent that requires handling at Biosafety Level 2 containment. Such agents have been associated with human disease. This cell line has NOT been screened for Hepatitis B, human immunodeficiency viruses or other adventitious agents. Cell lines derived from primate lymphoid tissue may fall under the regulations relating to blood-borne pathogens.

SECTION IV
Fire and explosion:
Not applicable.

SECTION V
Reactivity data:
Stable. Hazardous polymerisation will not occur.

SECTION VI
Method of disposal:
Spill: Contain the spill and decontaminate by using suitable disinfectants, such as chlorine bleach or 70% ethyl alcohol or isopropyl alcohol.
Waste disposal: Dispose of cultures and exposed materials by autoclaving at 121°C for 20 minutes.
Follow all national and local regulations.

SECTION VII
Special protection information:

For Biosafety Level 1 Cell Lines[b]
Handle as a potentially biohazardous material under at least Biosafety Level 1 containment. Cell lines derived from primate lymphoid tissue may fall under regulations relating to blood-borne pathogens.

For Biosafety Level 2 Cell Lines[b]
Handle as a potentially biohazardous material under at least Biosafety Level 2 containment. Cell lines derived from primate lymphoid tissue may fall under regulations relating to blood-borne pathogens.

SECTION VIII
Special precautions or comments:
Recommended that appropriate safety procedures be used when handling all cell lines, especially those derived from human or other primate material. For detailed discussions of laboratory safety procedures see references 15–18.

[a]This generalised example of a material safety data sheet is based on one that can be found at http://www.atcc.org/pdf/msds_animal.pdf; [b]Biosafety Levels 1 and 2 are broadly equivalent to European Containment Levels 1 and 2.

updates, see www.wfcc.info). Before arranging transport, the various legal requirements for export and import into the recipient country should be considered, including ethical issues (such as the use of human cells or tissues of embryonic origin), disease transmission, endangered species regulations (www.cites.org/), and bioterrorism regulations (see http://www.bt.cdc.gov/).

A cell culture may fall into any one of the classes of biological material used for shipping purposes, namely:

— diagnostic specimens;
— infectious specimens;
— biological products; or
— GMOs.

Principle 5: Compliance with relevant laws and regulations, and with ethical principles

From an ethical and legal point of view, it is desirable that high standards for cell and tissue culture should be established and maintained worldwide, so that accountability, safety and ethical acceptability can be universally guaranteed, as far as is reasonably practicable. The ethical and associated legal issues raised are extremely complex and beyond the scope of these GCCP guidelines. However, all concerned should maintain a sufficient level of awareness of the ethical issues related to cell and tissue culture work, and of public opinion and the relevant legislation at the national and international levels.

At present there are no ethical guidelines relating specifically to general cell culture practices, but various guidelines, regulations and laws are in place for dealing with cells and tissues of specific origin and/or use.

Before any studies are initiated, matters of ethical significance must be carefully considered. These can be subdivided, from a GCCP point of view, into general ethical considerations and more-specific considerations.

From a general perspective, diligence in legal and ethical matters leads to data of higher value, since it can help to avoid waste of effort and can increase confidence in the outcome of the study, to the benefit of all concerned, including the general public.

The more-specific considerations include the ethical implications of using material of animal and human origin, and GMOs.

5.1 Laws and Regulations

At present, there are no international laws specifically governing cell and tissue culture practices. However, any work involving animal or human pathogens has to be performed in compliance with national and international requirements. Some countries have, or are preparing, legislation or regulations to control specific areas, such as the use of material of human origin. New controls are also being drafted in response to the challenges and opportunities presented by transplantation, regenerative medicine, stem cell research and GMOs. Ownership of cell lines and patents must also be dealt with appropriately, and special conditions may apply where cell cultures are involved (see reference 9). In addition, there are international agreements relating to the provision of organisms and cell cultures that may be used for bioterrorism.

5.2 The Use of Animal Material

In general, any work involving animal material should be in compliance with local and national legislation on animal experimentation and the Three Rs (*reduction*, *refinement* and *replacement*) principles of Russell & Burch (10). In addition, other ethical issues may arise in certain circumstances. Examples include the use of cells derived from endangered species (http://www.cites.org/), the production of monoclonal antibodies by the ascites method (*see* References: Monoclonal antibodies and ethics), and the pretreatment of animals with chemical inducers to provide cells for culture with specific biochemical properties (for example, hepatocytes with elevated CYP450 enzyme levels).

In order to minimise pain and distress, donor animals should be handled according to the appropriate and approved procedures. As fetuses of many mammalian species can already feel pain long before birth (11), they should also be treated with the utmost care, again according to appropriate procedures.

Serum, and especially fetal bovine serum, is a commonly-used component of animal cell culture media. It is harvested from bovine fetuses taken from pregnant cows during slaughter. Here again, the current practice of fetal blood harvesting poses ethical problems (blood is usually taken via cardiac puncture, without any form of anaesthetic; 11, 12). Efforts are being made to reduce the use of animal serum and, where possible, to replace it with synthetic alternatives. A wide range of other cell culture materials derived from animals (such as tissue extracts, extracellular matrix materials) also raise ethical concerns.

Legal issues can also arise if animal-derived cells and tissues are found to be infected with viruses which could infect wildlife or species of agricultural importance. For this reason, the discovery of such viruses in cells and tissues may need to be notified to the relevant authorities and appropriate action taken.

5.3 The Use of Human Material

The use of human biological material is critical for medical research. It is particularly important that

researchers are aware of the need to handle such material in a responsible manner and in accordance with local and national requirements.

Those involved with the procurement, supply and use of human biological material should maintain proper records, to ensure appropriate traceability and control of the applications of the material in ways which are consistent with the nature of the consent given by, or on behalf of, the donor. All use of human tissue should be approved by the appropriate ethics committee, and copies of such approvals should be kept for reference. Where samples are provided to third parties, the custodian is responsible for the safe keeping of the code which enables samples to be linked to individual donors, where appropriate and when necessary.

Human material is usually procured either from specialised cell and tissue banks or from hospitals (13). Currently, most of the banks are run on a not-for-profit basis. Nevertheless, some of them have been set up by private industries, particularly for the production of engineered tissues. This raises serious ethical concerns (including the transfer of human material for profit), and has not yet been dealt with adequately at the national level in most countries or internationally. In Europe, this area will be regulated under the EU Human Tissues Directive (14).

Confidentiality with respect to the provision and use of human tissue is governed both by law and by professional guidelines. A legal requirement in most countries is that, when dealing with human material, informed consent must be sought either from the donor or from the donor's family.

Human tissue banks should be recognised as the most legally and ethically acceptable approach to the procurement and distribution of donated non-transplantable human tissue for research, as they are best equipped to deal with, and advise on, the complex issues involved, including ethics, consent, safety and logistics, as well as scientific questions.

The removal of blood samples from human volunteers should only be performed by qualified personnel, and particular precautions should be followed to minimise any risks. Such volunteers should also be considered to be donors, and documented informed consent will be required.

The use of human embryonic stem cells involves serious ethical questions, because of their origins and their potential uses. This is a relatively new research area, and, while some countries already have strict controls, other countries are currently considering what laws and regulations should be introduced in the public interest.

The procurement of stem cells from early embryos and fetuses is a particularly sensitive issue, because of the circumstances in which such embryos and fetuses become available. Stem cells can also be obtained from adult tissues and from umbilical cord blood, where the ethical considerations to be taken into account are similar to those involved in obtaining other human tissues, but it is the use of the stem cells which requires effective regulation.

Before any human material is used for the establishment of a new cell line, ethical approval should be obtained from the relevant authority.

5.4 Genetically Modified Cells

The creation, storage, transport, use and disposal of genetically engineered cells are currently subject to the requirements that apply to GMOs. This is a rapidly expanding field, and its long-term consequences are as yet unknown. It involves manipulating genes and cells in ways that do not occur in nature, and for this reason, it raises sensitive ethical issues. The above activities are regulated in many countries, where, before any work is initiated, relevant approval must be sought.

Principle 6: Provision of relevant and adequate education and training for all personnel, to promote high quality work and safety

The range of applications for cell culture is expanding rapidly and involves an ever-broadening range of technical manipulations (such as chemically induced and genetic modifications) for use in basic and applied science, manufacturing, diagnosis, and efficacy and safety testing procedures, as well as for providing therapeutic materials.

The competence of staff to perform their duties in a laboratory is central to ensuring that work is performed according to the standards of the organisation in relation to its scientific, legal and safety requirements and obligations. This requires education and training, as well as the regular monitoring of performance (Table 9).

A good basic education should be given in the nature and purposes of cell and tissue culture which is an essential basis for any future training programme. The basic principles of *in vitro* work, aseptic technique, cell and tissue handling, quality assurance, and ethics should be included. It is also important that those working with material of animal or human origin should have a sufficient understanding of any additional laws or regulations that will apply.

Training should be seen as an ongoing process for improving and developing practical skills, and maintaining competence. Given its critical importance to the success of any laboratory work, there should be a formally documented training programme for all members of staff, including training records and regular reviews of training needs. To ensure the quality of work in the long term, it

Table 9: Culture techniques, procedures and regulations that should be included in a cell culture laboratory training programme

Basic laboratory procedures
Understanding of the nature and purpose of SOPs
Microscopy
Centrifugation
Autoclave operation
Use and maintenance of laminar air flow or microbiological safety cabinets, incubators, cryostorage facilities
Maintenance of essential equipment
Laboratory design and safety
Risk assessment and risk management of *in vitro* work
Quality control
Waste disposal
Disinfection, fumigation and cleaning regimes
Basic culture procedures
Sterile technique and aseptic manipulation, including disinfection and sterilisation
The preparation, storage and monitoring of culture media
Cell and tissue culture isolation techniques
Cell viability testing and cell counting
Subculturing
Sterility or bioburden tests
Mycoplasma testing
Cryopreservation, storage and recovery of cells and tissues
Advanced and special culture procedures
Cell characterisation and authentication
Cell isolation and purification methods
Cell and tissue banking
Induction of differentiation
Complex culture techniques (for example, co-culture, culture on filter inserts, perfusion cultures)
Transfection and selection of stable cell lines
Use of bioreactors
Documentation and record keeping
General information and policies of the organisation responsible for the laboratory (operational issues, safety, quality standards)
Laboratory data, equipment records, storage records
Occupational health and training records
Safety records
Quality assurance records, manuals and information
Laws and regulations
All laboratory staff should be made familiar with the institutional, national and international procedures, guidelines, regulations and laws relevant to their work, such as the following:
— rules and policies of the organisation/institute
— allocation of responsibilities
— the containment of microorganisms;
— regulations on the use of animals and of animal cells and tissues; and
— regulations on the use of human cells and tissues.

is also important to link training with personal development programmes for technical and scientific staff, in order to ensure they are progressively trained and educated in line with changing laboratory activities and demands.

When new staff join a laboratory, their skills and experience should be assessed, and the need for further training procedures in relation to their new jobs should be identified. These needs may include a variety of general and specific procedures, covering SOPs, general laboratory maintenance, and safety and emergency procedures.

Training can be provided in-house by experienced members of staff and/or visiting experts, via accredited on-line programmes and/or through attendance at external courses. For certain applications including product manufacture and testing, and processing of cells and tissues for clinical use, training must be formally recorded and reviewed.

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Web-links

Cell banks, cell line authentication and identification

DSMZ – German Collection of Microorganisms and Cell Cultures:

http://www.dsmz.de

European Collection of Cell Cultures (ECACC): http://www.ecacc.org.uk

American Type Culture Collection (ATCC): http://www.atcc.org

CABRI – Common Access to Biotechnological Resources and Information: http://www.cabri.org

Italian Cell Line Collection: http://www.iclc.it

UK Stem Cell Bank: http://www.ukstemcellbank.org.uk

Alternatives to fetal bovine serum

http://www.focusonalternatives.org.uk

http://www.zet.or.at

Safety aspects

http://www.dh.gov.uk/PolicyAndGuidance/HealthAndSocialCareTopics/fs/en (UK Department of Health policy and guidance on prions, Creutzfeld-Jakob Disease [CJD] Hepatitis A, B and C, and HIV)

http://www.osha-slc.gov/SLTC/laboratories/ (US guidelines on occupational health and laboratory safety)

http://biosafety.ihe.be (Belgian Biosafety Server)

http://www.cdc.gov/od/ohs/safety/labsafetyhm.htm (Centre for Disease Control and Prevention [CDC] laboratory safety primer)

http://www.phppo.cdc.gov.nltn/pdf/lrawwh.pdf (CDC risk assessment resource)

http://www.hse.gov.uk/pubns/indg163.pdf (UK Health & Safety Executive resource for general risk assessment)

http://www.who.org (World Health Organisation)

http://www.osha-slc.gov/SLTC/laboratories/ (US Occupational Safety and Health Administration resource for laboratory safety)

http://www.bocgases.ie/saftey/othersaftey/pdf/cryogenics.pdf (guidance on the safe use of liquid nitrogen and other liquid gases)

http://www.iata.org/whatwedo/dangerous_goods

Appendix 1

Liquid Nitrogen

Work with liquid nitrogen probably poses the greatest single threat to the safety of cell culture workers (as gauged by the number of individuals using it and the potential severity of any accident), and for this reason is dealt with in greater detail here.

Details of general hazards, precautions, and first aid can be obtained from the suppliers of liquid nitrogen and of liquid nitrogen vessels (see, for example, http://www.bocgases.ie/saftey/othersaftey/pdf/cryogenics.pdf). Such relevant information must be obtained, and its contents must be taken into account in the relevant risk assessments. A printed version should be placed in a readily accessible location where it can be rapidly referred to, *before* any work using liquid nitrogen is undertaken.

A serious hazard in the use of liquid nitrogen is the risk of asphyxiation due to the displacement of air by nitrogen gas within a confined area. Areas where liquid nitrogen is stored or handled must therefore be well ventilated. In addition, oxygen-depletion monitors (wall-mounted and/or worn by staff), which can provide an early warning that the level of oxygen is declining below a safe level, should be used in areas where large numbers of storage vessels are held and/or significant amounts of liquid nitrogen are handled.

Liquid nitrogen is frequently stored in pressurised vessels. Many countries have regulations governing the design, construction, use, maintenance, testing and other aspects of such pressurised vessels (for example, the UK *Pressure Systems Safety Regulations 2000*). In countries where no such regulations exist, similar precautions should be taken. In particular, cell culture workers should ensure that they know how to operate such vessels safely (see the user's manual) and must have their vessels maintained and tested on a regular basis. Further useful information can be found at: http://www.hse.gov.uk/hid/land/comah/level3/5c9a7bd.htm.

Because of the ultra-low temperature of liquid nitrogen (–196°C), it can cause severe frostbite to exposed tissues, particularly if it is caught in loose clothing or shoes, or spilled down the cuff of an insulated glove. Therefore, appropriate clothing should always be worn (open-toed footware is not permissible, and clothing with loose cuffs, pockets and turn-ups should be avoided), with eye protection and insulated gloves (ideally, these should be loose-fitting for ease of removal, be made of impermeable material, and have close-fitting, elasticated cuffs).

The other hazard associated with liquid nitrogen is that it can enter storage vials (due to inadequate sealing) when they are immersed in the liquid phase, and this may cause the vials to explode upon thawing. Therefore, steps must be taken to protect workers from the effects of such an explosion. As a *minimum*, workers must wear a full-face visor, insulated gloves and a long-sleeved laboratory coat when thawing vials from liquid nitrogen, and other individuals must be kept clear of the immediate area. The vessel containing the liquid in which the vial is being warmed, if judiciously chosen, can be used to further protect the worker by containing any flying debris and/or directing the force of the blast away from the worker.

Such explosions could be particularly dangerous if the vials contained pathogenic material. Therefore, material known to be pathogenic *must not* be stored in the liquid phase of liquid nitrogen, but instead should be stored in the vapour phase. Another reason for this is that transfer of pathogenic material between containers stored in the liquid phase has been documented.[1] Clearly, the greatest care must be taken to ensure that storage vessels containing pathogenic material are fully sealed before placing them in storage, and that they will stay fully sealed under the intended storage conditions.

[1]Tedder, R.S. Zuckerman, M.A., Goldstone, A.H., Hawkins, A.E., Fielding, A., Briggs, E.M., Irwin, D., Blair, S., Gorman, A.M., Patterson, K.G., Linch, D.C., Heptonstall, J. & Brink, N.S. (1995). Hepatitis B transmission from contaminated cryopreservation tank. *Lancet* **346**, 137–40.

Appendix 2

Class II Biological Safety Cabinets

A Class II Biological Safety Cabinet (BSC) is designed to perform two functions: a) to protect the work materials (i.e. cell and tissue cultures in this case); and b) to protect the worker from infectious agents that may be contained in the work materials. Other cabinets (Class I and III) have been developed, each to serve only one of these functions [1], but, in addressing both requirements, the Class II cabinet is less robust if misused, and for safe and reliable performance of its dual function, requires careful installation, maintenance and practical use. Accordingly, the operators of BSCs require careful instruction as to their use. Supervisors must inform staff that Class II BSCs are not substitutes for good aseptic technique; in particular, the airflows will not provide protection in cases of gross spillage, high energy aerosols (for example, from centrifuges, or sprays) and physical disturbance. The following are some typical precautions to help ensure the correct and safe use of a Class II BSC.

— Before using the BSC, ensure that it is working correctly. Check the airflow indicators or the negative pressure gauges. Most BSCs are fitted with alarms to indicate any unsafe operation conditions.

— Use appropriate disinfection to decontaminate surfaces before commencing work.

— Ensure that all essential materials and equipment are placed in the BSCs before work is started; this will reduce the risk of interruptions to the BSC air flow during use, and will reduce the risk of contamination.

— Do not place too many items in the BSC at any one time, as cluttering the work area may affect the air flow.

— Ensure that a vessel of appropriate disinfectant is on hand, in case of spillages.

— Bear in mind that once the work has started, all materials within the BSC are potentially contaminated and should not be removed until after appropriate disinfection. This includes gloved hands.

— Do not subculture or otherwise manipulate more than one cell or tissue culture system in the BSC at any one time. This is essential, to avoid mislabelling or cross-contamination.

— Use separate bottles of growth medium for each cell or tissue culture system, as this will prevent the transfer of microbial agents between culture systems or possible cross-contamination.

— Avoid rapid movements, which may interrupt the air flow.

— When the work is completed, ensure that all materials and equipment are made safe. Place all materials that need to leave the BSC in appropriate transport containers, and disinfect by either spraying or wiping. Disinfect the work area in case of spillage and splashes.

— Depending on the work being carried out, the BSC may need to be decontaminated with formaldehyde prior to further work being undertaken.[1]

— Leave the BSC running for at least 10 minutes before switching it off, in order to remove any aerosols generated during the work.

— Class II safety cabinets should be sited, installed and commissioned according to national regulations.

[1] Jones, B.P.C. (1998). Laboratory practice. In *Safety Cell and Tissue Culture* (ed. G. Stacey, A. Doyle & P. Hambleton), pp. 64–86. Dordrecht, The Netherlands: Kluwer Academic Publishers.

Appendix 3

Classification of Infective Microorganisms by WHO Risk Group[1]

Risk Group 1
(no or low individual and community risk)

A microorganism that is unlikely to cause human or animal disease.

Risk Group 2
(moderate individual risk, low community risk)

A pathogen that can cause human or animal disease, but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available, and the risk of spread of infection is limited.

Risk Group 3
(high individual risk, low community risk)

A pathogen that usually causes serious human or animal disease, but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available.

Risk Group 4
(high individual and community risk)

A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available.

[1]World Health Organisation. (2004). *Laboratory Biosafety Manual*, 3rd edn, p. 1. Geneva, Switzerland: WHO. Website http.//www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf (Accessed 8.06.2005)