Risk assessment of animal cell culture procedures

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INTRODUCTION

The generation of continuous cell lines from animal and human tissues has yielded valuable tools for biological studies. Some of these cell lines have also proved important hosts for expression of recombinant DNA. The fact that cell culture is used in diverse disciplines has meant that to a large extent it has evaded specific attention regarding risk assessment. The primary concern relating to safety in cell culture is that animal cells can provide a suitable medium in which microorganisms, notably viruses, can multiply. There are a number of levels at which the culture and manipulation of animal cells should be considered from the point of view of safe handling. This overview attempts to outline the type of approach which should be adopted in risk assessment along with some practical suggestions to promote safe handling of cell cultures.
**RISK ASSESSMENT**

When using any new procedure or modifying an existing protocol it is essential to carry out a risk assessment of all aspects of the work process, namely starting materials, culture procedures, product purification and waste disposal. For most chemicals and reagents used in cell culture there are standard texts and sources of information, most obviously the manufacturer, which enable rapid assessment of risk based on the properties of the reagent, its physical form, the quantities used and the procedures to which it is to be subjected. However, there are a number of factors unique to the manipulation and culture of animal cells which make risk assessment a more difficult and sometimes uncertain process. The following paragraphs aim to identify the safety concerns specifically relating to cell culture and to give some general guidance for risk assessment in these areas.

(1) **Undefined Components of Growth Media.**

Numerous growth medium supplements, notably foetal bovine serum, can provide a potential source of virus contamination (Erickson *et al.* 1991). In general such supplements cannot be readily sterilised since this often results in their inactivation or degradation. Thus, it is important to obtain such materials from suppliers which guarantee that they stock only from uninfected sources or accredited virus free animal herds. Some manufacturers may carry out tests for the detection of adventitious agents, but this is limited to specific organisms (eg. mycoplasma and Bovine Viral Diarrhoea Virus). Obviously, if human (and primate) sources of undefined reagents are avoided then the risk of infection in laboratory workers by this route is minimised. There are a number of serum free defined culture media and sources of recombinant growth factors which eliminate the possibility of virus transmission via culture media components. Unfortunately, these are not yet widely used and they can be very expensive. However, the search for a cheaper culture medium may lead to the use of less pure reagents subjected to lower standards of quality assurance. Thus, in the long term, the use of less expensive culture media may be counterproductive both in terms of safety and quality.

2) **Cells and Adventitious Agents**

Risk assessment of animal cell cultures is a potentially confusing area since the cells are essentially undefinable and prone to variation. However, the primary cause for concern, in relation to laboratory work with cells, is the potential of cell cultures to sustain virus or other organisms which might infect laboratory workers. Thus practical approaches to risk assessment of animal cell cultures have been based on the virological risk represented by the species and tissue of origin (Frommer *et al.*, 1993). Human and primate cells derived from blood and lymphoid tissue are of greatest concern as carriers of serious human pathogens. Whilst non-human, non-primate sources of cells in general represent a much lower risk to laboratory workers some rodent viruses can cause human infection (eg. Hay,
Many cell lines undergo multiple passages in different laboratories. The history of such lines is rarely recorded and thus the risk of cross-infection with infectious agents from other cell lines and contaminated reagents cannot be assessed. For primary cells isolated directly from tissue the risk of infection is related directly to contamination from the tissue of origin (i.e., microbiological status of the animal or colony of origin) and the culture medium used. Probably the most likely organisms to pass between cell lines and establish an infection are mycoplasmas and acholeplasmas. These organisms survive well in the environment and unfortunately are commonplace in animal cell cultures and can be extremely difficult to eradicate. Some of the most common contaminant species in cell cultures (e.g., Mycoplasma orale, M. salivarum, M. hominus, M. fermentans) probably originate from the commensal flora of laboratory workers and fetal bovine serum (Del Giudice and Gardella, 1984). While mycoplasma infection can have drastic effects on cells, the species identified in cell culture are not generally associated with human disease except for M. pneumoniae. Thus, although the presence of adventitious agents can have serious consequences for infected cell lines, they are unlikely to represent a serious health threat to laboratory workers using good aseptic technique.

A more insidious problem is the potential for cell lines to harbour and secrete virus while showing no overt signs of infection. This unrecognised factor and the need to prevent the spread of contaminants between cultures are the primary indicators for care in cell lines containment (see below). The situation is simplified (in terms of risk assessment only) when a hazardous virus is used to infect cells. The culture as a whole then assumes the higher hazard level for the virus. Published literature and the experience of other workers regarding a particular cell culture of interest may also be valuable in risk assessment. However, direct transcription of specific data (e.g., virus testing) should be supported by proof of the common origin of cells in use and those in the literature utilized in risk assessment.

3) Cell products

Cell products naturally released from animal cell cultures do not usually represent a hazard. Generally, this is a problem relating to direct exposure to purified products which can be alleviated if direct exposure of the body surfaces and respiratory tracts of laboratory workers is minimised. Cell products produced at high concentration, for example from some recombinant organisms, demand a higher priority in risk assessments and should be assessed for their toxic properties, persistence in the environment and their ability to cause adverse immune reactions. The potential for exposure to transforming proteins, such as E1A adenovirus protein and SV4 T antigen, is also an important consideration. It should be borne in mind that scale-up procedures that are aimed to produce high and concentrated yields of product may represent significant hazards which were of lesser consequence at research and development stages. Control of products released inadvertently into the
laboratory environment should be provided for by adequate cleaning and decontamination procedures. This is important both as a routine procedure as well as for the containment of spillages. Together, these approaches will prevent the build up of any cell culture product in the working environment.

4) Cell processing procedures

Once all the various components of a particular process have been assessed individually it is important to review the proposed physical processes to be used and assess the level of containment required at each stage. Any procedures where aerosols are generated or materials may be accidentally transferred directly to an operator's tissues and/or blood stream (eg use of hypodermic needles) should be reconsidered. In such cases, it is important to identify alternative procedures or, if this is not possible, to ensure that reasonable precautions are taken to contain aerosols adequately and protect the operator. In procedures whereby cells are lysed aggressively it will be necessary to consider not only the proteins but also the DNA (recombinant or genomic) which is being released. Whilst the hazards of non-viral naked DNA are as yet not clearly quantified it is prudent to limit its spread.

5) Decontamination procedures

It is important that the decontamination procedures are chosen specifically for each process. Selected disinfectants should be checked for their efficacy against those microorganisms likely to be present and the use of mixtures of disinfectants and/or cleaning agents should be checked for their compatibility. Decontamination procedures should be recorded as laboratory protocols with instructions for the preparation, use and regular replacement of `in use dilutions' of disinfectants and cleaning agents. Cell culture contaminated materials should be treated to ensure that they cannot carry any infectious particles when leaving the laboratory. In the case of materials likely to be heavily contaminated this is readily achieved by destructive autoclaving (ie in an atmosphere of pure steam at 134C and 1 atm pressure for 30 mins). The mechanism of waste disposal should also be assessed to prevent outgoing waste contaminating new reagents and to prevent build up of waste which in addition may then become a secondary source of contamination within the laboratory.

PRACTICAL APPROACHES TO SAFE HANDLING OF ANIMAL CELLS

1) Use of Safety Cabinets.

As discussed above, cell lines may carry undetected microorganisms. Therefore all cell cultures should be handled within an appropriate microbiological safety cabinet. Where no human pathogen exceeding category 2 (see below) has been identified a Class II cabinet
may be used for the purpose of containment. It is vital that such equipment is installed, monitored and maintained correctly (eg as given in the British Standard BS5726 (2)). In addition all staff involved in tissue and cell culture work should receive training in the correct use of safety cabinets.

2) Organisation of work

Work practices should be designed to ensure that infected and uncharacterised cultures do not contaminate culture media and other "clean" (characterised) cultures. This can be readily achieved where there is the facility for separate areas for media preparation, "clean" cell cultures and infected or uncharacterised cells. Where this is not the case work can be organised into sessions of increasing risk of contamination through each day followed by stringent decontamination. Such approaches, while important for safety, are also of scientific benefit in preventing the transmission of agents, which may lead to altered cell characteristics, between cell lines. This chronological form of quarantine is absolutely dependent on a high level of training in aseptic technique amongst the laboratory staff (see below).

3) Characterisation of Cell Cultures.

In order for risk assessment of a cell culture procedure to be accurate, it is important to confirm the authenticity of the cells in use. When dealing with cell lines with unique characteristics or primary cells isolated directly from tissue there is little chance that the wrong cells might be used. However, for cell lines obtained from another laboratory there may be no proof of identity. Thus, it is necessary at least to have evidence of the species of origin of a cell line and a number of techniques are available which have been used for confirming the identity of cell cultures (Stacey et al 1992). It is also important to realise that the same cell line obtained from different sources can show phenotypic variation due to different culture histories.

4) Training

Probably the most important protection that the laboratory worker has against infection is aseptic technique. This prevents transmission of contaminated particles between the manipulation procedure and the environment (which includes the operator). Thus, while primarily intended to protect research material from contamination, aseptic technique is an important element in containment of infectious organisms. It is vital that all laboratory staff should receive training in aseptic technique which will benefit the productivity and quality of their work as well as their safety. Staff should also be trained in the correct practices for disinfection, sterilisation (ie autoclaving) and fumigation which provides a secondary level of containment. Further training in the manipulation of special hazards (eg cytotoxic drugs, oncogenic materials, genetically modified organisms) should also be
NEW DIRECTIVES ON SAFETY AND BIOLOGICAL AGENTS

Within Europe, a directive from the European Commission (90/679/EEC) has lead to a unified classification of biological agents. This has been prepared with requirements and recommendations for containment of each class of agent. This classification of biological agents is based on those used in a number of member states of the European Union, for example that of the UK Advisory Committee on Dangerous Pathogens (ACDP, (1)), as are the requirements for containment of pathogens. As undefined complex biological systems, which may contain adventitious agents, cell cultures represent uncertain hazards and should be treated as potentially infectious (ie as category 2 agents) even when an infectious agent has not been identified (ie the cells have not been tested for contamination). Class II containment requires the use of an appropriate safety cabinet, spill resistant benching, documented disinfection procedures and a restricted access to the laboratory area. Containment of organisms being transported between laboratories is extremely important since it introduces the possibility of direct transmission of organisms to the general public. Regulations relating to the transportation of biological agents within Europe have been recently updated (eg CHIPS II 1994 for UK (3)) and for global transportation IATA (8) have also prepared new regulations. Laboratories distributing biological material also have responsibilities to the recipient of cells or other potentially infectious material. The person receiving the culture should be supplied with adequate information; firstly, to enable them to recognise that the material is correct (ie a detailed physical description of the package contents) and secondly, to assist them in the preparation of their own risk assessments. This information can be provided as a materials safety data sheet (a statutory requirement in the UK) which not only identifies hazard but also describes the physical appearance of the material supplied. Thus, loss of material in transit or provision of the wrong material can be identified immediately. In addition the distributor of the culture must ensure that all recipients have appropriate facilities and trained staff for its safe handling.

CONCLUSION

In general, establishing relative theoretical risks of animal cell cultures in use is straightforward. This will be adequately addressed in most cases by the use of the European containment level 2 precautions and the use of a safety cabinet which protects the operator. Infection of a cell line with hazardous organisms automatically requires that containment should be appropriate for the organism in question. Additional assessment of manipulation and disposal procedures is important to identify and contain any aspect which exposes the operator to unreasonable risks. The application of good safety procedures can be used to give benefits within the laboratory far beyond the obvious requirement for healthy staff. Careful risk assessment respecting scale of work and the whole procedure (in addition to individual assessment of agents and reagents) will ensure safe working conditions for laboratory staff and if applied properly also encourages clean,
efficient and well documented work procedures which are synonymous with good science and economical use of time and resources.

REFERENCES

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