Guidance on Working with Cell Cultures

Guidance

1. Uncontaminated Cell Cultures

Uncontaminated cell cultures do not appear to present a significant hazard as even direct dermal inoculation may result in only local inflammation. However, the long-term consequences of direct inoculation are uncertain. The main risk presented by cell cultures is as a result of their ability to sustain the survival and/or replication of a number of adventitious agents. The major agents of concern are viruses, but other agents, e.g. mycoplasmas such as *Mycoplasma pneumoniae*, should also be considered. See section 4 for several recent examples of inadvertent culture contamination.

2. Table 1 and much of the text is adapted from guidance given by the UK Scientific Advisory Committee on Genetic Modification in their Compendium of Guidance (http://www.hse.gov.uk/biosafety/gmo/acgm/acgmcomp) and the UK Advisory Committee on Dangerous Pathogens document "Biological agents: Managing the risks in laboratories and healthcare premises" (http://www.hse.gov.uk/biosafety/biologagents.pdf). The recommendations are based on both the intrinsic properties of the cell culture and the possibility that the culture may be, or inadvertently become, contaminated with pathogens. Where a cell line is deliberately infected with a biological agent, or where it is likely that the cell line is contaminated with a particular agent, the containment level used must be appropriate for work with that agent.

3. Mammalian and insect cells have very stringent requirements for growth and are very susceptible to dehydration and to exposure to ultraviolet radiation. Outside of the animals from which they are derived, growth and survival requirements can only be met by using specialized media, the correct temperature range, optimum pH and an adequate oxygen concentration. **These constraints mean that cell lines will pose minimal risk to both human health and the environment**. In addition, due to immune rejection of non-self tissue, it is highly improbable that accidental exposure would result in survival and replication in normal healthy individuals (with the possible exception of some tumour cells). **Therefore, workers should not conduct work on their own cells and the use of cells derived from other laboratory workers should be avoided where possible**.

Table 1. Recommended baseline containment measures for work with cell cultures.

Hazard	Cell type	Baseline containment
Low	Well characterised or authenticated of the 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	BSL1
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, e.g. blood borne viruses	BSL2
High	Cell lines with endogenous biological agents or cells that have been deliberately infected	Containment level appropriate to the agent. For example, T-cells infected with HIV would require Biosafety Level 3
	Primary cells from blood or lymphoid cells of human or simian origin	Containment level appropriate to the risk
NB: Any work that could give rise to infectious aerosols must be carried out in suitable containment, e.g. a microbiological safety cabinet		

4. Adventitious Infection of Cell Lines - the Cautionary Tale of XMRV

Primary cell lines, especially those derived from blood or neural tissue, and cell lines that have not been fully authenticated or characterised are more likely to harbour adventitious agents than other cell lines. Where adventitious agents (or gene sequences from them) may be present in the cells, containment measures should be applied which are commensurate with the risks.

The possibility of adventitious infection of cell lines should not be underestimated. This is highlighted by several recent reports of laboratory cultured cell lines infected with murine retroviruses e.g. (Paprotka *et al* 2011, Recombinant Origin of the Retrovirus XMRV, Science 333, 97-101) which involves a report of contamination with the gammaretrovirus XMRV (xenotropic murine leukemia virus–related virus). Xenotropic retroviruses have the curious property that they can infect foreign cells, such as human cells, but do not re-infect murine cells. The paper indicates that the XMRV others found in human prostate cell lines passaged in nude mice is very likely to have been derived from a recombination event between two prophages present in the nude mice. The recombination event generated XMRV which was able to infect the human prostate cancer cells being passaged in the nude mice but not infect the mice themselves. They conclude that it is likely that the findings reported by several authors of XMLV in human prostate tumors and in blood samples from patients with chronic fatigue syndrome was a laboratory artifact.

A second report involves a number of lymphoid cell lines commonly used by EBV researchers (Akata, JY, DG75, Ramos, and P3HR1 Cl.13) which were found to be contaminated with a murine leukaemia virus. (Zhen Lin *et al*, 2012, Detection of Murine Leukemia Virus in the Epstein-Barr Virus-Positive Human B-Cell Line JY, Using a Computational RNA-Seq-Based Exogenous Agent Detection Pipeline, PARSES J Virol. 86: 2970–2977).

Further examples of inadvertent infection of cell culture can be seen in a number of incidents in the Biopharmaceutical industry which are summarized in Table 2. Even here where extremely stringent control measures are in place and the consequence of infection can be catastrophic for the company, infections occur on a regular basis.

Table 2. Examples of virus contamination in the Biopharmaceutical Industry (data from Michael E. Wiebe, Ph.D. Quantum Consulting)

Virus	Year	Company	Reported By
EHDV	1988	BioferonGmbH	Bioferon
MMV	1993	Genentech	Genentech
MMV	1994	Genentech	Genentech
Reovirus	1999	Abbott Labs	FDA
Cache Valley	2000	?	BioReliance
Vesivirus2117	2003	Boehringer-Ingelheim	BI
Cache Valley	2004	?	BioReliance
Human Adenovirus	?	Eli Lilly	Eli Lilly
Vesivirus2117	2008	Genzyme,	BelgiumGenzyme
Vesivirus2117	2008	Genzyme,	USAGenzyme
Vesivirus2117	2009	Genzyme,	USAGenzyme
PCV 1	2010	GlaxoSmithKline	GSK
PCV 1&2	2010	Merck	Merck

While many infections are obvious and cause cell destruction others may be more benign and result in inapparent infections. For example, human herpesviruses can become latent in very small percentages of various cell types without obvious signs, similarly retrovirus and mycoplasma infections are not always easily detected. Hence macroscopic examination of a culture is not always reliable evidence for the absence of an infection.

5. Human Tumour Cells

Many tumour cell cultures fall into the category of ‘well characterised continuous cell lines’ and will therefore require minimal containment. There are however some concerns over primary human tumour cells that have led to recommendations that all work with such cells should be carried out at a minimum of Biosafety Level 2. In addition to the potential for

adventitious agents to be present there is the possibility that some tumour cells may escape from normal immune surveillance, survive and replicate following accidental inoculation.

There is one report in the literature [a] of a tumour which grew in a laboratory worker accidentally inoculated with cells of a human tumour cell line and cancers have been transferred between people during transplantation [b]. Although the growth of tumour cells from the cell lines is highly unlikely in healthy individuals, anyone with a compromised immune system is at greater risk.

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[a] Gugel EA, Sanders ME. Needle-stick transmission of human colonic adenocarcinoma. New Engl J Med 315: 1487, 1986.

[b] Southam CM. Homotransplantation of human cell lines. Bull NY Acad Med 34: 416-423, 1958.

6. Cells Modified by Retroviruses e.g. Lentiviruses

The use of retrovirus vectors for modulating gene expression in cell lines is widespread in the University. For most current vector systems the resulting cell lines are not capable of producing infectious virus and consequently the manipulated cell line should be viewed in the same light as any other continuous cell line i.e. in much the same way as the parental line. However it is possibly worth pointing out that the same parental cell line transformed by the same retrovirus at different times may exhibit different properties simply due to the fact that the vector has inserted into a different genomic location

The design of some of the early retrovirus vector systems means that a producer cell line is generated and this cell line produces infectious retrovirus vectors. If researchers wish to use this type of vector please consult the University Biological Safety Officer and carry out a formal risk assessment which will need to be approved by the University Biosafety Committee before work can start.

7. Stem Cells and Induced Pluripotent Stem Cells (iPSC's)

The defining characteristics of undifferentiated human or murine embryonic stem cells (hESCs or mESCs) and induced pluripotent stem cells (iPSC's) are the potential for self-renewal i.e. unlimited expansion and the ability to generate cells of all three germ layers - endoderm, mesoderm, and ectoderm which can be further differentiated into many specific cell lineages. Because of this ability, their use has been proposed in a variety of clinical applications and as a tool for the study of human cellular and developmental systems.

These characteristics are also why there has been some concern over safety – could tumours or be formed in the event of accidental inoculation? In healthy individuals the hESC's or iPSCs from donors would normally be rejected and as stated in 3 above it is important that researchers do not conduct work on their own cells or cells derived from other laboratory workers as this would potentially bypass immune rejection.

The clinical use of hESCs (or iPSCs for that matter) is also complicated by the fear of spontaneous teratoma formation. At least two factors are important in decreasing the chance of this happening: differentiating the cells to a high purity endpoint and injecting smaller cell volumes and thus far in the few clinical trials of

hESCs tumour formation appears not to be a problem.

Work with iPSCs is a fast moving area of research and there are now various ways of generating iPSCs that don't involve the use of retrovirus vectors permanently transforming the cells. These include transient DNA transfection approaches using transposons, episomal vectors or plasmid minicircles, protein transduction and RNA transfection approaches. Several small molecules have also been found to augment iPSC derivation efficiency, allowing the use of a fewer number of genes during induction of pluripotency. The relative risk varies with the different ways of generating iPSCs so in any risk assessment it is important to detail how the lines will be generated.

8. Expression of Highly Potent Secreted Proteins

Where cells are genetically modified to express highly potent biologically active molecules such as cytokines, control measures may be required to minimise the risk of exposure to those molecules particularly if there is accumulation of such molecules in the cell medium.

9. Contamination Versus Containment

Many users will automatically use a microbiological safety cabinet and wear protective gloves to protect the cells from contamination. Similarly, there may be restricted access to culture facilities in order to minimise the possibility of contamination. These measures are specified in the list of controls required for Biosafety Level 2 but are a separate issue from the containment required to protect human health and the environment from the risks associated with the cells. Consequently a risk assessment may indicate that a particular cell lines requires only Biosafety level 1 but in practice it is handled at Biosafety level 2 because of a desire to prevent contamination. It is permissible to use higher containment than indicated by the risk assessment. However, where there is a disparity between the containment level actually being used and the risk assessment this should be documented.

Example of risk assessment for culture of Epstein-Barr virus positive cell lines (e.g. those shown in Table 3 below)

The experimental design involves growth of several litre's of cells in the case of the lymphoblastoid cell lines and approximately 10 T150's of each of the NPC derived lines followed by cell harvesting, concentration and lysis of the cells to extract RNA.

Table 3. Summary of cell lines – see Appendix 1 for details

Cell name	Morphology	Derived from (and approximately when)	EBV status	Virus production?
B95-8 (1)	Lymphoblastoid	Cotton top tamarin - B-cell (1972)	+ve	Yes
P3-HR1(2)	Lymphoblastoid	Burkitt lymphoma (1967)	+ve	Yes (non-transforming)
Raji (3)	Lymphoblastoid	Burkitt lymphoma (1963)	+ve	No
Namalwa (4)	Lymphoblastoid	Burkitt lymphoma (1972)	+ve	No
C666-1 (5)	Epithelial	NPC (1999)	+ve	No
HONE-1 (6)	Epithelial	NPC (1989)	+ve	No
HK-1 (7)	Epithelial	NPC (1980)	+ve	No

(1) Miller et al (1972). Epstein-Barr virus: transformation, cytopathic changes and viral antigens in squirrel monkey and marmoset leukocytes. Proc. Natl. Acad. Sci. U.S.A. 69:383-387

(2) Hinuma et al (1967). Immunofluorescence and herpes-type virus particles in the P3HR-1 Burkitt lymphoma cell line. J. Virol. 1:1045-1051.

(3) Pulvertaft JV. Cytology of Burkitt's tumour (African lymphoma). Lancet 1: 238-240, 1964. PubMed: 14086209

(4) Klein G, et al. Sensitivity of Epstein-Barr virus (EBV) producer and non-producer human lymphoblastoid cell lines to superinfection with EB-virus. Int. J. Cancer 10: 44-57, 1972. PubMed: 4122458

(5) Cheung et al. (1999) Nasopharyngeal carcinoma cell line (C666-1) consistently harbouring Epstein-Barr virus. Int J Cancer: 83:121-6.

(6) Glaser et al (1989) Two epithelial tumor cell lines (HNE-1 and HONE-1) latently infected with Epstein-Barr virus that were derived from nasopharyngeal carcinomas. Proc Natl Acad Sci U S A. 86:9524-8.

(7) Huang et al (1980) Establishment of a cell line (NPC/HK1) from a differentiated squamous carcinoma of the nasopharynx. Int J Cancer. 1980 Aug;26(2):127-32.

Identify the Hazards

Potential adventitious contamination of cell lines (The main risk from cell cultures is as a result of their ability to sustain the survival and/or replication of a number of adventitious agents e.g. *Mycoplasma pneumoniae*)

The cell lines contain Epstein-Barr virus (EBV). It might be possible for individuals to become infected with the virus. This might result in infectious mononucleosis or one of several associated lymphoma's or even nasopharyngeal carcinoma.

Following accidental inoculation tumour cell lines might continue to grow and subsequently form a tumour/lymphoma.

Identify who might be harmed

Those most at risk are individuals carrying out the work. Others sharing the facilities including the centrifuges, biosafety cabinet and cell culture incubator might be exposed to virus containing aerosols /media splashes and spills

Evaluate the risks (likelihood that harm will occur and consequences) and decide if existing precautions are adequate or whether more should be done.

As a general point all of the cell lines in the table have been in culture for a long period of time and can be considered as having a long history of safe use.

Risk 1:- Potential adventitious contamination

There is always the possibility of contamination of cell lines with adventitious agents for example *Mycoplasma pneumonia* that can be passed on to staff, however, for a number of reasons these cultures are highly unlikely to have been contaminated. All the cell lines being worked with have been obtained from reliable sources and where possible a culture collection. All cultures are tested on a regular basis for mycoplasma contamination and the cells from culture collections are certified free from contamination with a spectrum of biological agents. Liquid N_2 glycerol stocks are kept that are as close to the original authenticated passage as possible and repeated sub–culturing is limited reducing risks of the cultures being contaminated in the laboratory.

The possibility of adventitious infection of cell lines is highlighted by the recent report that a number of lymphoid cell lines commonly used by EBV researchers (Akata, JY, DG75, Ramos, and P3HR1 Cl.13) were contaminated with a murine leukaemia virus. (Zhen Lin *et al*, 2012, Detection of Murine Leukemia Virus in the Epstein-Barr Virus-Positive Human B-Cell Line JY, Using a Computational RNA-Seq-Based Exogenous Agent Detection Pipeline, PARSES J Virol. 86: 2970–2977).

The P3HR1 cell line used in these studies was screened by PCR and shown to be MuLV negative.

Risk 2:- Infection with EBV

All the cell lines being studied contain Epstein-Barr virus (EBV) and it might be possible for individuals to become infected with the virus. While this is theoretically possible there are a number of reasons why this is highly unlikely to be an issue. The vast majority of the population are infected asymptomatically at a young age and while laboratory workers may indeed be seronegative there are only anecdotal reports of laboratory acquired infection with EBV.

Of the cell lines being cultured only one, the B95-8 line, can produce virus and only very small amounts are produced in the absence of induction.

Risk 3:- Accidental inoculation

Following accidental inoculation tumour cell lines might continue to grow and subsequently form a tumour/lymphoma. Again while this is a theoretical possibility it is highly unlikely despite there being one record in the literature [8] of a tumour which grew in a laboratory worker accidentally inoculated with cells of a human tumour cell line and cancers have been transferred between people during transplantation [9].

In the event of a needlestick the individuals handling these cell lines are highly likely to reject the cells due to mismatched HLA. This fact underlines the importance of ensuring staff and students do not use their own cells (or cells of anyone else who is working in the laboratory) for experimental purposes. This could have

potentially serious consequences if individuals used their own cells as these cells would essentially circumvent the normal protection of the immune system if accidentally injected. The lab has banned staff and students from using their own cells or cells of others in the department.

[8] Gugel EA, Sanders ME. Needle-stick transmission of human colonic adenocarcinoma. New Engl J Med 315: 1487, 1986.

[9] Southam CM. Homotransplantation of human cell lines. Bull NY Acad Med 34: 416-423, 1958

Are existing precautions adequate?

From the table in the guidance it is apparent that the appropriate containment level is that of EBV i.e. BSL-2. The BMBL (Biosafety in Microbiological and Biomedical Laboratories) 5th edition containment recommendations for human herpesviruses including EBV are for "BSL-2 practices, containment equipment, and facilities for activities utilizing known or potentially infectious clinical materials or cultures of indigenous viral agents that are associated or identified as a primary pathogen of human disease. Although there is little evidence that infectious aerosols are a significant source of LAI, it is prudent to avoid the generation of aerosols during the handling of clinical materials or isolates, or during the necropsy of animals. Primary containment devices (e.g., BSC) should be utilized to prevent exposure of workers to infectious aerosols."

The measures outlined above (under risk 1) to minimise contamination along with culture at BSL-2 are those employed in the laboratory. Consequently our current containment conditions are appropriate and are expected to control any of the risks identified.

Record findings

See above. Primary record kept by laboratory superintendent, copies can be found in all laboratories of the department carrying out the culture work and all those involved have signed a record that they have read the assessment and will abide by the measures identified.

Review after a set time or following any incidents, near misses or spills.