



# Animal Cell Cultures: Risk Assessment and Biosafety Recommendations

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## Abstract

*During the last three decades, animal cell culturing has been essential for biomedical research and biotechnological activities in general. Along with this increasing importance, biosafety concerns have pointed to the risks of manipulating animal cell cultures for human health and the environment. A maximal reduction of these risks necessitates a thorough risk assessment of the cell cultures used. It involves an evaluation of both the intrinsic properties of the cell culture, including subsequent properties acquired as a result of genetic modification, and the possibility that the cell culture may inadvertently or deliberately become contaminated with pathogens. The latter is a major hazard associated with the manipulation of animal cell cultures, as adventitious agents may be pathogenic and have a better capacity to survive in unfavorable conditions. Consequently, most of the containment measures primarily aim at protecting cells from adventitious contamination. Therefore, a comprehensive evaluation of the risks encountered during the handling of cell cultures should include considerations regarding the type of manipulation as well. As a rule, cell cultures known to harbor an infectious etiologic agent should be manipulated in compliance with containment measures recommended for the etiologic agent. With the exception of very well-characterized cell cultures for which the use of a type II biosafety cabinet depends on the origin of the cells, work with cell cultures from human or primate origin should generally and minimally be performed under containment level 2 using a type II biosafety cabinet. In every case, containment measures should minimize adventitious contamination of the cell cultures and offer a maximal protection of human health and the environment.*

## Keywords

Risk assessment, animal cell cultures, contained use

## Introduction

The use of animal and human cell cultures has become very beneficial for diverse applications in the fields of biotechnology, medicine and veterinary. Originally used as substrates for the production of viral vaccines (Salk polio vaccine on monkey kidney cells, rabies, mumps and rubella vaccine using the WI-38 cell line), animal and human cell cultures became an indispensable tool to study intra- or intercellular responses and to serve as *in vitro* model for research. It has also been used for *in vitro* diagnosis of viruses or for the production of a wide range of biological products (hormones, interleukins, interferons and growth factors), including potential diagnostic and therapeutic products. More recently, cellular entity features associated to human and mammalian cell cultures have also gained interest in the search of new therapeutic approaches such as allo-/xeno- transplantation or cell-based gene therapy.

Along with the increasing importance of manipulating animal and human cell cultures *in vitro*, biosafety concerns have pointed to the risks with respect to human (as well as animal or plant) health and environmental considerations. A maximal reduction of these risks necessitates a thorough risk assessment of the given cell cultures, taking into account the type of manipulation and the implementation of appropriate containment measures.

Depending on the purpose or the type of activity, the use of animal cell cultures may fall within the scope of several regulatory provisions. In Europe, for example, as the manipulation of animal cell cultures may pose a risk related to the exposure of the worker to biological agents, this type of activity is covered by the European Directive 2000/54/EC. In many cases, tissue culture work will also involve the use of genetically-modified cell lines, in which case a risk assessment should be made in accordance with the provisions of the Directive 98/81/EC related to the contained use of genetically-modified organisms. Moreover, cell culturing activities aiming at manufacturing

biopharmaceuticals are covered by the Regulation (EC) No 726/2004 laying down procedures for the authorization and supervision of medicinal products for human and veterinary use, whereas activities that involve the use of human cells and tissues for application to the human body fall within the scope of the Directive 2004/23/EC which provides standards for the use of human cells and tissues in the category of cell therapy.

Since the use of cell cultures for therapeutic purposes addresses more extended considerations including quality, efficacy, safety, ethical, social and regulatory issues, it should be emphasized that the scope of this paper is limited to the risk assessment and risk management of diagnostic and research activities involving cell cultures. While biosafety recommendations (as outlined hereafter) are principally aimed at providing maximal protection of human health and environment, it is recognized that many of the precautionary measures would directly benefit the quality of research activities involving animal cell cultures as cross-contamination (Drexler et al., 1999) or inadvertent contamination with biologic agents are plaguing many researchers, often leading to inaccurate data, misinterpretation of results and a considerable waste of time and energy.

## Risk Assessment

Risk assessment of animal cell cultures is based on both the intrinsic properties of the cell culture—including subsequent properties acquired as a result of genetic modification—and the possibility that the cell culture may inadvertently or deliberately become contaminated with pathogens. In addition, the risks encountered during handling of animal cell cultures should be evaluated with a careful consideration of the type of manipulation.

## Deliberate Infection of Cell Cultures

Many biologic agents take advantage of a cell's machinery in order to survive or to complete their life cycles. Therefore, the study of a pathogens' life cycle or immunity escape mechanism may involve the deliberate infection of animal cell cultures. The determination of potential hazards related to infected cell cultures requires an examination of cell properties (as discussed below) and the inherent properties of the infecting pathogen. The latter implies an assessment of a number of criteria specific to the pathogen along with aspects such as the existence of effective therapies or prophylaxis. An evaluation of these criteria has been used to classify pathogens into classes of biological risk, also called Risk Groups. The four risk categories range from Risk Group 1, where a biologic agent is unlikely to cause human disease, to Risk Group 4, where the agent causes severe human disease and present serious hazard to workers with a potential of spreading to the community. Contrary to agents of lower

Risk Groups, there are usually no effective prophylaxes or treatments available for Group 4 Biological Agents. Some reference lists originating from international authorities are covering natural biological agents (not genetically modified) as well as the group under which they are classified (2000/54/EC, Switzerland) or the biosafety level under which these should be manipulated (BMBL). As a rule, the biological risk of infected cell cultures will depend on the biological risk of the infecting pathogen(s). For example, cell cultures deliberately infected with Hepatitis C virus (HCV) in order to produce virus particles are assigned to risk group 3, as HCV is a risk group 3 viral pathogen. Nevertheless, as discussed below, the appropriate level of containment to be adopted will also depend on the type of manipulation. Another 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 (schizonts) and induces host cell transformation and clonal expansion of the cell. These infected bovine leukocytes may be categorized under risk group 2, while the biosafety level (BSL 1 or 2) appropriate for handling is determined by the presence or absence of the infectious form of the parasites.

## Adventitious Contamination of Cell Cultures

Adventitious contamination of cell cultures is a major drawback for any activity that involves cell culturing (for a review see Langdon, 2004). In addition, one of the main biosafety concerns when manipulating animal cell cultures is the fact that animal cell cultures may provide a support for contaminating agents that cause harm to human health. Causative agents of cell contamination include bacteria, fungi, mycoplasmas, parasites, viruses, prions and even other animal cells.

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 color), slower growth of the cells and cell destruction. Antibiotics may be used to prevent cell 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 undetected for many passages and can change several cell properties such as growth, metabolism, morphology and genome structure (Paddenberg et al., 1996; McGarrity,

1985). It has also been reported to influence the yield of virus production in infected cells (Hargreaves et al., 1970). Hence, mycoplasmal contamination is also a biosafety concern, because some of the contaminating *Mycoplasma spp.* belongs 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. hyorhinae* (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. hyorhinae* are usually animal donors of tissues and biological constituents used for cell culture, e.g., calf serum and trypsin (Razin & Tully, 1995).

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 papilloma viruses. Although cell cultures from non-human origin may pose less risk, it should be emphasized that many viruses have a broad host range and can cross species barriers. Well-known viral contaminants of primate tissues or cells from non-human origin that can cause human disease are listed in Table 1. While contamination with some viruses may be associated with changes in cell morphology or behavior—such as the formation of syncytia (HIV, herpes viruses), swelling of cells (adenoviruses) or hemagglutination 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).

Adventitious contamination with parasites may be an issue when handling primary cell cultures 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 recognized that most

of the parasitic laboratory-acquired infections are caused by needle stick injuries (Herwaldt, 2001).

Finally, another class of agents that may contaminate cell cultures include unconventional agents that cause transmissible spongiform encephalopathies (TSE), the so-called prions (Solassol et al., 2003; Cronier et al., 2004; Vorberg et al., 2004). Neuroblastoma cell lines and primary cultured neurons and astrocytes have been shown to serve as hosts (Butler et al., 1988). 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. However, most recently, non-neuronal cells have been demonstrated to 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). Contrary to most of the infectious agents, TSE agents are resistant to most of the physical and chemical methods commonly used for decontamination of infectious agents and may form a matter of concern in case bovine-derived products are used as tissue culture supplements.

Animal cell cultures can also harbor pathogens which are not known or whose tropism has not been defined yet. For example, viruses that were not known until recently include 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).

Contamination of cell cultures is caused by different sources. Infected living organisms or infected cells or tissues from which a cell line has been established are the primary source of contamination. Contamination also occurs by the material used for cell culturing including glassware, storage bottles and pipettes due to improper maintenance or operation of sterilization of autoclaves. Presently, the use of disposable and sterile pipettes has considerably decreased the likelihood of adventitious contamination. In the past, the lip of the culture flask and the outside of the used pipette were found to be particularly heavily contaminated with mycoplasma agents (McGarrity et al., 1976). A third source of contamination resides in culture media and its components such as serum, basal cultural media, enzymes (trypsin, pronase and collagenase) and basic salt solutions. For example, media and additives derived from bovine sources are often contaminated with bovine viral diarrhoea virus (BVDV) (Levings et al., 1991). Finally, air supply, clothing, personnel and floor can be a source of airborne contamination (Hay et al., 1991).

### **Genetic Modification of Animal Cell Cultures**

The genetic engineering of animal cell cultures has benefited a number of applications in the biomedical and biotechnological fields. For instance, recombinant gene

expression in animal cells allows overproduction of biopharmaceuticals or biological products whose *in vivo* activity strongly depends on complex post-translational modifications of higher eukaryotic cells. Recombinant cells may also be chosen for their increased suitability or utility, i.e., for the replication of defective recombinant or wild type viruses.

The risk assessment of recombinant cells may be a complex task. Not only should properties that recombinant cells acquire following genetic modification be deter-

mined, an evaluation of each individual step in the process of genetic modification should be performed as well. 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. The purpose of a biological risk assessment is to define biological hazards in order to be able to eliminate or prevent risks to both human health and the environment. However, despite the fact that cell cultures may harbor pathogens and pose serious biological risks to human health (as

**Table 1**

Main viral contaminants of animal cell cultures or tissues that can cause human disease.

<b>Virus</b>	<b>References</b>
<b>In Human tissues</b>	
• Hepatitisviruses: HBV, HCV, HDV, HEV, HGV	Simmonds, P. (2001)
• Human Retroviruses: HIV-1, HIV-2, HTLV-1, HTLV-2	Popovic, M. et al. (1984); Clavel, F. et al., (1986); Poiesz, B. J. et al. (1980); Kalyanaraman, V. S. et al. (1982); Bhagavati, S. et al. (1988); Hjelle, B. et al. (1992)
• Herpesviruses: EBV, CMV, HHV-6, HSV-1, HSV- 2	Whitley, R. J. et al. (2001)
• Popovaviruses: different HPV sequences, no virus production	
<b>In Primate tissues</b>	
• Flaviviruses: Yellow Fever virus, Kyasanu forest Virus	Tomori, O. et al. (2004)
• Filoviruses: Marburg, Ebola	Shou, S., et al. (2000); Mahy, B. W. et al. (1991); Mahy, B. W. (1998); Peters, C. J. et al. (1992)
• Simian hemorrhagic virus	Mahy, B. W. (1998)
• Rabies virus	Brown, D. W. (1997)
• Hepatitis A virus	Dienstag, J. L. et al. (1976)
• Poliovirus	
• Herpesviruses (Herpes B Virus and others)	Davidson, W. L. et al. (1960); Weigler, B. J. (1992); Hummeler, U. et al. (1959)
• SV40 (non pathogenic for humans)	Vilchez, R. A. et al. (2004); Dang-Tan, T. et al. (2004)
• Simian Immunodeficiency virus (infection but also disease in humans?)	Hahn, B. H. et al. (2000); Khabbaz, R. F. et al. (1994)
• Monkeypox	Likos, A. M. et al. (2005)
• Simian Foamy virus	CDC, Delelis, O. et al. (2004)
<b>In Rodent tissues</b>	
• Lymphocytic Choriomeningitis virus (LCMV)	Mahy, B. W. et al. (1991); Hinman, A.R., et al. (1975); van der Zeijst, B. A. et al. (1983)
• Hantaan virus (hemorrhagic fever with renal syndrome)	Lloyd, G. et al. (1986); Mahy, B. W. (1998)
• Monkeypox	Likos, A. M. et al. (2005)

discussed above), it is unlikely that recombinant properties obtained by genetic modification may have an adverse effect upon release of the recombinant animal or human cells. Cells (genetically modified or not) have difficulties to survive in non-optimized conditions of growth, that is 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. Therefore, independent of the possibility that genetic modification could confer an expanded life-span, immortalization or increased capacity for tumor induction, the survival of such primary cells or cell lines outside of proper conditions is unlikely to occur.

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 (non-immunocompromised) host will recognize foreign cells and eventually destroy them. This is also one of the main reasons why the culturing of cells originating from the experimenter is not allowed for research and diagnostic activities.

Particular attention should be paid to the use of packaging cell lines. These are established cell lines which are deliberately and stably transfected with “helper constructs” to ensure the production of 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 viral vector but are crucial for viral replication. One of the most important 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 following transfection with viral vectors. Therefore the engineering of safer generations of retroviral packaging cell lines consists in minimizing the likelihood of generating replication-competent viruses by 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 is never completely ruled out.

Clearly, the risk group of the transfected packaging cell line will depend on the risk group of the retroviral 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 infec-

tivity, spectrum of host range, capacity of integration (insertional mutagenesis), stability and physiological role of the insert if expressed (for review see “Current Gene Therapy” Volume 3, Number 6).

### **Intrinsic Properties of Cell Cultures**

Good knowledge and characterization of the intrinsic properties of cells constitute the key to successful and safe culturing. With respect to the biological risks related to the manipulation of animal cell cultures, three properties intrinsic to cell cultures should be considered specifically while performing risk assessments.

First, the species of origin should be taken into account. Based on the fact that pathogens usually have specific species barriers, the closer the genetic relationship of the cell culture is to humans, the higher the risk is to humans. The incidence to harbor organisms that could cause harm to human health is higher in human or primate cells in general, compared to cells of non-human origin (Brown et al., 1997). This means that mammalian cells (other than human or primate cells) are considered to represent fewer risks, 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. 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 and SARS-associated coronavirus (SARS-CoV). Although increased growth, mobility and altered food patterns of the human population has been generally recognized as the main cause for the increased incidence of cross-species transfer of mammalian viruses, the occupational risks related to exposure to infected animal tissues or cell cultures should not be underestimated (Louz et al., 2005; Mahy et al., 2000).

Second, the cell type or type of tissue from which the cell lines are derived should be considered. Cell types dramatically differ in their *in vivo* half life: intestinal and certain white blood cells have a half-life of a few days, human red blood cells have approximately a 100-day half-life, and healthy liver cells rarely die, whereas, in adults, there is a slow loss of brain cells with little or no 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 which occur by culturing cells for a longer period. It is a known fact 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 cul-

tures 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 or being fully neoplastic upon accidental or deliberate introduction into the human body. Therefore, taking the tumorigenic potential into account, the following cell types may be ranked in decreasing order of risk: hematogenous (e.g., blood, lymphoid) cells and tissue, neural tissues, endothelium, gut mucosa, epithelial and fibroblast cells.

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). 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 characterize cell cultures. For this purpose, a number of techniques are available such as cytogenetic analysis, DNA fingerprinting, PCR, flow cytometry and isoenzymatic analysis.

Another 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 characterized by a finite life span, the time available for characterization 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, but for a great number of passages. These immortalized cells are obtained by isolating cells from tumors, by mutating primary cells with mutagens, by using viruses or recombinant DNA to generate indefinitely growing cells or by cell fusion of primary cells with a continuous cell line. Due to their increased life span, the time left for thorough characterization and detection of contaminating agents is considerably increased. Within this respect, well-characterized cell lines present the lowest risks compared to primary cultures or less characterized cell lines as the origin, the source and suitability are well-known and well-defined.

### Type of Manipulation

Apart from assessing the properties of the cell culture, risk assessment related to handling cell cultures necessitates an evaluation of the type of manipulation, because processes, methods and/or equipment involved

may increase or decrease the potential risks. For instance, though most of the established cell lines involved in large-scale operations are associated with low risks, the culturing of large volumes could give rise to new hazards due to inadequate containment measures. This may be the case for 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. On the other hand, there may be less risk associated with the manipulation of "high risk" cell cultures or cells known to harbor infectious agents once they are fixed by glutaraldehyde or formaldehyde/acetone for immunostaining.

The following procedures and/or manipulations should be considered among research and diagnostic activities involving the manipulation of animal cell cultures:

- Procedures generating aerosols: pipetting, vortexing, centrifugation, opening of wet caps, etc.;
- Handling cells outside of a type II BSC: flow cytometric analysis and sorting of cell populations constitute a special case of cell manipulation in which cells are handled outside of a biosafety cabinet. 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 FCM laboratories (Schmid et al., 1997). Some procedures and methods have also been described for assuring the sorting of cell material under optimal biosafety conditions (Perfetto et al., 2004; Lennartz et al., 2005).
- Altering culture conditions: Changing the availability of cell-specific nutrients, growth factors, signal molecules or adopting co-culture techniques may have significant effects on handling of 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 inevitable use of needles, sharps: due to injuries, cell material may be accidentally transferred directly to an operator's tissue and/or blood stream.
- Animal studies: major risks are self-inoculation (needle-stick injury) and exposure to aerosols.

Finally, the purpose of cell culturing should be taken

into consideration as many clinical approaches such as stem cell therapy, gene therapy, xeno- or allo-transplantation involve cell culturing *ex vivo* for therapeutic purposes. The latter clearly justifies more careful consideration regarding safety, ethical, social and regulatory issues, which cannot be addressed in this paper (FDA, EMEA, ICH, Stacey, 2005).

## Biosafety Recommendations and Containment Measures

The examination of biological risks related to animal cell cultures and the type of manipulation allows the determination of a necessary and sufficient containment level in order to protect human health and environment. The set-up and implementation of an appropriate containment level include a list of general and more specific work practices and containment measures. Table 2 lists precautionary measures that should be applied whenever handling animal cell cultures. Many of these measures basically aim at reducing the risk of contamination with adventitious agents by ensuring protection of both operator and cell culture. It should be emphasized that the most important measure relies upon an adequate training of the operator. It should limit the possibility of contamination by the operator, benefiting the safety, as well as the productivity and quality of work.

As a general rule, cell cultures known to harbor an infectious etiologic agent should be manipulated in com-

pliance with containment measures recommended for the etiologic agent. When cell cultures are not known to harbor 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-characterized cell lines or controlled cell sources for primary cells such as specified pathogen-free (SPF) animals. If no well-characterized 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 (refeeding 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 biosafety cabinet (BSC). If there is likelihood of the presence of adventitious agents of a higher risk group, the cell line should be handled under the appropriate containment level until tests have proven the absence of such organisms. Good documentation of the history of cell cultivation is mandatory.

The extent at which cell cultures should be con-

**Table 2**

Precautionary measures for handling cell cultures\*.

- respect good microbiological practices, especially those that are aimed at avoiding accidental contamination.
- 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.
- treat each new culture that is manipulated for the first time in the laboratory facility as potentially infectious.
- clean up any culture fluid spills immediately with a validated disinfectant.
- work with one cell line at a time and disinfect the work surfaces between two handlings involving cell lines.
- aliquot growth medium so that the same vessel is not used for more than one cell line.
- avoid pouring actions, which are a potential source of cross-contamination.
- proceed to an adequate use of the biosafety cabinet, this is turn on for a period before and after use, thoroughly disinfect BSC surfaces after each work session and do not clutter the BSC with materials.
- restrict the use of antibiotics in growth media.
- quarantine new cell cultures to a dedicated BSC or separate laboratory until the culture has been shown negative in appropriate tests.
- carry out a quality control of cells demonstrating the absence of likely contaminating pathogens on a regular basis or whenever necessary.
- handle cell cultures from undefined sources as risk group 2 agents. If there is a reasonable likelihood of adventitious agents of higher risk class, the cell line should be handled under appropriate containment level until tests have proven safety.

\*Adapted from Doblhoff-Dier, O., Stacey, G., et al., 2000.

trolled on the likelihood of contaminants strongly depends on the nature of activity. The World Health Organization and regulatory authorities, both in United States and in Europe, have formulated guidelines designed to minimize any potential risk for transmission of infectious agents in case animal cell cultures are used for the industrial production of biological with therapeutic purposes (EMEA, FDA, ICH, WHO). It includes extensive testing of the cell banks, the unpurified bulk material, as well as the final product with particular attention for viral clearance processes (Darling, 2002). 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. It is recognized that there is no single test suitable for detecting all possible contaminants. Therefore, the choice of detection technique depends on the contaminating pathogen and often a combination of methods is necessary to enhance detection in important samples such as master cell banks. Table 3 gives an overview of the use, detection capability and limitations of assays that might be used to detect and/or identify adventitious agents.

Work with cell cultures from human or primate origin should generally be performed under BSL2 conditions. Containment level 1 may be considered if all manipulations occur in a Type II biosafety cabinet and the

**Table 3**

Use, detection capability, and limitations of assays that can be used to detect adventitious agents.

Test	Detection capability	Advantage	Disadvantage
<b>Direct detection</b>			
· Electron microscopy studies	· Virus and virus-like particles		· Low sensitivity
· Sterility tests (a)			
<b>Indirect detection</b>			
· infectivity assays with sensitive or indicator cell lines (b)		· Capability of screening a broad range of viruses	· Detects only <b>infectious</b> viruses and those that result in CPE, HA and HAD = not appropriate to detect retroviruses
· <i>in vivo</i> tests (c)		· Highly sensitive	· Expensive and time consuming
<b>Immunodetection methods</b>			
· Mouse and hamster antibody production test (MAP, RAP and HAP) (d)	· Contaminants of rodent cells.		· Use of living animals, time consuming, some viral antigens may not lead to antibody production
· Immunofluorescence and enzyme immunoassays.	· Non-cytopathic viruses (like BVDV).		
<b>Molecular detection methods</b>			
· PCR	· Broad range of pathogens.	· Specific and powerful test	· Cross-reaction of primer sequences with cell specific DNA; does not indicate whether virus is infectious
· Reverse transcriptase detection (e)	· Retroviruses		· Only detects enzymes with optimal activity under preferred conditions, interpretation may be difficult due to presence of cellular enzymes

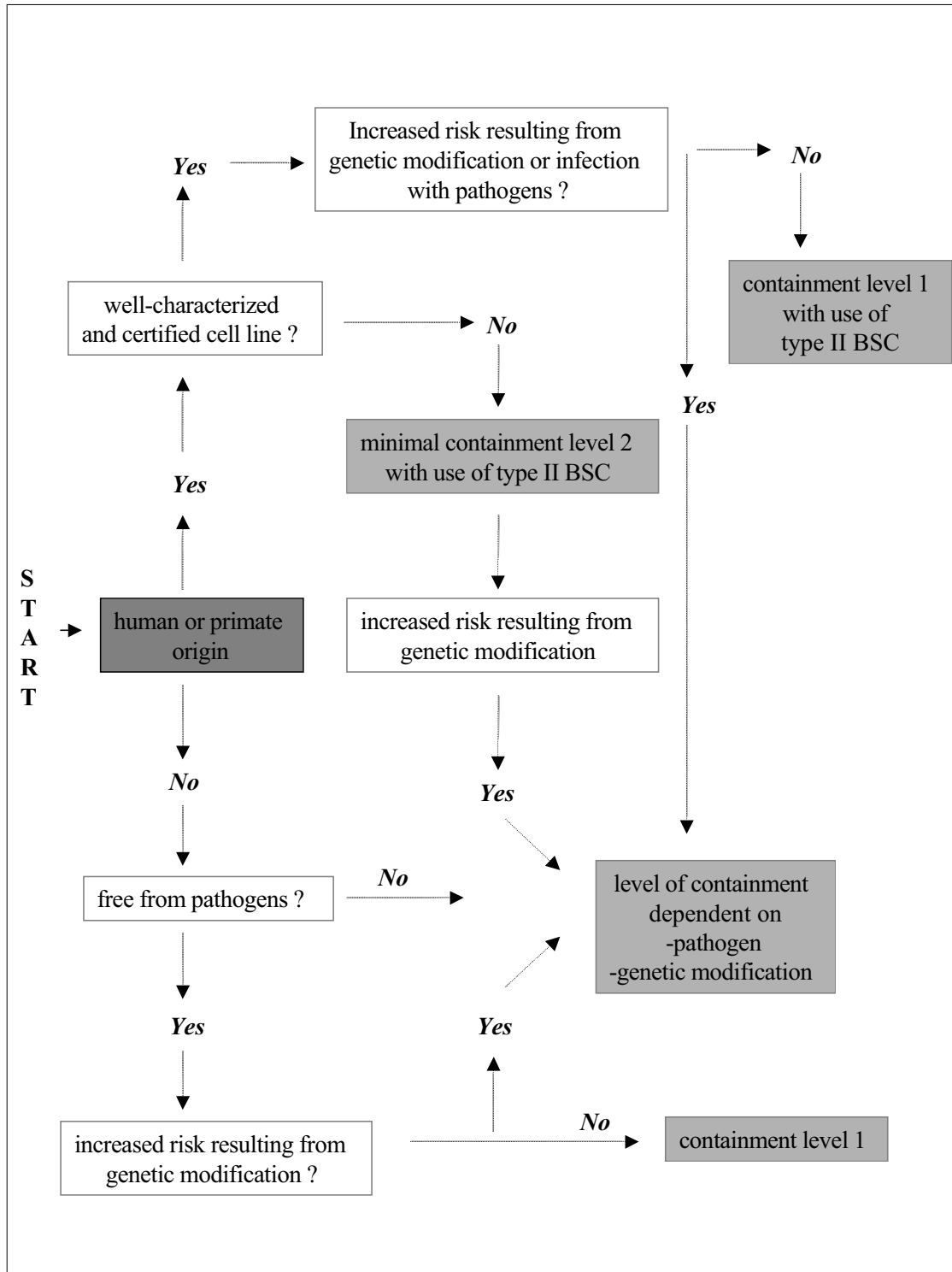
- (a) Samples are directly transferred on mycoplasma broth or rich broth and incubated at room temperature and at 37°C. Samples are then screened on signs of microbial growth.
- (b) Detector cells are observed for cytopathic effects (CPE), hemadsorption (HA) or hemagglutination (HAD).
- (c) inoculation of lysate of cells in live animals
- (d) the lysate of cells and their culture medium are inoculated into SPF mice, rats or hamsters; the developing of antibody response reveals the presence of a virus
- (e) based on activity test of the reverse transcriptase

cell culture is a well-characterized and certified cell line that presents no increased risk resulting from genetic modification or contaminating pathogen. However, as the implementation of good laboratory practices and the

use of a BSC is usually the norm in most laboratories dealing with cell culturing, those laboratories can be upgraded to BSL 2 facilities by implementing a restricted number of simple additional safety measures. As biosafety

**Figure 1**

Guidance for the assignment of the containment level.



measures aim at providing a maximal protection of human health and environment, it should be emphasized that horizontal laminar air flows and clean benches offer no protection for the manipulator or the environment, though it may guarantee protection of cell cultures from adventitious contamination. Therefore the 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, Figure 1 presents a schematic guidance for the assignment of an appropriate containment level when manipulating cell cultures. This flowchart is only indicative and should be applied and/or reconsidered according to case specific conditions and risk assessments.

## Conclusions

Bearing in mind the restricted survival capacity of animal cell cultures in non-optimized conditions of growth and recognising that many cell lines have a long history of safe use, it may seem unlikely that these cells may cause harm to humans, animals or plants. Actually, the main hazard associated with the manipulation of cell cultures resides in the fact that they may harbor adventitious agents, which are often hard to detect and, hence, less controllable. In contrast to their host cells, adventitious agents may have the capacity to survive in more hostile conditions and may present risks for human health or the environment in case they are pathogenic. Consequently, a risk assessment of cell cultures will often lead to a risk assessment of the potential adventitious contaminants; the agents used for immortalization of the cells (viruses, viral sequences, etc.) and/or the agents that have been used for deliberate infection of these. Therefore, though the assignment of containment requirements cannot be generalized and should be performed on a case-by-case basis, it is recognized that most of the containment measures primarily aim at protecting cells from adventitious contamination in order to minimize potential risks for the manipulator.

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