Cell culture guidance

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Contents

1. Scope

This document is provided as guidance for anyone proposing to work with eukaryotic cell culture systems at the University of Bristol. It is intended to help you to comply with the requirements of the Control of Substances Hazardous to Health Regulations (COSHH) and in the preparation of your risk assessment for this work.

2. Introduction

The Control of Substances Hazardous to Health regulations (COSHH) requires employers to control exposure to hazardous substances in order to prevent ill health. In addition to general requirements, COSHH specifically classifies biological agents into hazard groups (1-4) based on the risk to human health upon exposure and specifies the minimum control and containment measures to be used to protect human health and safety when handling such agents. Furthermore, COSHH includes cell cultures in the definition of a biological agent. Work with cell cultures may also be covered by the requirements of the Genetically Modified Organisms (Contained Use) regulations.

2.1 Genetic modification.

If it is intended to genetically modify the cell culture in anyway, including the immortalisation of the line, (or your cell line has already been modified before receipt) then the requirements of the Genetically Modified Organisms (Contained Use) Regulations 2000 (GMO(CU)) will also have to be considered in addition to COSHH and a specific type of risk assessment undertaken. Further guidance on genetic modification work (including guidance on what constitutes genetic modification under the Regulations) can be found in the SACGM Compendium of Guidance on Genetic Modification. Part 2 pp66-67 of that guidance specifically relates to risk assessment of genetically modified cell cultures. Your risk assessments will also need to be reviewed by the University's Biological and Genetic Modification Safety Committee (BGMSC).

2.2 Human blood and primary human tissue.

If the culture work involves using blood or primary human tissue then additional requirements relating to health and safety are set out in the University Biological Safety and Genetic Modification Code of Practice and requirements relating to consent and licensing for the storage and use of human tissue under the Human Tissue Act 2004 are set out in the Human Tissue Authority Codes of Practice. The University Biological Safety Officer (and departmental deputies) or Designated Individuals (appointed in departments undertaking human tissue work under the Human Tissue Act) should be consulted for further guidance.

3. Risk assessment.

COSHH includes cell culture in the definition of a biological agent as they may be infected with adventitious biological agents such as mycoplasmas (e.g. *Mycoplasma pneumoniae*) or viruses, sometimes they may also have been infected intentionally. The nature of cell culture systems also means that they are often able to sustain and allow amplification of such agents during use. In addition, cells may also present other hazards to human health, such as the ability to produce toxins or allergens. An assessment of the risks must therefore be undertaken before work involving any cell cultures commences.

Suitable risk assessment forms can be found on the biological safety section of the Safety and Health Services website (www.bristol.ac.uk/safety/biosafety/#forms). COSHH risk assessments indicating a requirement for containment level 2 or 3 conditions to control the risks and all GM risk assessments must be approved by the Biological and Genetic Modification Safety Committee before work can start or certain materials can be stored on Bristol University premises (see forms for details). These should be submitted to the Biological Safety Officer (DBSO) covering your school or unit in the first instance.

Note that if you are undertaking genetic modification work then a detailed risk assessment made under the GMO(CU) Regulations can also satisfy the risk assessment requirements of COSHH often without the need for additional paperwork. Conversely, as the GMO(CU) Regulations require you to consider risks to the environment as well as to humans a COSHH risk assessment alone will not normally satisfy the GMO(CU) regulations.

3.1 Identifying the hazards.

The following should be considered in your risk assessment.

- **Cell/tissue origin** - Cells and tissues of primate origin are more likely to contain biological agents classified as hazardous to human health until they have been authenticated and fully characterised. In general non-primate animal and plant cells can be considered of low infection risk, however it is possible that these cells may still present risks to human health and safety. The risk from any cell line should be considered in terms of the likelihood of contamination and the ability of the cell line to support growth of the contaminant.
- **Cell/tissue source** – This will indicate the potential contaminants and the potential for expression or reactivation of latent viruses. Cells derived from peripheral blood and lymphoid cells present the greatest likelihood of contamination with human pathogens of greatest risk (higher COSHH hazard groups).
  Researchers MUST NOT use their own cells (or cells of anyone else who is working in the laboratory) for experimental purposes. This presents a particular hazard as any self-inoculation injury could have potentially serious consequences, as cells would essentially circumvent the normal protection of the immune system.
- **Cell/tissue type** – Primary cell cultures should be considered the greatest risk for contamination. However, established cell lines should not be considered low risk unless fully authenticated as some established cell lines have been shown to be persistently infected (e.g. B95-8 with EBV, MT4 with HTLV). The potential for contamination of cells with blood-borne viruses including hepatitis B, hepatitis C, HIV, SIV and HTLV should be considered. In particular, the continuous culture of permissive (CD4+) primate cell lines must be carefully assessed and the possibility of contamination with and inadvertent co-culture of HIV or SIV taken into account. Appropriate containment measures must be applied in such cases based upon the likelihood of contamination (also see table below). If HIV or SIV are known to be present then a minimum of Containment level 3 will be required.
- **Culture media** – Products of animal origin may be a source of microbial contamination.

Materials sourced from the originator, an established supplier or national cell culture collection, such as ATCC or ECACC, will normally be supplied with extensive information regarding these hazards and should have been screened for human pathogens. Peer-reviewed publications describing the cells will also be a useful source of similar information. Judgements regarding risk can then be made with more confidence. Cells from other sources may have a high passage number and this may not have been accurately recorded making judgements about infection risk hard to make. Of course, the potential for your own culture conditions to amplify any contaminating agents must be assessed and measures taken to control and monitor this where necessary.

3.2 Assess the nature of your work.

The risk assessment should also take into account the work procedures to be used and the control measures supplemented accordingly. You should also take into account the route(s) of infection for any biological agent. For example, consider whether the work is likely to generate significant aerosols and therefore requires the use of a microbiological safety cabinet, or whether splashing is likely such that eye or face protection is required. Also consider whether sharps are really necessary and if there is no suitable alternative what precautions will be taken; whether the culture conditions might amplify any contaminants; the volumes of cultures; and the number of samples in use.

3.3 Determining an appropriate containment level.

The minimum containment level required to protect human health from a biological agent is related to its hazard grouping as defined by COSHH. However, these containment measures must also be supplemented by measures to control any other hazards presented by the cell lines and/or introduced by the work procedures. Similar containment conditions to control **animal pathogens** are also defined by DEFRA in order to protect the environment. The Anti-Terrorism, Crime and Security Act also specifies security controls for certain pathogens and toxins listed in Schedule 5 of the Act. All of these containment measures should be taken into account and the higher standard applied where an agent is classified differently under several regulations. The University Biological Safety Officer can be consulted for further guidance on this and containment laboratory standards.

Hazard	Cell type	Baseline containment level
Low	Well characterised or authenticated finite or continuous cell lines of human or primate origin with a low risk of endogenous infection with a biological agent presenting no apparent harm to laboratory workers and which have been tested for the most serious pathogens	Containment Level 1
Medium	Finite or continuous cell lines/strains of human or primate origin not fully characterised or authenticated, except where there is a high risk of endogenous biological agents, eg blood borne viruses	Containment Level 2
High	Cell lines with endogenous biological agents or cells that have been deliberately infected	Containment level appropriate to the agent. For example, if infected with Hepatitis B virus, Containment Level 3 would be required
	Primary cells from blood or lymphoid cells of human or simian origin	Containment appropriate to the potential risk. A minimum of Containment Level 2 is recommended
NB: Any work that could give rise to infectious aerosols such as with medium or high risk cell lines must be carried out in suitable containment, eg a microbiological safety cabinet		

Table 1. Cell culture and containment; taken from 'SACGM Compendium of Guidance' (HSE, 2007)

Table 1 can be used as a guide to selecting the containment requirements for different cell types.