Safety Services Guidance

Working with Cell Cultures

Key word(s):	Cell cultures, cell type, adventitious agents
Target audience:	PIs and workers in biological containment labs

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Introduction

1. The guidance sets out the factors to consider when assessing and controlling the risks for work activities with cell cultures.

Assessing the risks

2. You need to consider the properties of the cells/cell lines themselves, risks from any infectious agents (either adventitious or deliberately infected), and the nature of the work undertaken with the cell cultures including whether the cells have been/or are going to be genetically modified.

Properties of cells/cell lines

3. Source of cells – material sourced from humans or non-human primates presents the greatest risk in terms of being able to support the growth of agents capable of infecting humans and also being contaminated with infectious agents. But remember that some agents can cross species barriers eg LCMV, BSE, H5N1. You must not culture your own cells or those of your immediate colleagues as you could provide a vehicle for self-infection of your self or colleagues with adventitious agents which your immune system would not recognise.

4. Cell type – certain cell types can be more tumourigenic than others. Cell type also gives an indication of how likely they are to contain contaminants including latent viruses. Changing culture conditions can result in expression of otherwise latent viruses.

5. Culture type – primary cell lines/cultures present the greatest risk. Continuous cell lines present a lesser risk (although certain lines are known to be persistently infected).

Adventitious agents

6. Bacteria and fungi – contamination with these agents should be easy to detect as they tend to overgrow the cell culture.

7. Mycoplasma – these can go undetected for multiple passages and can damage the host cell. Certain Mycoplasma spp are infectious for humans (eg pneunoniae).

8. Viruses – contamination with viruses may not result in any obvious cytopathic effect or else infection may be latent. Although cells sourced from human and non-human primates present the greatest risk, other cell types can harbour zoonotic agents.

Controlling the risk

9. The Advisory Committee on Dangerous Pathogens (ACDP) have issued guidance on appropriate controls when working with different types of cells/cell line and this is summarised in the table below. The need for any additional measures should be addressed in the risk assessment. If, as is likely, work is carried out in a Class 2 microbiological safety cabinet to protect the cells as well as those carrying out the work, users should understand the differences and ensure that regular safety checks are carried out on the cabinet as required by COSHH.

Risk Assessment Form and Authorisation

10. For work where the hazard is deemed as high, then the Application to Handle Biological Materials and COSHH Risk Assessment form should be used and processed according to University procedures.

11. Where the hazard is deemed to be medium, the standard risk assessment form may be used but must be approved by your local BSO. However, your local rules may require that the above application form should be used instead.

12. Where the hazard is deemed to be low, the standard risk assessment form may be used.

Containment measures for work with cell cultures

13. On page 68 of the guidance document from HSE "Biological agents: managing the risk in laboratories and healthcare premises", there is a description of the base line containment requirements for low, medium and high hazard cell lines. A more detailed criteria for the hazard classification of cell lines is provided below.

Criteria for Hazard Categorization of Cell Lines

14. A cell line which is described as being of a low hazard in HSE guidance is defined as being well-characterized or authenticated, finite or continuous cell lines of human or non-human primate origin with a low risk of endogenous

infection with biological agents presenting no apparent harm to laboratory workers and which have been tested for the most serious pathogens. However, HSE's guidance does not define a number of the key words used in the definition such as well-characterized, authenticated or most serious pathogen.

15. A fully authenticated cell line used in biopharmaceutical production is one which is subject to extensive analysis including karyotyping, species of origin determination, screening tests for viral adventitious agents, mycoplasma testing and testing for retroviruses. This level of characterization is not pragmatic for cell lines where the issue is to address worker safety. A set of criteria for meeting the definition of low hazard is required and the following text is intended to define a set of criteria with practical advice on how to achieve the requirements.

16. A well characterized or authenticated cell line is one which is obtained from a reputable supplier whose production methods includes a quality management programme (such as ATCC, ECACC or other recognized cell culture depository). The supplier must provide a product description including propagation details and any characterization analysis.

17. To maintain the above status, propagation records should be such that it allows traceability to the originally supplied material. These records must also be linked to the product description supplied with the original starting material. There should be standard operating procedures describing the propagation procedures and controls used to prevent cross-contamination with other cell lines and microbial contamination.

18. The cell line should have the typical morphology associated with the cell type and there should be no obvious abnormal features or characteristics. The split ratio for the propagation of the cell line and other growth requirements should be consistent with that described in the supplier's product description.

19. Routine mycoplasma testing should be carried out as it can be used as an indicator of good tissue culture practice. The frequency of mycoplasma testing should be described in the standard operating procedure. Routine microscopic examination can be used to confirm the absence of cytopathic adventitious agents.

20. One criterion for a low hazard cell line in HSE guidance is that it should be tested for the most serious pathogens. The most serious pathogens in most cases would be human blood-borne viruses (BBVs), however, if the cell line is not permissive for BBVs, then no testing is required.

21. Where the cell line is permissive for BBV agents, then testing to confirm the absence of relevant BBV is necessary unless a certificate is supplied with the starting material indicating its BBV-free status. Alternatively if there is no safe history of use, then confirmation of the authenticity of the cell line is required. Additionally, reagents used in the culturing of the cell line must be free of human BBVs or unlikely to be contaminated with such agents. Documentary evidence regarding the status of the reagents should be available.

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