Good Cell Culture Practice

ECVAM Good Cell Culture Practice Task Force Report 1

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Background

The maintenance of high standards is fundamental to all good scientific practice, and is essential for ensuring the reproducibility, reliability, credibility, acceptance and proper application of any results produced.

The aim of this Good Cell Culture Practice (GCCP) initiative is to reduce uncertainty in the development and application of *in vitro* procedures, by encouraging the establishment of principles for the greater international harmonisation, rationalisation and standardisation of laboratory practices, nomenclature, quality control systems, safety procedures and reporting, linked, where appropriate, to the application of the principles of Good Laboratory Practice (GLP), as recently interpreted for *in vitro* studies (1).

The ECVAM Task Force on GCCP was established in response to proposals made at a workshop on the standardisation of cell culture procedures (2), held during the Third World Congress on Alternatives and Animal Use in the Life Sciences (Bologna, Italy, 29 August–2 September 1999; 3). In a subsequent plenary session, the Congress participants as a whole endorsed the following statement:

> "The participants in the Third World Congress on Alternatives and Animal Use in the Life Sciences call on the scientific community to develop guidelines defining minimum standards in cell and tissue culture, to be called Good Cell Culture Practice (GCCP), analogous to the OECD Principles of Good Laboratory Practice (GLP), which cannot normally be fully implemented in basic research, on the grounds of cost and lack of flexibility. Such guidelines should facilitate the interlaboratory comparability of *in vitro* results. One of the intentions of this statement is to encourage journals in the life sciences to adopt these guidelines as a condition for the publication of work involving cell and tissue culture. This statement should be reviewed and updated at further World Congresses on Alternatives and Animal Use in the Life Sciences."

These initiatives resulted from recognition of the rapidly expanding use of *in vitro* systems, not only in basic research, but also to meet regulatory requirements for chemicals and products of various kinds. Indeed, some of the recent major developments in the use of *in vitro* systems have been in the fields of toxicity testing and product quality control, and experience gained here and in the validation of alternative (i.e. non-animal) procedures has much to offer to other users of *in vitro* technology.

In vitro systems are also finding increasing application as production systems for various kinds of materials, including monoclonal antibodies, vaccines, hormones, drugs and nutrients, for use in research, diagnosis and therapy. In addition, there is an increasing use of genetically modified human and animal cells, and of cells and tissues derived from genetically modified animals. New therapies, based on cell and gene therapy and tissue engineering, in which *in vitro* methodologies play a vital role, are also becoming more widely used.

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

The *in vitro* systems themselves are also becoming more varied and more sophisticated, with the development of co-culture, sandwich culture, perfusion culture, aggregate culture, hanging-drop methods, air–liquid interface maintenance, long-term

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maintenance, controlled cell differentiation, and tissue-reconstruction approaches, including organotypic cultures.

The ECVAM Task Force on GCCP was established in the autumn of 1999 under the chairmanship of Thomas Hartung (University of Konstanz, Germany). The remit given to the task force was to define the scope of GCCP guidance and to further its elaboration. The task force has met three times, in Zurich, Switzerland; Innsbruck, Austria; and Ispra, Italy. The proposals and comments presented in this report provide a basis for the drafting of a GCCP guideline document at an ECVAM workshop to be held in the near future.

The scope of this report has deliberately been broadly defined, to include systems based on cells, tissues and organs obtained from humans and animals, and issues related to: nomenclature; labelling and reporting; characterisation and maintenance of essential characteristics; quality assurance; culture media; storage; cell culture collections; human tissue banks; patenting; education and training; safety; and ethics.

The intention of the ultimate guidelines will be to foster a consensus among all concerned, in any way, with the use of *in vitro* systems, in order to assist scientists involved in research, testing, biotechnology, bioreactor production and clinical applications, to establish and maintain best laboratory practices, to promote effective quality control systems, to facilitate education and training, to support journal editors and editorial boards, to assist research funding bodies, and to help any authorities who need to interpret and apply conclusions based on *in vitro* data.

The Inherent Variation of *In Vitro* Systems

Cultured human and animal cells are increasingly used as the basis for simplified, direct test systems that have the potential to be more controllable and more reproducible than test systems employing laboratory animals. However, if a biological test system is simplified to fundamental levels, it is paramount that the essential components of such a reduced system are closely defined and are reproducible. The standardisation of *in vitro* systems begins with control of the starting materials.

The initiation of an *in vitro* system essentially involves the donor, the cells or tissue, the culture medium, and the substratum. These components interact, and the properties of the total system and any variation in them are undoubtedly a result of this interaction. However, the potential for variation can also be considered for each separate component.

Starting Materials

The cells or tissues used in test systems may be freshly isolated from animal or human donors (primary cells) or, at the other extreme, may comprise a laboratory-adapted strain or line that has been serially propagated and maintained in continuous culture for long periods (continuous cell lines). Freshly isolated primary cells will rapidly dedifferentiate in culture, and they have a limited capacity to multiply. The ability to proliferate *in vitro*, as shown by continuous cell lines, is associated with genetic and phenotypic modifications of the type commonly associated with tumour cells.

Continuous cell lines are poorly differentiated, and lose many of the phenotypic characteristics of the original cell type *in vivo*. They have a variable capacity for serial propagation, and some cell lines show senescence, and progressively lose the ability to multiply, typically after 15–20 population doublings. The potential for cell-associated variability for primary cells is different from that of continuous cell lines and cell strains (which have a limited life-span of up to 50 population doublings).

Primary cell and tissue cultures

Primary cultures isolated from animals or humans represent heterogeneous populations with respect, for example, to differences in cell types and states of differentiation. Each isolate will be unique and impossible to reproduce exactly. The process of dedifferentiation commences the moment that cells are separated from their parent tissues, so a primary cell culture is a dynamic system in a constant state of change. Primary cell cultures commonly require complex nutrient media, supplemented with animal serum and other non-defined components. Consequently, primary cell culture systems are extremely difficult to standardise.

Continuous cells and cell strains

Immortalised cell lines and cell strains are able to multiply for extended periods *in vitro*, and can be expanded and cryopreserved as cell bank deposits. Most of the fundamental phenotypic changes that occur shortly after the original isolation from the parent animal tissue have been completed, so a continuous cell line is more homogeneous, more stable, and so more reproducible than a heterogeneous population of primary cells. The outstanding disadvantage of most continuous cell lines is that they retain little phenotypic differentiation, and poorly represent the *in vivo* situation.

The opportunity for variability of continuous cell lines stems from the very fact that they can often be cultured indefinitely. Continuous cell lines have

been distributed worldwide in a totally uncontrolled manner, creating a massive potential for identification errors and the introduction of contaminants. Therefore, it is recommended that authenticated stocks of a continuous cell line are purchased from a recognised, national or international animal cell-culture repository, such as the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) in Germany, the American Type Culture Collection (ATCC), the Riken Gene Bank in Japan, or the European Collection of Animal Cell Cultures (ECACC) in the UK.

Nevertheless, it is possible that even samples of an authenticated continuous cell line with the same nominal identity, but purchased from different sources, may exhibit phenotypic differences that reflect divergence arising from their different culture histories. It is even possible that different banks of the same cell line, manufactured within the same repository, may exhibit minor differences that could be significant in the context of a specific study.

Culture media

Cell culture medium usually comprises a defined base solution, which includes salts, sugars and amino acids, mixed with a variety of supplements, depending on the culture requirements of the cell type. Medium supplements, particularly sera from various animal species or from different suppliers, are inevitably complex and cannot be defined. In fact, all the components of culture media comprise a significant potential source of variability.

A batch of animal serum is often a pool of donations taken from a large number of animals. Such a pooling strategy can result in an acceptable degree of homogeneity between different batches of animal serum produced by the same manufacturer. However, there are likely to be qualitative differences between sera collected in different geographical regions.

The concentration/activity of growth factors must not be expressed in μg/ml but in Units/ml where "Unit" corresponds to a consistent (WHO) standard.

Reporting on Cells, Other Materials and Equipment

To produce a high-quality scientific report, various components have to be incorporated, namely, generation of ideas, planning and experimental design (including statistical aspects), execution of the study, data collection and analysis, and discussion and conclusions.

The final report is the ultimate goal of all scientific work, which (if not restricted for internal use) will be communicated to the scientific community in the form of a scientific publication.

Where the work needs to be carried out routinely, a procedure should be written down. In the context of regulatory acceptance, this procedure should be capable of being developed into a proper GLP-compliant, stepwise Standard Operating Procedure (SOP).

Many guidelines are available concerning what a scientific report for academic, industrial or regulatory purposes should contain. To achieve a wide acceptance of the GCCP guidelines, it is therefore crucial to encourage implementation of these practices at all levels where cell and tissue culture work is conducted and reported.

The recommended content of a GCCP-compliant study report, including a study plan and a GLP-compliant SOP, should form an integral part of GCCP guidelines.

Cells

Any report on cell culture experiments should include a basic description of the cells cultured, including, where appropriate, the following.

1. Nomenclature of cell type or line in use (code, such as ATCC number, alias); the lot number of the cell bank represents the ultimate definition of a cell.

2. a) Origin and mode of culture initiation (species, organ, tissue, lineage, mode of transformation, genetic modification, sublines/hybrid cells; and, in the case of human cells: donor characteristics, such as race, sex, age, health status, medication, disease, biopsy, and tumour status).

   b) Methods of cell isolation (for example, mechanical, enzymic); transport and storage of biopsies and explants.

3. Source, such as cell bank (ATCC, ECACC, DMSZ, Riken Gene bank, etc.) or depositor, and laboratory of origin, as well as information on original publication/patent; shipping of cells, i.e. frozen or in liquid medium.

4. Basic morphological description of cultured cells, including the phenotype and its stability and expected doubling time.

5. Culture conditions, including basic medium and supplements/additives, additional buffer systems, composition of incubator atmosphere, maintenance temperature.

6. Subcultivation intervals (cell density, confluent/subconfluent cultures, cell harvest, split

ratio, initial passage number, number of passages in culture).

7. Conditions for freezing/thawing, including cryoprotectant, storage conditions, viability, plating efficiency.

8. Indicators of the state of differentiation and expression of specific activities, where available; precise definition of measures undertaken to maintain or induce differentiation and activity

9. Test method, dose and test intervals for *Mycoplasma* contamination, and other appropriate controls.

Other materials, equipment and procedures

The quality of equipment and cell culture materials should be sufficient to maximise the generation of reproducible and reliable results.

1. The compositions of culture media for routine cultures (maintenance media) and/or experimental cultures, and supplements/additives (for example, serum, heat-inactivation or irradiation of serum, growth factors, hormones, antibiotics) should be defined.

2. Non-defined preparations (for example, serum replacements, growth factor mixtures) should not be considered acceptable, except in specific circumstances.

3. Volumes of media used, and feeding cycles should be defined.

4. Culture vessels (for example, flasks, Petri dishes, bottles, roller cultures) should be defined.

5. Culture substrata, coating materials (for example, collagen, fibronectin, laminin), and coating procedures should be defined.

6. Names and addresses of the manufacturer/suppliers of culture media supplements, vessels and substrata should be given.

Quality Assurance

The aim of a quality assurance regimen is to assure consistency, traceability and reproducibility. In each laboratory, a person responsible for the quality assurance of cell culture work should be appointed.

When *in vitro* systems are used to model the *in vivo* situation, all possible efforts should be made to approximate *in vivo*-like cell behaviour. In general, cell proliferation and differentiation are conflicting aims of cell culture. This implies the following.

1. The impact of variation of these materials should be monitored and documented.

2. Equipment and instruments should be properly maintained and calibrated (for example, control of temperature and CO_2 levels of incubators).

3. All materials employed should be stored under appropriate conditions to protect them from damage, infestation or contamination.

4. Changes of batches of material should be monitored with regard to their influence on principal endpoints in use in a study.

5. Measures should be taken to assess the status of cells before any experiment is undertaken.

6. Suitable measures should be employed for cell line identification and verification, and for testing for cross-contamination.

7. The differentiation state/phenotype of cells should be monitored (for example, by morphology, histochemistry, enzyme/gene expression, growth rate, viability, sensitivity to toxins, ability to stimulate cell functions, surface markers, adherence to substratum, DNA/chromosome analysis, or fingerprinting).

8. The appropriate measure of differentiation should be independent of the cellular function under study, and should be assessed at least at the beginning and at the end of each series of experiments.

9. Positive and negative controls should be included in all experiments.

The value of studies on cell cultures is endangered by contamination and/or infection. Therefore, laboratory animals from an outside source, to be used as donors, should be kept in quarantine for an appropriate period. Similarly, appropriate measures should be taken when a cell line is introduced into the laboratory, to ensure that no infection/contamination of cell lines already present can occur (certificate from supplier, testing for the more common contaminants [for example, *Mycoplasma*], growth on antibiotic-free medium for a specified period). Tests for frequent contaminations (such as *Mycoplasma*) should be performed on a regular basis, and results should be discarded in the event of any evidence of contamination of materials. If an infection has been eradicated, the regimen should be defined and reported.

The onus is on a laboratory to confirm that incoming materials are suitable for the intended purpose, by:

1. identifying the critical materials;
2. defining the test and criteria/markers related to suitability;
3. either introducing a regimen to test incoming materials/lots for these markers, or obtaining certification from manufacturers/suppliers; and
4. keeping reference samples of previously used lots for comparison with incoming materials.

In addition, it is vital to introduce an adequate system of record keeping, and to maintain appropriate records concerning all those conducting the work.

Safety

Some safety issues connected with cell and tissue culture are summarised in Table 1.

Potentially infectious materials are widely used, and all work with human materials should be performed in Class II cabinets.

Whenever possible, testing for viral infections should be performed at the very beginning of cell/tissue isolation. A primary consideration in the research use of human tissues is the safety of all the personnel who will be, or could be, exposed to such tissues, or any product obtained from them, such as cell cultures or cell fractions. The most important aspects of ensuring safety are awareness, suitable handling and disposal procedures, and the provision of adequate training for all users. Although it is possible to test for a number of viral and bacterial pathogens, there are significant residual risks when this testing has been performed. Therefore, such testing does not absolve either the provider of non-transplantable tissue or the end-user from taking appropriate precautions against known and unknown hazards. As the cases of HHV8 and Kaposi's sarcoma, and of Coxsaeckie 4 and juvenile diabetes have recently demonstrated, new human viruses linked with pathogenic conditions have been discovered as often as every 5–10 years, and more are likely to be found in the future. Therefore, whatever the results of specific testing, all human tissue must be considered to be hazardous at all stages of its procurement, experimental use and disposal. Personnel should be vaccinated whenever possible (for example, against hepatitis B). Although the risk for the experimenter may be relatively low during standard procedures, the proper handling and disposal of waste material is critical.

Table 1: Some safety issues connected with cell and tissue culture

Primary cultures	Primary cultures of human origin may be a source of viral infection (for example, HIV, hepatitis B, hepatitis C), and precautions should be agreed with the tissue-supplying agency. Primary cultures of animal origin may also be a source of infection or allergy.
Cell lines	Cell lines may contain endogenous viruses, genetically manipulated material, be of tumour origin and/or be tumorigenic.
Procedures	Procedures should be performed in suitable facilities, according to local legal regulations (for example, sterile/aseptic working place, use of laminar-flow cabinets and cell culture incubators). Experimental procedures and downstream processing of culture should be clearly defined (for example, cell harvesting, isolation of cell culture products, virus propagation, vaccine production, induction of differentiation). Proper handling of liquid nitrogen during cryopreservation of cells and retrieval of vials from frozen storage is essential.
Disposal	All waste should be treated properly, to minimise any threat to humans (for example, toxicity, mutagenicity, teratogenicity), as well as to other cells and animals under study.
Infections	The main safety concern is the potential for worker infection (viruses, bacteria, fungi, mycoplasmas and parasites are potential pathogens). Potential exists for continuous cell lines to carry latent viruses and for transformed lines to spontaneously produce viruses with oncogenic potential in humans.

The assessment of risk requires a knowledge of the history and status of the cell population: risk is low when the work involves cells derived from pathogen-free animals, or cell lines that have been determined to be free of adventitious agents. The potential for infection increases when the work involves the use or the production of pathogenic agents. In cells from pathogen-free sub-primate species, the principal safety concern is probably the potential for injury associated with handling liquid nitrogen during the cryopreservation of cells and the retrieval of vials from frozen storage. In laboratories where glass pipettes are used, breakage of these can also pose a significant threat.

The main safety concern is the potential for infection of workers from continuous cell lines carrying latent viruses, and for transformed lines spontaneously to produce viruses with oncogenic potential in man. Because human cells and body fluids can carry infectious agents such as HIV and hepatitis viruses, cells of human origin must be considered a potential risk. It is important to remember that HIV has been isolated from human cells and tissues, cell extracts, whole blood, and body fluids, including semen, vaginal secretions, cerebrospinal fluid, tears, breast milk, and urine.

All work with human cells should be carried out on the assumption that the specimen may carry an infectious agent. Significant viral pathogens that could be encountered include hepatitis B, hepatitis C, HIV and cytomegalovirus (CMV), all of which have specific known pathogenicities, and the recently identified HHV8 (Kaposi's sarcoma virus), which may cause tumours under certain circumstances. In addition, the possibility of bacterial hazards, such as various *Mycobacterium* species, and antibiotic-resistant organisms, such as methicillin-resistant *Staphylococcus aureus*, should not be ignored, as they are becoming increasingly frequent among hospital cases.

There is also a potential risk that certain human or primate cell lines, if able to enter the body, may have oncogenic potential. A classification of cell lines as aetiological agents can be consulted.

The safe use of human tissue that might contain known and unknown infectious agents depends on secure containment during collection, transport and use (to minimise exposure of laboratory personnel and transportation couriers who handle such tissues directly), effective methods of sample decontamination prior to release for disposal from the laboratory (to eliminate risk to the environment and to the general public), and strict protocols for handling and disposal as waste. It is essential that the experiments are performed in suitable facilities, according to the legal regulations (sterile/aseptic working place, use of laminar-flow cabinets and cell culture incubators). Full descriptions of experimental procedures and downstream processing of cultures are essential.

The number of people directly exposed to any risks should be kept to an absolute minimum, and such exposure should be strictly limited to those who have sufficient knowledge and training to be considered "informed" as to their personal safety. All such personnel should be provided with whatever effective and safe immunisations are available at the time. Tracking methods that clearly identify samples of human origin, especially when this is not obvious by inspection, for example, tissue homogenates or DNA extracts, which may contain viral DNA, are essential, to ensure that the appropriate containment is maintained at all times, up to and including the final disposal and decontamination of all samples.

Specific procedures that may amplify a hazard and thus greatly increase risk, such as the *in vitro* culture of a susceptible cell type from a potentially infected donor, must be clearly identified, and the risks posed should be separately assessed before the work is undertaken.

Education and Training

In successful cell and tissue culture, living material from various tissues should proliferate and/or function under appropriate culture conditions, while preserving sufficient differentiated characteristics, which closely resemble those of their ancestors *in vivo*.

Additional aims can include permitting cells to be manipulated either genetically or chemically, transfecting cells for the expression of foreign genes and proteins, or the use of cultured cells for virus propagation or vaccine production.

Therefore, the proper training of all personnel in basic cell culture practice, in the application of specific procedures, and in general and specific safety precautions, depending on the types of cells (primary cultures, transformed and/or transfected cells, etc.) and on the aims of the work, should be viewed as mandatory. Some aspects of training are summarised in Table 2.

Ethical Issues

From an ethical and a legal point of view, it is desirable to establish high standards for cell and tissue culture worldwide, so that ethical acceptability, safety and accountability can be guaranteed, insofar as that is possible.

Two ECVAM workshops have considered ethical and quality control aspects related to cells and tissues obtained from human donors (4, 5). These aspects extend from the initial contact with the donor or their next-of-kin, through isolation and supply procedures, to use in the research facility. It was strongly felt that human tissues should be sup-

Table 2: Aspects of training in the use of basic culture techniques and examples of special culture procedures

Basic culture technique
Principles of sterile technique
Handling of culture media
Feeding of cultures (media changing)
Cell counting
Subcultivation (trypsinisation)
Detection and elimination of contamination
Growth parameters, growth curves
Viability assays
Storage and freezing/thawing of cells
Special culture procedures
Primary cell and tissue cultures
Toxicity testing, viability assays
Cloning
Transfection, expression cloning
Cell transformation and immortalisation
Virus propagation and isolation

plied through officially recognised human tissue banks, and collected according to acceptable ethical standards and in compliance with national laws.

The members of the ECVAM Task Force on GCCP endorse the recommendations made in the reports on ECVAM workshops 32 (1), 37 (4) and 44 (5).

Conclusions and Recommendations

1. An internationally agreed set of guidelines or principles on GCCP should be produced and published.

2. The guidelines should be adopted by editorial boards of journals, in order to improve the reporting of essential information.

3. The guidelines should be adopted by research funding bodies, in order to define quality controls and ensure the overall quality of *in vitro* studies.

4. The guidelines should be used in education and training in the use of *in vitro* techniques.

5. A harmonised nomenclature system for cell lines should be introduced.

6. The authentication of cell lines according to standards laid down by independent bodies would be useful.

7. A central registration of cell characteristics would be useful.

8. Awareness of the widespread use of potentially infectious materials in cell and tissue culture should be improved.

9. Many aspects of the patenting of cell lines need clarification. Access to patented cell lines should be regulated and harmonised.

10. The Declaration of Bologna should be updated at future World Congresses on Alternatives and Animal Use in the Life Sciences.

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