

# Chapter 22

## Biosafety Recommendations on the Handling of Animal Cell Cultures

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**Abstract** The first steps in tissue culture are dating back to the beginning of the nineteenth century when biosafety measures did not yet exist. Later on, animal cell culture became essential for scientific research, diagnosis and biotechnological activities. Along with this development, biosafety concerns have emerged pointing to the risks for human health and in a lesser extent for the environment associated to the handling of animal cell cultures. The management of these risks requires a thorough risk assessment of both the cell cultures and the type of manipulation prior the start of any activity. It involves a case-by-case evaluation of both the intrinsic properties of the cell culture genetically modified or not and the probability that it may inadvertently or intentionally become infected with pathogenic microorganisms. The latter hazard is predominant when adventitious contaminants are pathogenic or have a better capacity to persist in unfavourable conditions. Consequently, most of the containment measures primarily aim at protecting cells from adventitious contamination. Cell cultures known to harbour an infectious etiologic agent should be manipulated in compliance with containment measures recommended for the etiologic agent itself. The manipulation of cell cultures from human or primate origin necessitates the use of a type II biosafety cabinet. The scope of this chapter is to highlight aspects relevant for the risk assessment and to summarize the main biosafety recommendations and the recent technological advances allowing a mitigation of the risk for the handling of animal cell cultures.

**Keywords** Biosafety • Risk assessment • Risk management • Contained use • Genetically modified organisms • Pathogenic organisms

### Abbreviations

BSC Biosafety cabinet  
BSL Biosafety level  
GMO Genetically modified organism  
LAIs Laboratory-acquired infections

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SPF	Specified-pathogen-free
TSE	Transmissible spongiform encephalopathies

## 22.1 Introduction

Biosafety is a concept that refers to the need to protect human health and the environment from the possible adverse effects of pathogenic and/or genetically modified organisms and micro-organisms used in basic research, research and development (R&D) and modern biotechnology. To this end, a case-by-case risk assessment is conducted which consists in the identification and characterisation of potential effects, which may be intended or unintended, together with an assessment of the likelihood and consequences should any effect occur. Depending on the risks identified, risk management measures can be proposed.

The use of cell cultures may fall within the scope of one or several regulatory provisions which consider an assessment of biological risks. For example in Europe, tissue culture work will in many cases involve the use of genetically modified cell lines as well, in which case a risk assessment should be made in accordance with the provisions of the Directive 2009/41/EC related to the contained use of genetically modified micro-organisms (European Commission 2009). Cell culturing activities aiming at manufacturing biopharmaceuticals are covered by the Regulation (EC) No 726/2004 and its amending acts laying down procedures for the authorisation and supervision of medicinal products for human and veterinary use (European Commission 2004a), whereas activities that involve the use of human cells and tissues for application to the human body falls within the scope of the Directive 2004/23/EC and its amending acts (European Commission 2004b). The manipulation of animal cell cultures also exposes the worker to potential biological risks which are considered under the provision of the European Directive 2000/54/EC (European Commission 2000). It should be pointed out that guidelines aiming at mitigating the biological risks for the laboratory workers, public health and the environment have been issued by some scientific advisory bodies or competent authorities (World Health Organization 2004; Centers for Disease Control and Prevention 2009a; Swiss Expert Committee for Biosafety 2011).

While biosafety recommendations (as outlined hereafter) are principally aimed at providing maximal protection of human health (including laboratory workers) and the environment, it is recognised that many of the precautionary measures will also directly benefit the quality of research activities involving animal cell cultures. Indeed, cross-contamination (Lucey et al. 2009; Capes-Davis et al. 2010; Stürzl et al. 2013; Jäger et al. 2013; Johnen et al. 2013; MacLeod et al. 2013) or inadvertent contamination with infectious micro-organisms (bacteria, fungi, yeasts, virus, and prion) are plaguing many researchers, often leading to unproductive data, misinterpretation of results and a considerable waste of time and energy (Mahy et al. 1991; Drexler and Uphoff 2000; Mirjalili et al. 2005; Cobo et al. 2007; Pinheiro de Oliveira et al. 2013). It should also be emphasized that even if Good

Manufacturing Process (GMP) aim at protecting the product, some of the GMP measures are compatible with biosafety measures and reveal to be complementary.

The objective of this chapter will be to address and review biorisk assessment and management considerations of diagnostic and research activities involving cell cultures.

## 22.2 Biological Risk Assessment

From an historical perspective, the assessment of biological risks has an empirical basis and has resulted from the awareness of the scientific community with regards the risks associated with the handling of pathogenic organisms, as demonstrated through many reported cases of laboratory-acquired infections, followed by the potential risks associated with experiments involving recombinant DNA. In the 1970s, some initiatives for implementing measures guaranteeing the safe use of recombinant DNA were linked to the safety measures which were at that time already successfully applied in microbiology for the containment of pathogenic organisms (National Institute of Health 1979). The conjunction of these two aspects constitutes two pillars of biosafety and has led to a classification system of organisms into risk classes or risk groups. So far, many risk assessments have been carried out among the scientific community on the use of pathogenic organisms (genetically modified or not), regardless of the scale or the purpose of the activity. The basis for this risk assessment methodology takes into account the most recent scientific, technical data and uses a scientifically sound approach.

The methodology of biological risk assessment of contained use activities involving pathogenic and/or genetically modified organisms (GMO) identifies and takes into account the probability of occurrence and the severity of a potential negative effect on public health (including the exposed workers) and/or the environment. As a result of this methodology, a well characterised risk will lead to the choice of appropriate preventive measures encompassing the adoption of an appropriate containment level, the use of safety equipment including personal protective equipment, work practices and waste management.

The risk assessment methodology is commonly used and its 4-step approach is described in Fig. 22.1. The first step (1) takes into account the characteristics of the organism(s) used and, in the case of genetic modification, the genetic material introduced and the resulting GMO. Based on information relative to their harmful characteristics, natural pathogenic micro-organisms can be categorised in several classes of risk or risk groups. This classification takes into account the severity of the disease that pathogenic organisms may cause to human or animal health, their ability to spread amongst the population and the availability of prophylaxis or efficient treatment (World Health Organization 2004). For zoonopathogens, the classification system is mainly based on the definitions of the World Organisation of Animal Health (OIE), which categorises animal pathogens into four groups according to their risk to animal health, and since 2008, their risk to human health

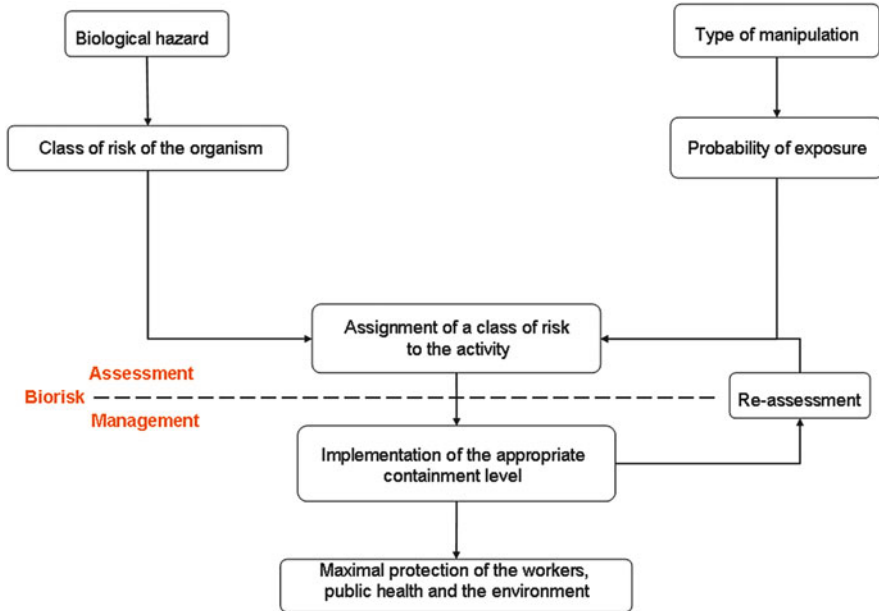


Fig. 22.1 Flow diagram summarizing the Biorisk assessment and management methodology

as well (World Organisation for Animal Health 2009). Micro-organisms that are unlikely to cause disease are classified into class of risk 1 while etiologic agents responsible for severe diseases with a high potential of transmissibility and for which no prophylaxis or treatment is available are assigned to class of risk 4. As such, pathogenic organisms will be categorised from class of risk 2 to class of risk 4. Some periodically revised reference lists issued by international and national authorities or advisory committees classify natural biological agents (not genetically modified) into risk groups or assign the biosafety level under which these should be manipulated (World Health Organization 2004; European Commission 2000; Swiss Agency for the Environment, Forests and Landscape 2005; Centers for Disease Control and Prevention 2009a; Belgian Biosafety Server 2010; Advisory Committee on Dangerous Pathogens 2013).

In the second step (2), the magnitude of the identified negative effects such as human diseases, including allergenic or toxic effects or the transfer of genetic material to other organisms are characterized. In a third step (3), an assessment is performed of the exposure of the laboratory worker, the population and/or the environment to the considered organism and the consequences of each negative effect should it occur. In the fourth step (4), a characterization of the risk is performed resulting in the assignment of the risk level associated with the contained use involving the use of the organism(s). On this basis, the containment measures and other protection measures (e.g. safe work practices, safety equipment and biological waste management) to be adopted are determined. There are four levels

of risk to which contained uses can be assigned, with level of risk increasing from 1 to 4. The final step consists in definitively classifying the contained use activity by conducting a re-assessment of the whole procedure before starting the research, diagnosis or production activity.

In this chapter, the risk assessment methodology applied to animal cell cultures is developed and illustrated by examples. It is important to mention that such a risk assessment is always performed on a case by case basis by the scientist (s) responsible for the activity and the biosafety officer (or biosafety professional) in compliance with local guidance and regulatory requirements.

### ***22.2.1 Risk Assessment of Cell Cultures***

The risk assessment applied to animal cell cultures relies on a thorough evaluation of both the intrinsic properties of the cell culture – including subsequent properties acquired as a result of genetic modification(s) – and the possibility that the cell culture may inadvertently be contaminated or deliberately infected with pathogenic micro-organisms. It also includes an exposure assessment which means that the type of manipulation carried out with the cell cultures is taken into account.

The assessment of cell cultures harbouring pathogens follows the same principles as the assessment of the pathogens itself. First, the main organism characteristics (a comprehensive description of the pathogen) is considered by taking into account the following parameters (not by order of importance): (1) the pathogenicity and, when available, the infectious dose (2) the mode of transmission, (3) the host range, (4) the epidemiology (assignment of appropriate risk group may depend on the geographic localisation), possible reservoir and vector(s), and the ability to zoonosis (5) the stability and the persistence of the organism in the environment (i.e. survival outside the host). In addition, information related to the physicochemical properties of the pathogenic organism is considered such as: (1) susceptibility to disinfectants, (2) physical inactivation and (3) drug susceptibility (e.g. sensitivity and known resistance to antibiotics or antiviral compounds). Finally, aspects related to the disease caused by the pathogen are also to be taken into consideration. This includes (1) the availability of an effective prophylaxis, (2) the availability of an efficient therapy and (3) any reported case of laboratory-acquired infection (s) (LAIs).

Although underestimated, many cases of LAIs related to the handling of cell cultures and/or containing virus suspension have been reported. Among them are the reported laboratory worker's exposure to (recombinant) vaccinia viruses amplified in cell culture resulting into infections (Jones et al. 1986; Openshaw et al. 1991; Mempel et al. 2003; Moussatché et al. 2003; Wlodaver et al. 2004; Lewis et al. 2006; Centers for Disease Control and Prevention 2009b). Recommendations to work safely with vaccinia virus have been reviewed recently together with an overview on the reported cases of LAIs involving this virus (Isaacs 2012).

The risk assessment of cell cultures that are genetically modified basically follows a comparative approach: the characteristics of the GMO are compared to those of the non-modified (wild-type) organism from which it is derived under corresponding situations of use. The distinctive feature of the risk assessment of genetically modified cell cultures which consists of the evaluation of the recipient cell, the vector, the donor organisms and the inserted genetic material (insert) is developed in Sect. 22.2.5.

### 22.2.2 *Intrinsic Properties of Cell Cultures*

Good knowledge and characterisation of the intrinsic properties of cells are key to successful and safe cell culturing. With respect to the biological risks and the risk assessment associated with the manipulation of animal cell cultures, three properties intrinsic to cell cultures should be considered : the species of origin, the cell type or type of tissue, organ from which the cell line is derived and the status of the culture (Fig. 22.2).

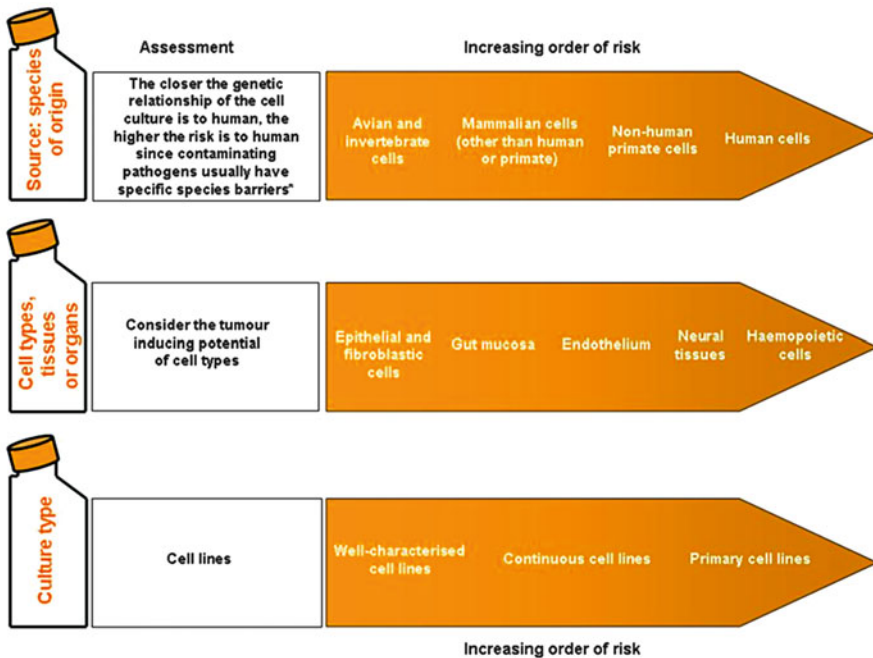


Fig. 22.2 The three intrinsic properties of animal cell cultures distributed in increasing order of risk. \*Some contaminating organisms might cross the usual species barrier (e.g. H5N1 influenza, BSE, SARS, etc.)

With respect to the species of origin and based on the fact that pathogens usually have specific species barriers, it is considered that the closer the genetic relationship of the cell culture is to humans, the higher the risk is to humans. The incidence to harbour organisms that could cause harm to human health is therefore considered higher in human or primate cells compared to cells of non-human origin (Brown 1997). Accordingly, mammalian cells other than human or primate cells are considered to represent less risk, followed by avian and invertebrate cells. However, it should be kept in mind that some infectious agents are able to cross the species barrier and to persist in new host species, leading to zoonotic diseases. It is acknowledged since many years that more or less 70 % of the emerging infectious diseases are zoonotic (Chomel et al. 2007). Well documented cases of viruses that have crossed the species barrier from animal reservoirs to humans include hantavirus (murine reservoir), haemorrhagic fever viruses (Ebola, Marburg) (Peters et al. 1992), avian Influenza virus (Reperant et al. 2012) and Severe acute respiratory syndrome (SARS) associated coronavirus (Ksiazek et al. 2003; Herman et al. 2004). These examples show that incidences of cross-species transfer can occur and that occupational risks related to exposure to infected animal tissues or cell cultures should not be underestimated (Mahy and Brown 2000; Louz et al. 2005).

Cells may dramatically differ in their *in vivo* half-life depending on the cell type or type of tissue from which these are derived. For example, intestinal and certain leukocytes have a half-life of a few days, human erythrocytes have approximately a 55–60-day half-life, healthy liver cells have a life span of several months, whereas, in adults, there is a slow loss of brain cells with little replacement. Partly due to this fact, some cell lines can be more readily obtained than others. The establishment of cell lines is often obtained by a series of (generally uncontrolled) mutations that occur by culturing cells for a longer period. It is known that cells cultured for extensive periods of time display changing growth properties. A reduction of the doubling time, as a result of transformation, may give cells the ability to overgrow the rest of the population and to survive for a large (infinite) number of passages compared to primary cells with a finite life span. Therefore, the establishment of cell cultures of a certain cell type upon extensive passage relies on the positive selection for cells that have a growth advantage. These transformed cells can have an increased tumorigenic potential and may present more risks of becoming/being fully neoplastic upon accidental (Gugel and Sanders 1986) or deliberate introduction into the human body. Therefore, taking the tumorigenic potential into account, the following cell types may be ranked in increasing order of risk: epithelial and fibroblast cells, gut mucosa, endothelium, neural tissues, haematogenous (e.g. blood, lymphoid) cells and tissue.

A third inherent property to consider is the status of cell culture. Diagnostic and research activities involve the manipulation of primary cultures or cell lines as well as continuous cell lines derived from primary cultures. Primary cell cultures and cell strains are produced directly from organs or tissues and are often the most accurate *in vitro* tool for reproducing typical cellular responses observed *in vivo*. However, as they are characterised by a finite life span, the time available for

characterisation and detection of contaminating agents remains limited. Also, because typical cell characteristics are often lost during the passage of cells, primary cell cultures are repeatedly obtained from fresh tissue, resulting in increasing risks for potential contaminating pathogens.

A feature that distinguishes continuous cell lines from primary cell cultures is the ability to survive if not infinitely, at least for a great number of passages. These immortalised cells are obtained by isolating cells from tumours, by mutating primary cells with mutagens, by using viruses or recombinant DNA to generate indefinitely growing cells or by cell fusioning of primary cells with a continuous cell line. Due to their increased life span, the time left for thorough characterisation and detection of contaminating agents is considerably increased. Within this respect, well-characterised cell lines present the lowest risks compared to primary cultures or less characterised cell lines as the origin, the source and suitability are well-known and well-defined.

For cell lines obtained from external sources (e.g. different laboratory), cross-contamination of cell-lines and/or a lack of proof of identity is actually a widespread problem (Buehring et al. 2004; Capes-Davis et al. 2010). In order to have at least evidence of the species of origin of a cell line and to be able to conduct a thorough risk assessment, it may be necessary to fully characterise the used cell lines. For this purpose, a number of techniques are available such as cytogenetic analysis, DNA fingerprinting, PCR, flow cytometry and isoenzyme analysis. (Matsuo et al. 1999; Cabrera et al. 2006).

### ***22.2.3 Intentional Infection of Cell Cultures***

Many micro-organisms benefit from a cell's machinery to complete their life cycles and to disseminate. Hence the study of a pathogens' lifecycle or immunity escape mechanism requires the intentional *in vitro* infection of animal (or human) cells. The identification of potential hazards associated with infected cell cultures requires a consideration of the intrinsic cell properties and the inherent properties of the infecting pathogen. The latter implies an assessment of a number of pathogen specific criteria along with aspects such as the existence of effective treatment or prophylaxis. On the basis of these criteria the WHO defines a classification system that enables the categorisation of micro-organisms into four risk groups (World Health Organization 2004). A fundamental rule is that the biological risk of infected cell cultures will depend on the infecting pathogen(s) class of risk. For example, cell cultures deliberately infected with Hepatitis C virus (HCV) in order to produce virus particles are assigned to class of risk 3, as HCV is a class of risk 3 virus. Human cells infected with an airborne pathogen like species of the *Mycobacterium tuberculosis* complex are also assigned to class of risk 3 and are requiring the adoption of Biosafety Level 3 containment. However, as discussed below, the class of risk to which the infected cell cultures are assigned will not necessarily indicate the level of containment to be implemented as the latter will also be determined by



the nature of the work carried out with these cells. An example is the infection of bovine leukocytes with *Theileria parva*, a tick-transmitted, intracellular protozoan of veterinary importance and the causative agent of East Coast fever among domestic livestock. It is an animal pathogen of risk group 3, which is not pathogenic to humans. The sporozoite form (infective form) invades bovine lymphocytes where it develops into a non-infective form (shizonts) and induces host cell transformation and clonal expansion of the cell. These infected bovine leukocytes may be categorised under class of risk 2 for animals, while the biosafety level (BSL1 or 2) appropriate for handling is determined by the presence or absence of the infectious form of the parasites.

Cell culture can also be coupled with electron microscopy to identify viral diseases of unknown cause as shown in a recent study published by the Centers for Disease Control and Prevention (Goldsmith et al. 2013). In case of outbreaks the harvested tissues from dead or living infected patients are inoculated to a permissive cell line (generally on VERO E6 cells) and eventually subjected to electron microscopy for morphological analysis of the causal virus. Although alternatives methods such as high throughput DNA sequencing are available to identify a micro-organism without a prior *in vitro* expansion, cell culture followed by electron microscopy remains the complementary approach of choice to molecular methods for the unbiased diagnosis of ill-defined infectious disease. Some of the diagnostic activities presented by the authors required the adoption of BSL3 measures to handle the cell cultures. It illustrates how activities involving the *in vitro* amplification of unknown virus may represent a risk for the laboratory personnel and requires the adoption of an appropriate containment level and work practices.

#### 22.2.4 Adventitious Contamination of Cell Cultures

Adventitious contamination of cell cultures is a major drawback for any activity that involves cell culturing (Langdon 2004). Causative agents of cell contamination include bacteria, fungi, mycoplasmas, parasites, viruses, prions and even other animal cells. Beside the fact that contamination of cell cultures may place experimental results in question or may lead to the loss of cell cultures, one of the main biosafety concerns when manipulating animal cell cultures for research, diagnosis or production purposes is the fact that they may provide a support for contaminating agents that cause harm to human health.

Generally, bacterial or fungal contamination can be readily detected because of their capacity to overgrow cell cultures. Typically, these organisms cause increased turbidity, pH shift of media (change in media colour), slower growth of the cells and cell destruction. Antibiotics may be used to prevent cell bacterial contamination, however, continuous use of antibiotics in cultures may lead to development of resistant organisms with slow growing properties, which are much more difficult to detect by direct visual observation. Compared to bacterial or fungal infections, mycoplasma contamination gives more problems in terms of incidence,

detectability, prevention and eradication. *Mycoplasma*, an intracellular bacterium, is one of the most common cell culture contaminants. It may go unnoticed for many passages and can change several cell properties such as growth, metabolism, morphology and genome structure (Paddenberg et al. 1996; McGarrity and Kotani 1985). It has also been reported to influence the yield of virus production in infected cells (Hargreaves and Leach 1970). Mycoplasmal contamination is also a biosafety concern, because some of the contaminating *Mycoplasma* spp. belong to risk group 2. Together with *M. arginini*, *M. orale*, *M. pirum* and *M. fermentans*, pathogenic organisms like *M. gallisepticum* (risk group 3 for animals), *M. hyorhinis* (risk group 2 for animals), *M. pneumoniae* and *M. hominis* (risk group 2 for humans) account for more than 96 % of mycoplasma contaminants in cell cultures. Primary sources of contamination with *M. orale*, *M. fermentans*, and *M. hominis* in the laboratory are infected people who handle cell cultures and suspensions of viruses. Sources of *M. arginini* and *M. hyorhinis* are usually animal donors of tissues and biological constituents used for cell culture, e.g. calf serum and trypsin (Razin and Tully 1995; Pinheiro de Oliveira et al. 2013). It was already reported that the contamination of cell cultures by *Mycoplasma* occurs via aerosols (O'Connell et al. 1964).

Viral contamination merits particular attention because infected cells may pose a serious harm to human health, especially when infected cells are able to release infectious particles. Human cells may be infected by various viruses like hepatitis viruses, retroviruses, herpes viruses or papillomaviruses. Although cell cultures from non-human origin may pose less risk, it should be emphasised that many viruses have a broad host range and can cross species barriers. Since a number of non-human viruses are capable of infecting and/or replicating in human cells *in vitro*, their possibility to infect human cells *in vivo* if human exposure occurs should be carefully considered. Well-known viral contaminants of primate tissues or cells from non-human origin that can cause human disease are listed in Table 22.1. While contamination with some viruses may be associated with changes in cell morphology or behaviour – such as the formation of syncytia (HIV, herpes viruses), swelling of cells (adenoviruses) or haemagglutination or haemadsorption – viral contamination may be harder to detect when cytopathic effects remain absent. Viral contamination could also trigger adverse effects as a result of recombination events or phenotypic mixing between contaminating components and experimentally introduced agents, creating agents with new properties. For example, experimental results suggested that HTLV-I or HTLV-II undergo phenotypic mixing with HIV-1 in HTLV/HIV-1 co-infected cells, leading to an increase of the pathogenicity of HIV-1 by broadening the spectrum of its cellular tropism to CD4 negative cells (Lusso et al. 1990). Another example is the contamination of murine cell cultures by Lymphocytic choriomeningitis virus (LCMV). LCMV is an arenavirus that establishes a silent, chronic infection in mice but causes aseptic meningitis, encephalitis or meningoencephalitis to humans. The significance of LCMV contamination has been reinforced by the description of cases of laboratory-acquired LCMV infections arising from contaminated murine tumour cell lines (Mahy et al. 1991). Manipulation of LCMV infected material or material with an increased likelihood of LCMV contamination necessitates the

**Table 22.1** Main viral contaminants of animal cell cultures or tissues that can cause human infectious diseases

Virus	CR <sup>a</sup>	References
<b>In human tissues</b>		
Hepatitis viruses : HBV, HCV, HDV, HEV, HGV	3 <sup>b</sup>	Simmonds (2001)
Human Retroviruses : HIV-1, HIV-2, HTLV-1, HTLV-2	3 <sup>b</sup>	Popovic et al. (1984), Clavel et al. (1986), Poiesz et al. (1980), Kalyanaraman et al. (1982), Bhagavati et al. (1988), Hjelle et al. (1992)
Herpesviruses : EBV, CMV, HHV-6, HSV-1, HSV- 2	2	Whitley (2001)
Papovaviruses	2	Butel (1996)
<b>In primate tissues</b>		
Flaviviruses : Yellow Fever virus, Kyasanur forest Virus	3	Tomori (2004)
Filoviruses : Marburg, Ebola	4	Shou and Hansen (2000), Mahy et al. (1991), Mahy (1998), Peters et al. (1992)
Simian hemorrhagic virus	2 <sup>c</sup>	Mahy (1998)
Rabies virus	3	Brown (1997)
Hepatitis A virus	2	Dienstag et al. (1976)
Poliovirus	2	
Herpesviruses (Herpes B Virus and others)	2	Hummeler et al. (1959), Davidson and Hummeler (1960), Weigler (1992)
Cercopithecine herpesvirus <b>Simian</b>	3	Estep et al. (2010)
SV40 (non-pathogenic for humans)	2	Vilchez and Butel (2004), Dang-Tan et al. (2004)
Simian Immunodeficiency virus (SIV)	3 <sup>b</sup>	Hahn et al. (2000), Khabbaz et al. (1994)
Monkeypox	3	Likos et al. (2005)
Simian Foamy virus 1&3	2 <sup>c</sup>	Delelis et al. (2004)
<b>In rodent tissues</b>		
Lymphocytic choriomeningitis virus (LCMV)	2	Hinman et al. (1975), van der Zeijst et al. (1983), Mahy et al. (1991)
Hantaan virus (hemorrhagic fever with renal syndrome)	3	Lloyd and Jones (1986), Mahy (1998)
Murine leukemia virus (MuLV)	2	Stang et al. (2009)
Monkeypox	3	Likos et al. (2005)

<sup>a</sup>CR Class of risk for human, from available lists from different sources (Swiss Agency for the Environment and Landscape 2005; Belgian Biosafety Server 2010; Advisory Committee on Dangerous Pathogens 2013)

<sup>b</sup>Not infectious via the airborne route

<sup>c</sup>Animal only

implementation of a BSL2. However, it should be kept in mind that the handling of the neurotropic LCMV itself should be performed in a BSL 3 laboratory.

Adventitious contamination with parasites may be an issue when handling primary cell or organ cultures originating from a donor organism that is known or suspected to be infected with a specific parasite. As the life cycle of most parasites comprises distinct developmental stages, transmission and survival of the parasite will strongly depend on the ability of the invasive stage to recognize and invade specific host cells. But even with cells developing the non-infectious form of parasites, possible harmful effects remain to be considered since natural modes of transmission could be bypassed during the manipulation of infected cells. It is recognised that most of the parasitic laboratory acquired infections are caused by needle stick injuries (Herwaldt 2001).

Finally, the use of bovine-derived products as tissue culture supplements may also lead to the contamination with unconventional agents that cause transmissible spongiform encephalopathies (TSE), the so-called prions (Solassol et al. 2003; Cronier et al. 2004; Vorberg et al. 2004). Contrary to the majority of the infectious agents, TSE agents are resistant to most of the physical and chemical methods commonly used for decontamination of infectious agents. It has been shown that neuroblastoma cell lines, primary cultured neurons and astrocytes can serve as hosts (Butler et al. 1988). Although many studies have suggested that the risk of propagation of TSE agents in tissue culture cells cultivated in the presence of bovine serum potentially contaminated with TSE was restricted to neurons or brain-derived cell cultures, it has been shown recently that non-neuronal cells can also support TSE infection, suggesting that any cell line expressing normal host prion protein could have the potential to support propagation of TSE agents (Vilette et al. 2001; Vorberg et al. 2004). While the understanding of the transmission of prions is still in progress (natural transmission seems mainly to take place via oral route in human and animals), investigators using cell cultures need to take into account different routes by which these agents may be transmitted experimentally. Mouse scrapie aerosol transmission has been successfully obtained in mice (Stitz and Aguzzi 2011; Haybaeck et al. 2011). In cervids, Chronic Wasting Disease (CWD) was already proposed as a natural airborne pathogen (Denkers et al. 2010). In the case of Creutzfeldt Jacob Disease (CJD), there is to date no proof of release of prion into aerosols.

Animal cell cultures can also harbour unknown pathogens or whose tropism has not been defined yet. Examples described in the literature include viruses such as Hepatitis G (Linnen et al. 1996), HHV8 (Moore et al. 1996), TT virus (Nishizawa et al. 1997) or human pneumovirus (van den Hoogen et al. 2001).

Cell cultures can be contaminated by different sources. Infected organisms or infected animal cells or tissues from which a cell line has been established are the primary source of contamination. An accidental contamination can also occur through the material used for cell culturing including glassware, storage bottles and pipettes due to incorrect maintenance or sterilisation. Before the use of disposable material the lip of the culture flask and the outside of the used pipette were important sources of contamination with mycoplasma (McGarrity 1976).

Nowadays, the use of disposable and sterile pipettes has significantly decreased the likelihood of adventitious contamination. A third source of contamination resides in culture media and its components such as serum, basic culture media and salt solutions and enzymes (trypsin, pronase and collagenase). For example, media and additives derived from bovine sources are often contaminated with bovine viral diarrhoea virus (BVDV) (Levings and Wessman 1991). As mentioned above the relative resistance of TSE agents may also be an issue when using bovine-derived products as tissue culture supplements. Finally, non-filtered air supply, clothing, personnel and floor can be a source of airborne contamination (Hay 1991).

### ***22.2.5 Genetically Modified Animal Cell Cultures***

Genetically modified (GM) animal cell cultures are employed for a number of different activities. For example, the expression of transgenes and production of proteins of interest whose function depends on methylation, sulfation, phosphorylation, lipid addition, or glycosylation may necessitate the capacity of higher eukaryotic cells to perform post-translational modifications. GM animal cell cultures may also be chosen for the replication of defective recombinant or even wild type viruses. The risk assessment of GM cells should follow the five-step methodology as outlined in Fig. 22.1, which means that an evaluation of each individual aspect in the process of genetic modification should be performed. This includes an evaluation of the recipient cell, the vector, the donor organism properties and an assessment of the characteristics of the inserted genetic material. A comprehensive risk assessment of genetically modified cells expressing transgenes should also take into account the risk associated with the transgene products. A gene product may be intrinsically harmful (e.g. toxic properties) or could induce hazardous properties via its expression in GM cells, dependent upon the genome integration site, promoter activity and expression of regulatory sequences governing expression. The risk assessment for transgenes is not straightforward and demands appropriate consideration. Comprehensive reviews have specifically addressed this topic (Bergmans et al. 2008; van den Akker et al. 2013). Genetic modification may confer an expanded life-span, immortalisation or increased capacity for tumour induction. However, it is unlikely that recombinant properties obtained by genetic modification may have an adverse effect upon release of the recombinant animal or human cells into the environment. Cells (genetically modified or not) have difficulties to survive in a hostile environment where control of temperature and osmolality is lacking or where cell-specific nutrients (e.g. glucose, vitamins, lipids) are not balanced or missing. Hence, the survival of such primary cells or cell lines outside of proper conditions is unlikely to occur. Apart from the fact that GM cell cultures may harbour pathogens and pose serious biological risks to human health (as discussed above), recombinant cells are more likely to cause harm when entering the body of animals or humans. However, the extent of the harmful effect remains hard to predict. It should be kept in mind that the lack of histocompatibility

between recombinant cells and the host organism remains a major obstacle for these cells to survive and to multiply as the natural immune response of the healthy (immunocompetent) host will recognise foreign cells and eventually destroy them. This is also one of the main reasons why the culturing of cells originating from the laboratory worker is not allowed for research and diagnostic activities (risk associated with autologous cells).

Particular attention should be paid to the use of packaging cell lines. These are established cell lines that are deliberately and stably transfected with “helper constructs” to ensure the replication and packaging of replication deficient viral vectors. For example, in case of retroviral packaging cell lines, the expression of “helper genes” allows high-level constitutive production of viral proteins (e.g. gag, pol and env proteins), which are missing in the genome of the replication deficient viral vector but are crucial for viral replication. One of the main biosafety issues related to the use of packaging cell lines is the fact that replication-competent viruses may be generated as a result of (homologous) recombination between the replication deficient viral vector and viral sequences present in the packaging cell. These events could result in the formation of viruses with novel yet unwanted properties such as the generation of replication-competent viruses. One of the strategies to engineer safer generations of packaging cell lines consists in minimising the likelihood of generating replication-competent viruses by separating viral functional elements into different expression plasmids, thereby increasing the number of recombination events necessary to generate replication-competent viruses (Dull et al. 1998) or by reducing or eliminating the sequence homology between the viral vector and the helper sequences. However, endogenous retrovirus genomes expressed in safer generations of retroviral packaging cell lines may still give rise to unwanted recombination events (Chong et al. 1998). This means that the possibility to generate replication-competent viruses cannot be ruled out.

Clearly, the risk group of the transfected packaging cell line will depend on the risk group of the viral vector itself. Consequently, risk assessment of packaging cell lines should be based on the biosafety of the produced viral vectors, including an evaluation of their infectivity, spectrum of host range, capacity of integration (insertional mutagenesis), stability and physiological role of the transgene(s) if expressed (Baldo et al. 2013).

### ***22.2.6 Type of Manipulation***

Aside from the identification and characterisation of hazards intrinsic to the cell culture, a thorough risk assessment must also consider the exposure pathways through which cell cultures may present a risk to human health or the environment. This necessitates an evaluation of the type of manipulation, because processes, methods and/or equipment involved may increase or decrease the likelihood of exposure and hence the resulting potential risks. For instance, while established cell lines inherently present low risks, large scale operations involving the culturing of

large volumes (from 10 to 1,000 l) are prone to contamination when inadequate containment measures are applied. This is exemplified by continuous processes such as cell cultivation in bioreactors where an appropriate design of seals, valves, pumps and transfer lines is required to guarantee long-term sterility of the operation to avoid inadvertent contamination. At the opposite, the handling of cell cultures belonging to risk group 2 may present less risk once they have been fixed by glutaraldehyde or formaldehyde/acetone for immunostaining and may therefore require less stringent containment measures

Manipulations that are common to research and diagnostic activities and warrant consideration with respect to the risk assessment of animal cell cultures are described hereunder:

- Procedures generating aerosols: pipetting, vortexing, centrifugation, opening of wet cups, etc.;
- Handling cells outside of a class II BSC: flow cytometric analysis and cell sorting constitute a special case of cell manipulation in which cells are handled outside of a BSC. The use of a fixative is in many cases not appropriate (e.g. viable cell sorting for subsequent further cell culturing) and the risk of aerosol formation can be particularly high, especially during sorting experiments and upon instrument failure such as a clogged sort nozzle. All scientists in the field of flow cytometry must be aware of the potential hazards associated with their discipline and only experienced and well-trained operators should perform potentially biohazardous cell sorting. General recommendations approved by the International Society of Analytical Cytology should help to set a basis for biosafety guidelines in flow cytometry laboratories (Schmid 2012). Some standard operating procedures and methods have also been described for ensuring the cell sorting under optimal biosafety conditions even under BSL3 conditions (Lennartz et al. 2005; Perfetto et al. 2011).
- Altering culture conditions: changing the availability of cell-specific nutrients, growth factors, signal molecules or adopting co-culture techniques may have significant effects on animal cell cultures as it may result in altered neoplasia (Stoker et al. 1990), altered expression of (proto)onco-genes or cell surface glycoproteins and release of endogenous viruses (Cunningham et al. 2004). As a consequence, changing culture conditions may lead to altered susceptibility of cultured cells to biologic agents such as viruses (Anders et al. 2003; Vincent et al. 2004).
- Manipulations involving use of needles or sharps: due to injuries cell material may be accidentally transferred directly to an operator's tissue and/or blood stream.
- *In vivo* experiments involving animals: major risks are self-inoculation (needle-stick injury) and exposure to aerosols.

Laboratory workers handling infected rodents or cell cultures originating from infected animals expose themselves at risk by directly exposing cuts, open wounds or mucus membranes with infected body fluids or by inhaling infectious aerosolized

particles of rodent urine, faeces or saliva. The risk can be minimised by utilising animals or cell cultures from sources that are regularly tested for the virus.

Finally, the purpose of cell culturing should be taken into consideration as many clinical approaches such as stem cell therapy, gene therapy, xeno- or allotransplantation involve cell culturing *ex vivo* for therapeutic purposes. This latter clearly justifies more careful consideration regarding safety, ethical, social and regulatory issues, which will not be addressed in this chapter ([Food and Drug Administration](#); [European Medicines Agency](#); ICH 1997).

## 22.3 Biological Risk Management

### 22.3.1 *General Biosafety Recommendations*

The assessment of biological risks related to animal cell cultures and the type of manipulation allows the determination of an adequate containment level in order to optimally protect human health and the environment. The implementation of an appropriate containment level includes a list of general and more specific work practices and containment measures.

Table 22.2 lists precautionary measures that should be applied whenever handling animal cell cultures. Much of these measures focus on reducing the risk of contamination with adventitious agents. It should be emphasised that the drawn-up of an appropriate set of standard operating procedures and adequate training of the staff is of crucial importance.

As a general rule, cell cultures known to harbour an infectious etiologic agent should be manipulated in compliance with containment measures recommended for the etiologic agent. When cell cultures are not known to harbour infectious agents, cells may be considered free of contaminating pathogens as long as a number of conditions are fulfilled. This implies the use of well-characterised cell lines or controlled cell sources for primary cells such as specified-pathogen-free (SPF) animals. If no well-characterised cell lines or SPF are available, tests for detection of likely contaminating agents should be negative. Second, whenever cell cultures are manipulated, media sources should be pathogen free and appropriate containment measures should be adopted to reduce potential contaminations during sampling or subsequent manipulation of cells (re-feeding and washing steps).

As the history of a cell culture may be poorly documented when a given cell culture is manipulated for the first time in the laboratory, it often remains unclear whether all appropriate measures have been implemented regardless of the fact that it may have been manipulated for years in another laboratory facility. In this case, cell cultures should be considered to be potentially infectious and should be manipulated in a class II BSC. If the presence of adventitious agents of a higher risk group is considered likely, the cell line should be handled under the appropriate



**Table 22.2** Precautionary measures for handling animal cell cultures

To respect good microbiological practices, especially those that are aimed at avoiding accidental contamination.
To be in compliance with good cell culture practice (GCCP), especially in industrial settings for vaccine production.
To avoid opening of culture vessels or contact with culture fluid through a defective culture vessel, stopper or poor technique because of the ever present likelihood of contamination with airborne pathogens.
To treat each new culture that is manipulated for the first time in the laboratory facility as potentially infectious.
To clean up any culture fluid spills immediately with an appropriated and validated disinfection protocol.
To work with one cell line at a time and disinfect the work surfaces between two operations involving cell lines.
To aliquot growth medium and other substrates so that the same vessel is not used for more than one cell line.
To mitigate cross-contamination by avoiding pouring actions.
To proceed to the use of the biosafety cabinet (BSC) by adequately trained staff, t.i. turn on for a period before and after use, thoroughly disinfect BSC surfaces after each work session and do not clutter the BSC with unnecessary materials.
To restrict the use of antibiotics in growth media.
To quarantine new cell cultures to a dedicated BSC or separate laboratory until the culture has been shown negative in appropriate tests.
To carry out a quality control of cells demonstrating the absence of likely contaminating pathogens on a regular basis or whenever necessary.
To operate cell cultures from undefined sources as risk group/class of risk 2 organism. If the presence of adventitious agents of higher risk class is expected, the cell line should be handled under appropriate containment level until tests have proven safety.

Adapted from Pauwels et al. (2007)

containment level until tests have proven the absence of such organisms. Good documentation of the history of cell cultivation is mandatory.

The extent to which cell cultures should be controlled on the likelihood of contaminants strongly depends on the nature of activity. For example, guidelines have been issued aiming at minimising any potential risk for transmission of infectious agents with respect the use of animal cell cultures for industrial production of biopharmaceuticals ([European Medicines Agency](#); [Food and Drug Administration](#); [ICH 1997](#); [World Health Organization 1998](#)). Hardly any guidance has been provided for the extent of detecting possible contaminants in case animal cell cultures are used for *in vitro* research or diagnostic activities, or for purposes other than therapeutics or production of biopharmaceuticals. The choice of the detection technique depends on the contaminating pathogen and often a combination of methods is recommended for important samples such as master cell banks.

The implementation of BSL2 measures is adequate for most of the work carried out with cell cultures from human or primate origin. BSL1 measures may be considered provided that all manipulations occur in a class II BSC and the cell culture is a well-characterised and certified cell line that presents no increased risk resulting from genetic modification or contaminating pathogen. The contained use

in a BSL1 of viral infected cells could be envisaged if no viral particles are detectable in the supernatant of the infected cells. However, it should be emphasized that procedures for viral clearance are virus-host specific and that variables such as vector titre and the infection protocol can influence the degree and rate of clearance (Bagutti et al. 2012). Since the implementation of good laboratory practices and the use of a BSC is usually the norm in most laboratories dealing with cell culturing, we think that BSL1 laboratories can relatively easily be upgraded to BSL2 facilities by implementing a restricted number of simple additional safety measures. It is important to notice that horizontal laminar air flows and clean benches minimise the risk of adventitious contamination of the cell cultures, however they offer no protection for the manipulator or the environment. Bearing in mind that biosafety measures intend to provide a maximal protection of human health and the environment, it is important to note that the sole use of a horizontal laminar “clean bench” should be prohibited.

Based on, but not limited to, key features of risk assessment and the type of manipulation performed as discussed in former paragraphs, we developed a flow diagram providing the cell culture users a schematic guidance for the assignment of an appropriate containment level when manipulating human or primate cells *in vitro* (Fig. 22.3). This flowchart is indicative and should be applied and/or reconsidered according to case specific conditions and risk assessments proper to the activities performed.

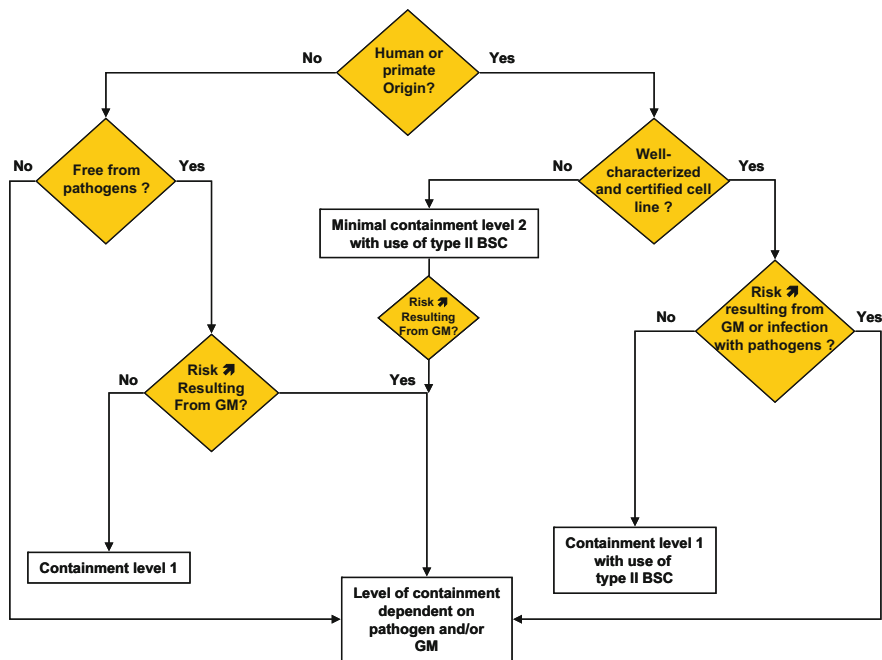


Fig. 22.3 Flow diagram helping for the assignment of the containment level to adopt as a function of the handled biological material

### ***22.3.2 Novel Approaches for Reducing Hazard and/or Exposure Associated with Handling of Cell Cultures***

Whenever possible approaches developed to *ab initio* reduce the risk associated with the handling of animal cell cultures should be favoured. Such approaches involve the use of dedicated instruments or safer biological material. The examples hereunder illustrate how the choice to apply one or several of these approaches is determined on a case-by-case basis, depending on the intrinsic characteristics of the biological material and the intended use.

During the last couple of years instruments enabling automated mechanical passaging and nutrient supply for *in vitro* cell expansion have been developed for primary cells, adherent and non-adherent mammalian cells (Kato et al. 2010; Thomas and Ratcliffe 2012). Some of these developments also provide a safer approach with respect to biosafety considerations by including a biosafety module in the design of such automated platforms.

For example a long-term cell culture device ensuring both the protection of the product and the operator has been designed for the culturing of embryonic stem cells in an antibiotic-free medium by means of an integrated automation platform using a class II BSC confining the microwells (liquid handling robot contained in a BSC) (Hussain et al. 2013)

An automation system has also been deployed for the production (140–1,000 ml) of HIV-1 pseudovirus for HIV vaccine trials in compliance with GCLP. This robust automated system for cultivation of 293 T/17 cells is contained in a class II BSC that guarantees the protection of the workers, environment and the product. This system can be implemented to produce other biological reagents under standardised large-scale conditions (Schultz et al. 2012).

Lowering the hazards associated with the cell cultures handled is another approach that was applied in the characterisation of pandemic influenza viruses under emergency situations. While such activities typically require BSL3 conditions, inactivation protocols applied to influenza virus culture allows for performing the virological and immunological assays under BSL2 conditions (Jonges et al. 2010).

Another example where the assignment of a less stringent containment level was enabled relates to the use of insect cells and GM bacteriophage Lambda capsids in cases where usually the maintenance of infectious stocks of highly pathogenic or emerging influenza viruses is involved (Domm et al. 2014). The bacteriophages are “decorated” by the viral glycoproteins of interest in an insect cell-derived system so as to use hemagglutinin displaying bacteriophages in hemagglutination-inhibition assay on pathogenic influenza viruses. The same approach was already applied for HIV-envelope protein (Mattiaccio et al. 2011).

With regards to HIV-1 *in vitro* testing, a non-infectious cell-based assay to assess the HIV-1 susceptibility to protease inhibitors was also developed recently (Buzon et al. 2012). Here again, activities that normally require relatively high containment level can be performed in facilities with less expensive infrastructure. It also shows that careful consideration of the material handled can lead to cost-effective choice while maintaining the same objectives.

### Concluding Remarks

Biosafety is an internationally recognized concept referring to the maximal protection of laboratory workers, public health and the environment from the possible adverse effects associated to the use of organisms and micro-organisms (genetically modified or not). Within this respect activities involving the use of animal cell cultures for fundamental research, R&D or *in vitro* diagnosis purposes pose biosafety considerations as well. Before starting any of such activities biosafety considerations should be addressed by performing a biological risk assessment allowing the identification and characterisation of any potential adverse effect together with an evaluation of the likelihood and consequences should any effect happen. Such an approach is made on a case-by-case basis, taking into account the type of manipulation and the type of cell culture handled. A risk assessment will result in biosafety recommendations in view of the implementation of an adequate containment level. Considering the very limited persistence capacity of animal and human cells outside non-optimised culture conditions and the fact that many cell lines have a long history of safe use, it is generally considered unlikely that cell cultures may inherently cause harm to humans or the environment. The main hazard associated with the handling of animal cell cultures resides in the presence of adventitious pathogenic micro-organisms, which are often difficult to detect and hence less controllable. In contrast to their host cells, adventitious organisms can persist in more hostile conditions and may present risks for human health or the environment in case they are pathogenic (Bean et al. 1982; Walther and Ewald 2004; Kallio et al. 2006; Kramer et al. 2006). For this reason, a risk assessment of cell cultures will frequently lead to a risk assessment of the potential adventitious contaminants, the organisms used for cell's immortalisation (viruses, viral sequences, etc.) and/or the micro-organisms intentionally used to experimentally infect them. Though the assignment of biosafety containment level requirements cannot be generalised and should be performed on a case-by-case basis, it is recognised that most of the containment measures primarily aim at protecting cells from adventitious contamination in order to mitigate potential risks for the laboratory worker. Except for authenticated cell lines proved to present no risk, the activities involving cell cultures from human or primate origin should generally be performed under containment level 2 involving the use of a class II Biosafety cabinet and Good Laboratory Practice.

Whilst the occurrence of adverse effects related to the handling of cell cultures cannot be excluded, a thorough risk assessment and the implementation of the appropriate containment level offer an optimal protection for the laboratory worker and by extension, public health and the environment. Continuous efforts have been made to mitigate the risk associated with the handling of cell culture in laboratory settings. These biorisk management

(continued)

measures (i.e. adoption of the appropriate containment level) can be coupled with the implementation of automation platforms allowing the reduction of cell culture cross-contamination and accidental contamination with infectious agents.

It is anticipated that future efforts contributing to the improvement of the quality of the data necessary for the risk assessment, the containment measures and the increased awareness of biosafety considerations within the scientific community will also directly benefit the quality of research activities involving animal cell cultures.

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## Glossary

**Authentication** Is the process by which the true origin and identity of cell lines are determined and should form an essential part of any cell culture operation.

**Biosafety** In the context of this chapter, biosafety relates to the evaluation of the potential risks to human health and the environment associated with the use of genetically modified organisms (GMOs) or pathogenic organisms.

**Biosafety Cabinet (Class II)** Safety cabinet with a front aperture through which the operator can carry out manipulations inside the cabinet and which is constructed so that the laboratory worker is protected, the product and cross contamination is low. The escape of airborne particulate contamination generated within the cabinet is controlled by means of an appropriate filtered internal airflow and filtration of the exhaust air (HEPA filters).

**Contained use** Any activity in which (micro)-organisms are genetically modified or in which such organism (pathogenic or not) are cultured, stored, transported, destroyed, disposed or used in any other way, and for which specific containment measures are used to limit their contact with the general population and the environment.

### Culture Type: Primary Cell Cultures, Diploid Cell Lines, Continuous Cell Lines

**Primary cell cultures** Are established directly from tissues of animals and are often the most appropriate *in vitro* tool for reproducing typical cellular responses observed *in vivo*. However, as typical cell characteristics are lost during passaging, these cultures must be obtained from fresh tissue that may contain or may become inadvertently contaminated with pathogens. Consequently, primary cell cultures may potentially present increased risks compared to continuous, established cell lines.

**Diploid cell lines** Are similar to primary cells, are considered non-tumourigenic and have a finite capacity for serial propagation. They are used for the preparation of viral vaccines and are from human or monkey origin.

**Continuous cell lines** Are immortalized cells that may survive almost infinite serial passages. These cells are obtained by either isolating cells from tumours (neoplastic origin), primary cells treated with mutagens, oncogenic viruses or recombinant DNA (oncogenes) or by cell fusioning of primary cells with a continuous cell line. As a consequence, the class of risk of these cell lines is often correlated to the class of risk of primary cells of whom they are derived. Due to the “immortality” of the continuous cell lines, their ability to induce tumours has also to be considered.

**Transfection** Gene transfer of DNA in eukaryotic cells using non-viral delivery methods.

**Transduction** Gene transfer of DNA in eukaryotic cells using viral vectors.

**Infection** Process occurring when a virus (wild type) that typically replicates and spreads into neighbouring cells mediates a gene transfer belonging to its own genome or extra genes it may carry.

**Viral vector** Is a protein particle derived from a replicative virus that contains genetic information in the form of RNA or DNA. A viral envelope may be present as well.

## References

- Advisory Committee on Dangerous Pathogens (2013) The approved list of biological Agents, 3rd edn. Health and Safety Executive, UK. <http://www.hse.gov.uk/pubns/misc208.pdf>. Accessed 31 Jan 2014
- Anders M, Hansen R, Ding RX, Rauen KA, Bissell MJ, Korn WM (2003) Disruption of 3D tissue integrity facilitates adenovirus infection by deregulating the coxsackievirus and adenovirus receptor. *Proc Natl Acad Sci U S A* 100(4):1943–1948
- Bagutti C, Schmidlin M, Mueller M, Brodmann P (2012) Washout kinetics of viral vectors from culture mammalian cells. *Appl Biosaf* 17(4):188–197
- Baldo A, van den Akker E, Bergmans HE, Lim F, Pauwels K (2013) General considerations on the biosafety of virus-derived vectors used in gene therapy and vaccination. *Curr Gene Ther* 13(6):385–394
- Bean B, Moore BM, Sterner B, Peterson LR, Gerding DN, Balfour HH (1982) Survival of influenza viruses on environmental surfaces. *J Infect Dis* 146:47–51
- Belgian Biosafety Server (2010) Belgian classifications for micro-organisms based on their biological risks. <http://www.biosafety.be/RA/Class/ClassBEL.html>. Accessed 31 Jan 2014
- Bergmans H, Logie C, Van Maanen K, Hermsen H, Meredyth M, Van Der Vlugt C (2008) Identification of potentially hazardous human gene products in GMO risk assessment. *Environ Biosafety Res* 7:1–9
- Bhagavati S, Ehrlich G, Kula RW, Kwok S, Sninsky J, Udani V, Poiesz BJ (1988) Detection of human T-cell lymphoma/leukemia virus type I DNA and antigen in spinal fluid and blood of patients with chronic progressive myelopathy. *New Engl J Med* 318:1141–1147
- Brown DW (1997) Threat to humans from virus infections of non-human primates. *Rev Med Virol* 7(4):239–246

- Buehring GC, Eby EA, Eby MJ (2004) Cell line cross-contamination: how aware are Mammalian cell culturists of the problem and how to monitor it? *In vitro cellular and developmental biology. Animal* 40(7):211–215
- Butel JS (1996) Chap. 66: Papovavirus. In: Baron S (ed) *Medical microbiology*, 4th edn. University of Texas Medical Branch at Galveston, Galveston
- Butler DA, Scott MR, Bockman JM, Borchelt DR, Taraboulos A, Hsiao KK, Kingsbury DT, Prusiner SB (1988) Scrapie-infected murine neuroblastoma cells produce protease-resistant prion proteins. *J Virol* 62(5):1558–1564
- Buzon MJ, Erkizia I, Pou C, Minuesa G, Puertas MC, Esteve A, Castello A, Santos JR, Prado JG, Izquierdo-Useros N, Pattery T, Van Houtte M, Carrasco L, Clotet B, Ruiz L, Martinez-Picado J (2012) A non-infectious cell-based phenotypic assay for the assessment of HIV-1 susceptibility to protease inhibitors. *J Antimicrob Chemother* 67:32–38
- Cabrera CM, Cobo F, Nieto A, Cortés JL, Montes RM, Catalina P, Concha A (2006) Identity tests: determination of cell line cross-contamination. *Cytotechnology* 51:45–50
- Capes-Davis A, Theodosopoulos G, Atkin I, Drexler HG, Kohara A, Macleod RA, Masters JR, Nakamura Y, Reid YA (2010) Check your cultures! A list of cross-contaminated or misidentified cell lines. *Int J Cancer* 127(1):1–8
- Centers for Disease Control and Prevention (2009a) *Biosafety in Microbiological and Biomedical Laboratories (BMBL)* 5th edn. <http://www.cdc.gov/biosafety/publications/bmbl5/>. Accessed 31 Jan 2014
- Centers for Disease Control and Prevention (2009b) Laboratory-acquired vaccinia virus infection, United States (Virginia) 2008. Morbidity and mortality weekly report, July 31. [http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5829a1.htm?s\\_cid=mm5829a1\\_e](http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5829a1.htm?s_cid=mm5829a1_e). Accessed 31 Jan 2014
- Chomel BB, Belotto A, Meslin FX (2007) Wildlife, exotic pets, and emerging zoonoses. *Emerg Infect Dis* 13(1):6–11
- Chong H, William S, Vile R (1998) A replication-competent retrovirus arising from a split-function packaging cell line was generated by recombination events between the vector, one of the packaging constructs, and endogenous retroviral sequences. *J Virol* 72:2663–2670
- Clavel F, Guetard D, Brun-Vezinet F, Chamaret S, Rey MA, Santos-Ferreira MO, Laurent AG, Dauguet C, Katlama C, Rouzioux C (1986) Isolation of a new human retrovirus from West African patients with AIDS. *Science* 233(4761):343–346
- Cobo F, Cortés JL, Cabrera C, Nieto A, Concha A (2007) Microbiological contamination in stem cell cultures. *Cell Biol Int* 31:991–995
- Cronier S, Laude H, Peyrin JM (2004) Prions can infect primary cultured neurons and astrocytes and promote neuronal cell death. *Proc Natl Acad Sci* 101(33):12271–12276
- Cunningham DA, Dos Santos Cruz GJ, Fernandez-Suarez XM, Whittam AJ, Herring C, Copeman L, Richards A, Langford GA (2004) Activation of primary porcine endothelial cells induces release of porcine endogenous retroviruses. *Transplantation* 77(7):1071–1079
- Dang-Tan T, Mahmud SM, Puntoni R, Franco EL (2004) Polio vaccines, Simian Virus 40, and human cancer: the epidemiologic evidence for a causal association. *Oncogene* 23(38):6535–6540
- Davidson WL, Hummeler K (1960) B virus infection in man. *Ann N Y Acad Sci* 85:970–979
- Delelis O, Lehmann-Che J, Saib A (2004) Foamy viruses – a world apart. *Curr Opin Microbiol* 7(4):400–406
- Denkers ND, Seelig DM, Telling GC, Hoover EA (2010) Aerosol and nasal transmission of chronic wasting disease in cervidized mice. *J Gen Virol* 91(6):1651–1658
- Dienstag JL, Davenport FM, McCollum RW, Hennessy AV, Klatskin G, Purcell RH (1976) Nonhuman primate-associated viral hepatitis type A. Serologic evidence of hepatitis A virus infection. *JAMA* 236(5):462–464
- Dom W, Brewer M, Baker SF, Feng C, Marinez-Sobrido L, Treanor J, Dewhurst S (2014) Use of bacteriophage particles displaying influenza virus hemagglutinin for the detection of hemagglutination-inhibition antibodies. *J Virol* 197:47–50

- Drexler HG, Uphoff CC (2000) Mycoplasma contamination of cell cultures. In: Sper RE (ed) *The encyclopedia of cell technology*. Wiley, New York, pp 609–627
- Dull T, Zuffery R, Kelly M, Mandel RJ, Nguyen M, Trono D, Naldini L (1998) A third-generation lentivirus vector with a conditional packaging system. *J Virol* 72:8463–8471
- Estep RD, Messaoudi I, Wong SW (2010) Simian herpesviruses and their risk to humans. *Vaccine* 28(Suppl 2):B78–B84
- European Commission (2000) Directive 2000/54/EC of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work. *Off J* 17.10.2000; L262/21
- European Commission (2004a) Regulation No. 726/2004 of the European Parliament and of the Council of 31 March 2004 laying down Community procedures for the authorization and supervision of medicinal products for human and veterinary use and establishing a European Medicines Agency. *Off J* 30.04.2004; L136/1
- European Commission (2004b) Directive 2004/23/EC of the European Parliament and of the Council of 31 March 2004 on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells. *Off J* 07.04.2004; L102/48
- European Commission (2009) Directive 2009/41/EC of the European Parliament and of the council of 6 May 2009 on the contained use of genetically modified micro-organisms. *Off J* 21.05.2009; L125/75
- European Medicines Agency (EMA) <http://www.ema.europa.eu/ema/>. Accessed 31 Jan 2014
- Food and Drug Administration (FDA). Centers for biologics evaluation and research. <http://www.fda.gov/>. Accessed 31 Jan 2014
- Goldsmith CS, Ksiazek TG, Rollin PE, Comer JA, Nicholson WL, Peret TCT, Erdman DD, Bellini WJ, Harcourt BH, Rota PA, Bhatnagar J, Bowen MD, Erickson BR, McMullan LK, Nichol ST, Shieh WJ, Paddock CD, Zaki SR (2013) Cell culture and electron microscopy for identifying viruses in diseases of unknown cause. *Emerg Infect Dis* 19(6):886–891
- Gugel EA, Sanders ME (1986) Correspondence. Needle-stick transmission of human colonic adenocarcinoma. *New Engl J Med* 315(23):1487
- Hahn BH, Shaw GM, De Cock KM, Sharp PM (2000) AIDS as a zoonosis: scientific and public health implications. *Science* 287(5453):607–614
- Hargreaves FD, Leach RH (1970) The influence of mycoplasma infection on the sensitivity of HeLa cells for growth of viruses. *J Med Microbiol* 3(2):259–265
- Hay RJ (1991) Operator-induced contamination in cell culture systems. *Dev Biol Stand* 75:193–204
- Haybaeck J, Heikenwalder M, Klevenz B, Schwarz P, Margalith I, Bridel C, Mertz K, Zirdum E, Petsch B, Fuchs TJ, Stitz L, Aguzzi A (2011) Aerosols transmit prions to immunocompetent and immunodeficient mice. *PLoS Pathog* 7(1):e1001257
- Herman P, Verlinden Y, Breyer D, Van Cleemput E, Brochier B, Sneyers M, Snacken R, Hermans P, Kerkhofs P, Liesnard C, Rombaut B, Van Ranst M, Van Der Groen G, Goubau P, Moens W (2004) Biosafety risk assessment of the severe acute respiratory syndrome (SARS) Coronavirus and containment measures for the diagnostic and research laboratories. *Appl Biosaf* 9(3):128–142
- Herwaldt BL (2001) Laboratory-acquired parasitic infections from accidental exposures. *Clin Microbiol Rev* 14(4):659–688
- Hinman AR, Fraser DW, Douglas RG, Bowen GS, Kraus AL, Winkler WG, Rhodes WW (1975) Outbreak of lymphocytic choriomeningitis virus infections in medical center personnel. *Am J Epidemiol* 101(2):103–110
- Hjelle B, Appenzeller O, Mills R, Alexander S, Torrez-Martinez N, Jahnke R, Ross GD (1992) Chronic neurodegenerative disease associated with HTLV-II infection. *Lancet* 339 (8794):645–646
- Hummeler K, Davidson WL, Herle W, LaBocetta AC, Ruch HG (1959) Encephalomyelitis due to infection with Herpesvirus simiae. *New Engl J Med* 261(2):64–68



- Hussain W, Moens N, Veraitch FS, Hernandez D, Mason C, Lye GJ (2013) Reproducible culture and differentiation of mouse embryonic stem cells using an automated microwell platform. *Biochem Eng J* 77(100):246–257
- ICH (1997) topic Q5A. Step 4 consensus guideline, quality of biotechnological products: viral safety evaluation of biotechnology products derived from cell lines of human or animal origin, CPMP/ICH/295/95. The European Agency for the Evaluation of Medicinal Products, Human medicines Evaluation Unit, Canary wharf
- Isaacs SN (2012) Working safely with vaccinia virus: laboratory technique and review of published cases of accidental laboratory infections. *Methods Mol Biol* 890:1–22
- Jäger W, Horiguchi Y, Shah J, Hayashi T, Awrey S, Gust KM, Hadaschik BA, Matsui Y, Anderson S, Bell RH, Ettinger S, So AI, Gleave ME, Lee IL, Dinney CP, Tachibana M, McConkey DJ, Black PC (2013) Hiding in plain view: genetic profiling reveals decades old cross contamination of bladder cancer cell line KU7 with HeLa. *J Urol* 190(4):1404–1409
- Johnen G, Rozynek P, von der Gathen Y, Bryk O, Zdrenka R, Johannes C, Weber DG, Igwilokuefuna OB, Raiko I, Hippler J, Bruning T, Dopp E (2013) Cross-contamination of a UROtsa stock with T24 cells—molecular comparison of different cell lines and stocks. *PLoS One* 8(5): e64139
- Jones L, Ristow S, Yilma T, Moss B (1986) Accidental human vaccination with vaccinia virus expressing nucleoprotein gene. *Nature* 319(6054):543
- Jonges M, Liu WM, van der Vries E, Jacobi R, Pronk I, Boog C, Koopmans M, Meijer A, Soethout E (2010) Influenza virus inactivation for studies of antigenicity and phenotypic neuraminidase inhibitor resistance profiling. *J Clin Microbiol* 48(3):928–940
- Kallio ER, Klingström J, Gustafsson E, Manni T, Vaheri A, Henttonen H, Vapalahti O, Lundkvist A (2006) Prolonged survival of Puumala hantavirus outside the host: evidence for indirect transmission via the environment. *J Gen Virol* 87:2127–2134
- Kalyanaraman VS, Sarngadharan MG, Robert-Guroff M, Miyoshi I, Golde D, Gallo RC (1982) A new subtype of human T-cell leukemia virus (HTLV-II) associated with a T-cell variant of hairy cell leukemia. *Science* 218(4572):571–573
- Kato R, Tejima D, Agata H, Asahina I, Okada K, Ueda M, Honda H, Kagami H (2010) A compact, automated cell culture system for clinical scale cell expansion from primary tissues. *Tissue Eng Part C Methods* 16(5):947–956
- Khabbaz RF, Heineine W, George JR, Parekh B, Rowe T, Woods T, Switzer WM, McClure HM, Murphey-Corb M, Folks TM (1994) Brief report, infections of a laboratory worker with simian immunodeficiency virus. *New Engl J Med* 330(3):172–178
- Kramer A, Schwebke I, Kampf G (2006) How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. *BMC Infect Dis* 6:130
- Ksiazek TG, Erdman D, Goldsmith CS, Zaki SR, Peret T, Emery S, Tong S, Urbani C, Comer JA, Lim W, Rollin PE, Dowell SF, Ling AE, Humphrey CD, Shieh WJ, Guarner J, Paddock CD, Rota P, Fields B, De Risi J, Yang JY, Cox N, Hughes JM, Le Duc JW, Bellini WJ, Anderson LJ (2003) A novel coronavirus associated with SARS. *New Engl J Med* 248(20):172–178
- Langdon SP (2004) Cell culture contamination: an overview. *Methods Mol Med* 88:309–317
- Lennartz K, Lu M, Flasshove M, Moritz T, Kirstein U (2005) Improving the biosafety of cell sorting by adaptation of a cell sorting system to a biosafety cabinet. *Cytometry* 66(2):119–127
- Levings RL, Wessman SJ (1991) Bovine viral diarrhea virus contamination of nutrient serum, cell cultures and viral vaccines. *Dev Biol Stand* 75:177–181
- Lewis FM, Chernak E, Goldman E, Li Y, Karem K, Damon IK, Henkel R, Newbern EC, Ross P, Johnson CC (2006) Ocular vaccinia infection in laboratory worker, Philadelphia, 2004. *Emerg Infect Dis* 12(1):134–137
- Likos AM, Sammons SA, Olson VA, Frace AM, Li Y, Olsen-Rasmussen M, Davidson W, Galloway R, Khristova ML, Reynolds MG, Zhao H, Carroll DS, Curns A, Formenty P, Esposito JJ, Regnery RL, Damon IK (2005) A tale of two clades: monkeypox viruses. *J Gen Virol* 86:2661–2672

- Linnen J, Wages J, Zhang-keck ZY, Fry KE, Krawczynski KZ, Alter H, Koonin E, Gallagher M, Alter M, Hadziyannis S, Karayiannis P, Fung K, Nakatsuji Y, Shih JW, Young L, Piatak M, Hoover C, Fernandez J, Chen S, Zou JC, Morris T, Hyams KC, Ismay S, Lifson JD, Hess G, Fong SK, Thomas H, Bradley D, Margolis H, Kim JP (1996) Molecular cloning and disease association of hepatitis G virus: a transfusion-transmissible agent. *Science* 27(5248):505–508
- Lloyd G, Jones N (1986) Infection of laboratory workers with hantavirus acquired from immunocytomas propagated in laboratory rats. *J Infect* 12(2):117–125
- Louz D, Bergmans HE, Loos BP, Hoeben RC (2005) Cross-species transfer of viruses: implications for the use of viral vectors in biomedical research, gene therapy and as live-virus vaccines. *J Gene Med* 7(10):1263–1274
- Lucey BP, Nelson-Rees WA, Hutchins GM (2009) Henrietta Lacks, HeLa cells, and cell culture contamination. *Arch Pathol Lab Method* 133(9):1463–1467
- Lusso P, Lori F, Gallo RC (1990) CD4-independent infection by human immunodeficiency virus type I after phenotypic mixing with human T-cell leukemia viruses. *J Virol* 64(12):6341–6344
- MacLeod RAF, Dirks WG, Drexler HG (2013) Where all the cell lines gone? *Int J Cancer* 132(5):1232–1234
- Mahy BW (1998) Zoonosis and haemorrhagic fever. *Develop Biol Standard* 93:31–36
- Mahy BW, Brown CC (2000) Emerging zoonoses: crossing the species barrier. *Rev Sci Tech* 19(1):33–40
- Mahy BW, Dykewicz C, Fisher-Hoch S, Ostroff S, Tipple M, Sanchez A (1991) Virus zoonoses and their potential for contamination of cell cultures. *Develop Biol Standard* 75:183–189
- Matsuo Y, Nishizaki C, Drexler HG (1999) Efficient DNA fingerprinting method for the identification of cross-culture contamination of cell lines. *Hum Cell* 12(3):149–154
- Mattiacio J, Walter S, Brewer M, Domm W, Friedman AE, Dewhurst S (2011) Dense display of HIV-1 envelope spikes on the lambda phage scaffold does not result in the generation of improved antibody response to HIV-1 Env. *Vaccine* 29(14):2637–2647
- McGarrity GJ (1976) Spread and control of mycoplasmal infection of cell cultures. *In Vitro* 12:643–648
- McGarrity GJ, Kotani H (1985) Cell culture mycoplasmas. In: Razin S, Barile MF (eds) *The mycoplasmas*, vol 4, *Mycoplasma pathogenicity*. Academic, Orlando
- Mempel M, Isa G, Klugbauer N, Meyer H, Wildi G, Ring J, Hofmann F, Hofmann H (2003) Laboratory acquired infection with recombinant vaccinia virus containing an immunomodulating construct. *J Investig Dermatol* 120(3):356–358
- Mirjalili A, Parmoor E, Moradi Bidhendi S, Sarkari B (2005) Microbial contamination of cell cultures: a 2 years study. *Biologicals* 33:81–85
- Moore PS, Gao SG, Dominguez E, Cesarman E, Lungu O, Knowles DM, Garber R, Pellett PE, McGeoch DJ, Chang Y (1996) Primary characterization of a herpesvirus agent associated with Kaposi's sarcoma. *J Virol* 70(1):549–558
- Moussatché N, Tuyama M, Kato SE, Castro AP, Njaine B, Peralta RH, Peralta JM, Damaso CR, Barroso PF (2003) Accidental infection of laboratory worker with vaccinia virus. *Emerg Infect Dis* 9(6):724–726
- National Institute of Health (1979) Laboratory safety monograph: a supplement to the NIH guidelines for recombinant DNA research. National Institute of Health, Public Health Service, U.S. Department of Health, Education and Welfare, pp 1–227
- Nishizawa T, Okamoto H, Konishi K, Yoshizawa H, Miyakawa Y, Mayumi M (1997) A Novel DNA virus (TTV) associated with elevated transaminase levels in posttransfusion hepatitis of unknown etiology. *Biochem Biophys Res Commun* 241:92–97
- O'Connell RC, Wittler RG, Faber JE (1964) Aerosols as a source of widespread mycoplasma contamination of tissue cultures. *Appl Microbiol* 12:337–342
- Openshaw PJ, Alwan WH, Cherrie AH, Record FM (1991) Accidental infections of laboratory worker with recombinant vaccinia virus. *Lancet* 338(8764):459

- Paddenberg R, Wulf S, Weber A, Heimann P, Beck LA, Mannherz HG (1996) Internucleosomal DNA fragmentation in cultured cells under conditions reported to induce apoptosis may be caused by mycoplasma endonucleases. *Eur J Cell Biol* 71(1):105–119
- Pauwels K, Herman P, Van Vaerenbergh B, Do Thi CD, Berghman L, Waeterloos G, Van Bockstaele D, Dorsch-Hasler K, Sneyers M (2007) Animal cell cultures: risk assessment and biosafety recommendations. *Appl Biosaf* 12(1):26–38
- Perfetto SP, Ambrozak DR, Nguyen R, Roederer M, Koup RA, Holmes KL (2011) Standard practice for cell sorting in a BSL-3 facility. In: Hawley TS, Hawley RG (eds) *Flow cytometry protocols*, vol 699, 3rd edn, *Methods in molecular biology*. Springer Science + Business Media LLC, Philadelphia, pp 449–469
- Peters CJ, Jahrling PB, Ksiazek TG, Johnson ED, Lupton HW (1992) Filovirus contamination of cell cultures. *Dev Biol Stand* 76:267–274
- Pinheiro de Oliveira TF, Fonseca AA, Camargos MF, de Oliveira AM, Pinto Cottorello AC, dos Reis Souza A, Garcia de Almeida I, Heinemann MB (2013) Detection of contaminants in cell cultures, sera and trypsin. *Biologicals* 41(6):407–414
- Poiesz BJ, Ruscetti FW, Gazdar AF, Bunn PA, Minna JD, Gallo RC (1980) Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. *Proc Natl Acad Sci U S A* 77(12):7415–7419
- Popovic M, Sarngadharan MG, Read E, Gallo RC (1984) Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. *Science* 224(4648):497–500
- Razin S, Tully JG (1995) *Molecular and diagnostic procedures in mycoplasmaology*, vol 1. Academic, San Diego, p 483
- Reperant LA, Kuiken T, Osterhaus AD (2012) Adaptive pathways of zoonotic influenza viruses: from exposure to establishment in humans. *Vaccine* 30(30):4419–4434
- Schmid I (2012) How to develop a standard operating procedure for sorting unfixed cells. *Methods* 57(3):392–397
- Schultz A, Koch S, Fuss M, Mazzotta AS, Sarzotti-Kelsoe M, Ozaki DA, Montefiori DC, von Briesen H, Zimmermann H, Meyerhans A (2012) An automated HIV-1 Env-pseudotyped virus production for global HIV vaccine trials. *PLoS One* 7(12):e51715
- Shou S, Hansen AK (2000) Marburg and Ebola virus infections in laboratory non-human primates: a literature review. *Comp Med* 50(2):108–123
- Simmonds P (2001) The origin and evolution of hepatitis viruses in humans. *J Gen Virol* 82:693–712
- Solassol J, Crozet C, Lehmann S (2003) Prion propagation in cultured cells. *Br Med Bull* 66:87–97
- Stang A, Petrasch-Parwez E, Brandt S, Dermietzl R, Meyer HE, Stühler K, Liffers ST, Überla K, Grunwald T (2009) Unintended spread of a biosafety level 2 recombinant retrovirus. *Retrovirology* 6(86):1–6
- Stitz L, Aguzzi A (2011) Aerosols: an underestimated vehicle for transmission of prion diseases? *Prion* 5(3):138–141
- Stoker AW, Hatier C, Bissell MJ (1990) The embryonic environment strongly attenuates v-src oncogenesis in mesenchymal and epithelial tissues, but not in endothelia. *J Cell Biol* 111(1):217–228
- Stürzl M, Gaus D, Dirks WG, Ganem D, Jochmann R (2013) Kaposi's sarcoma-derived cell line SLK is not of endothelial origin, but is a contaminant from a known renal carcinoma cell line. *Int J Cancer* 132(8):1954–1958
- Swiss Agency for the Environment, Forests and Landscape (2005) *Classification of organisms. Viruses* (Berne 2005), *Parasites* (Berne, 2003), *Fungi* (Berne, 2004), *Bactérie* (Berne, 2003). [http://www.umwelt-schweiz.ch/buwal/eng/fachgebiete/fg\\_biotechnologie/national/ouc/classi/](http://www.umwelt-schweiz.ch/buwal/eng/fachgebiete/fg_biotechnologie/national/ouc/classi/). Accessed 31 Jan 2014
- Swiss Expert Committee for Biosafety (2011) *Recommendation on the safe handling of human and animal cells and cell cultures*. (SECB) June 2011. <http://www.efbs.admin.ch/en/documentation/recommendations/index.html>. Accessed 31 Jan 2014

- Thomas R, Ratcliffe E (2012) Automated adherent human cell culture (mesenchymal stem cells). *Methods Mol Biol* 806:393–406
- Tomori O (2004) Yellow fever: the recurring plague. *Crit Rev Clin Lab Sci* 41(4):391–427
- van den Akker E, van der Vlugt CJB, Bleijs A, Bergmans HE (2013) Environmental risk assessment of replication competent viral vectors applied in clinical trials: potential effects of inserted sequences. *Curr Gene Ther* 13(6):395–412
- van den Hoogen BG, de Jong JC, Groen J, Kuiken T, de Groot R, Fouchier RA, Osterhaus AD (2001) A newly discovered human pneumovirus isolated from young children with respiratory tract disease. *Nat Med* 7(6):719–724
- van der Zeijst BA, Noyes BE, Mirault ME, Parker B, Osterhaus AD, Swyryd EA, Bleumink N, Horzinek MC, Stark GR (1983) Persistent infection of some standard cell lines by lymphocytic choriomeningitis virus: transmission of infection by an intracellular agent. *J Virol* 48(1):249–261
- Vilchez RA, Butel JS (2004) Emergent human pathogen simian virus 40 and its role in cancer. *Clin Microbiol Rev* 17(3):495–508
- Vilette D, Andreoletti O, Archer F, Madelaine MF, Vilotte JL, Lehmann S, Laude H (2001) Ex vivo propagation of infectious sheep scrapie agent in heterologous epithelial cells expressing ovine prion protein. *Proc Natl Acad Sci U S A* 98(7):4055–4059
- Vincent T, Pettersson RF, Crystal RG, Leopold PL (2004) Cytokine-mediated downregulation of coxsackievirus-adenovirus receptor in endothelial cells. *J Virol* 78(15):8047–8058
- Vorberg I, Raines A, Story B, Priola SA (2004) Susceptibility of common fibroblast cell lines to transmissible spongiform encephalopathy agents. *J Infect Dis* 189(3):31–39
- Walther BA, Ewald PW (2004) Pathogen survival in the external environment and the evolution of virulence. *Biol Rev* 79:849–869
- Weigler BJ (1992) Biology of B virus in macaque and human hosts, a review. *Clin Infect Dis* 14(2):555–567
- Whitley RJ (2001) Herpes simplex viruses. In: Knipe DM, Howley M, Griffin DE (eds) *Fields virology*, vol 2, 4th edn. Lippincott, Philadelphia, pp 2461–2509
- Wlodaver CG, Palumbo GJ, Waner JL (2004) Laboratory-acquired vaccinia infection. *J Clin Virol* 29(3):1740–1745
- World Health Organization (1998) WHO requirements for the use of animal cells as *in vitro* substrates for the production of biologicals. WHO technical report series, no. 878
- World Health Organisation (2004) Laboratory biosafety manual, 3rd edn. [http://www.who.int/csr/resources/publications/biosafety/WHO\\_CDS\\_CSR\\_LYO\\_2004\\_11/en/](http://www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/). Accessed January 2014
- World Organisation for Animal Health (2009) *Manuel of diagnostic tests and vaccines for terrestrial animals*. Chapter 1.1.2 biosafety and biosecurity in the veterinary microbiology laboratory and animal facilities. [http://web.oie.int/eng/normes/mmanual/2008/pdf/1.1.02\\_BIO\\_SAFETY.pdf](http://web.oie.int/eng/normes/mmanual/2008/pdf/1.1.02_BIO_SAFETY.pdf). Accessed 31 Jan 2014