STANDARD MATERIALS

Quality assessment of biobank biological materials and reference specimens

Tina Lombardo[1], Antonio Lavazza*[2], Guerino Lombardi[3], Giorgio Varisco[4], Stefano Cinotti[4], Nadia Andrea Andreani[1], Riccardo Villa[1], Maura Ferrari[1]

Abstract

Biobanks play a pivotal role in scientific progress and public health development as they can make available high-quality biological samples and provide access to associated data that are otherwise difficult to find for scientists in both human and veterinary medicine. The majority of relevant studies on epidemiology, pathogenesis, diagnosis and prevention of infectious diseases are based on obtaining biological specimens collected over long-term sampling. Moreover, the storage of specimens in biorepositories also offers the possibility of further evaluating samples with "next-generation" technologies that may not be available when the samples were originally collected. Furthermore, recent advances in molecular biology and genetics have increased the demand for properly preserved specimens and all relevant associated data on a large scale. Nevertheless, it is difficult to obtain samples with well-known features, except for those that are received from certified centres. The quality control assessment procedures used to evaluate the samples stored in the Biobank of Veterinary Resources (BVR) at the Lombardy and Emilia-Romagna Experimental Zooprophylactic Institute (IZSLER), have made it possible to ensure standard features and have improved the information related to stored biological materials.

Keywords

- Biobank
- Biological material
- Quality control
- One Health
- Veterinary medicine

[1] IZSLER, OIE Collaborating Centre for Veterinary Biologicals Biobank, 25124, Brescia, Italy.

[2] IZSLER, OIE Reference Laboratory for Rabbit Myxomatosis, 25124, Brescia, Italy.

[3] IZSLER, National Reference Centre for Animal Care, 25124, Brescia, Italy.

[4] IZSLER, 25124, Brescia, Italy.

* Corresponding author : antonio.lavazza@izsler.it

STANDARD MATERIALS

Introduction

About 75% of emerging and re-emerging human disease outbreaks throughout the world over the past two decades have been caused by pathogens of zoonotic origin (Hinchliffe, 2015). A wide range of animal species, both wild and domestic, could be reservoirs for these pathogens, which may be viruses, bacteria or parasites. Furthermore, increasing globalisation, livestock, pets and wildlife, as well as international trade and travel contribute to the spread of pathogens, and global warming is favouring the transmission of vector-borne diseases (Balogun *et al.*, 2016). Within this context, a transnational approach to zoonosis prevention and control programmes is required. In developing countries, contact between humans and animal populations in the surrounding environment is particularly close. Human health and animal health are inextricably linked; nevertheless, there is still separation between the human and animal health sectors (Rabinowitz and Conti, 2013). In order to overcome this gap, a global integrative concept, often referred to as “One Health”, has been developed and strongly endorsed in the last few decades, reflecting the need for collaboration in the field of surveillance (Capps and Lederman, 2015; Scotch *et al.*, 2009).

Biobanking is an essential tool for ensuring easy availability of high-quality biomaterial collections that include essential samples and their associated data that are otherwise difficult for researchers to access (Zielhuis, 2012). In fact, supply of biological resources with poorly described features and stored in inadequate conditions is a recurring problem because samples may lack the required quality for research purposes (Carter and Betsou, 2011). Furthermore, an increasing number of test methods rely on the use of certified, stable and validated biological materials. In order to overcome these problems and meet modern requirements for biological materials, the Organisation for Economic Co-operation and Development (OECD, 2001) introduced a new concept of repositories of high-quality samples and information. Subsequently, to address these issues, the OECD (2007) published the *Best Practice Guidelines for Biological Resource Centres* (BRCs) and developed the document *Best Practice Guidelines for the Microorganism Domain*, with the purpose of ensuring that microorganisms held and supplied by BRCs meet high standards and are authentic. Furthermore, the World Health Organization (WHO, 2010) published the *Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks*, to provide guidance to National Regulatory Authorities and National Control Laboratories and manufacturers on the basic principles and procedures for the characterisation of animal cells that are to be used in the manufacture of biological products. More recently, the World Organisation for Animal Health (OIE) provided guidelines on the preparation, validation and distribution of antibodies as International Reference Standards for antibody assays for infectious diseases of animals. Such standard preparations are designated by the OIE as primary reference standards for tests described in the *OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*.

The field of veterinary research is rapidly evolving with new technologies and new standards. The European Technology Platform for Global Animal Health (ETPGAH) has identified the lack of biological material as one of the main gaps in the development of new effective tools for the control and prevention of animal diseases. Biobanks play a pivotal role in improving epidemiological research, which relies on the availability and quality of samples and the associated data. In particular, for retrospective studies and longitudinal designs for evaluating the course of diseases, the requirements for obtaining time-specific data are even stronger. Furthermore, biological materials are an essential resource for genomic research. Significant research is being carried out in genomics to improve efficiency of selection for healthier animals with disease-resistance properties.

As the need to access high-quality materials has increased at the global level, within the Biobank of Veterinary Resources (BVR) of the IZSLER, we have established a panel of quality tests to evaluate the features of the stored samples. These assays are specific for the

STANDARD MATERIALS

different biological resources that are stored to be used as reference materials for research and other purposes. All these tests are performed in compliance with the UNI CEI EN ISO/IEC 17025 Standard and the biobank infrastructure is certified ISO 9001:2008. The migration to ISO 9001:2015 is currently in progress. The purpose of this paper is to briefly describe the quality controls performed on the major requested resources at the BVR, including cell cultures, bacteria, mycoplasmas and viruses, and to present various practical considerations for proper storage of biological materials.

1. Cell cultures

Cell lines are critical components of experiments and should be considered as standard reagents for research like other commercial laboratory products (Hughes *et al.*, 2007). In fact, cell line misidentification and contamination with microorganisms (such as bacteria, mycoplasmas, fungi and viruses), together with both genetic and phenotypic instability, are among the recurrent problems that can arise in cell culture laboratories. Contamination with microorganisms is quite simple to detect and is well regulated from the normative point of view. On the contrary, in the past, cell line authentication was not considered a real concern by the scientific community and it was not routinely performed.

As reported by Nardone (2007), misidentification and inter- and intra-specific cross-contamination of cell cultures represent a frequent and widespread problem with an estimated incidence of 18–30% cross-contaminated continuous cell lines, that makes scientific results unreliable and jeopardises the validity of data in literature (Hughes *et al.*, 2007; Parodi *et al.*, 2002). Frequently, the invading cells are better adapted to the culture conditions and grow faster than the original cells. Because of the morphological similarities of different cell lines, it is impossible to rely only on microscopic observations to screen for cross-contamination. In fact, with the progress made in karyotyping methods, it became apparent that about one third of all cell lines used in research were misidentified (Reid and Mintzer, 2012). Human cell lines are most frequently contaminated by HeLa cells, but also by a number of other rapidly-growing cell lines (Kniss and Summerfield, 2014). Nowadays, authentication testing should be considered an essential part of good cell culture practice to assure researchers that the cell line used is a valid experimental model (Capes-Davis *et al.*, 2010). In addition to authentication, there are other issues that should be considered when assessing the quality of a cell line (Almeida *et al.*, 2016). For these reasons, all cell types in the BVR undergo quality testing in order to evaluate the suitability of their features for their purpose. These controls are carried out using different techniques, including cell characterisation, authentication and microbiological testing.

■ 1.1 Characterisation and authentication

Cell characterisation includes viability tests that are performed on all cell cultures before freezing and after thawing, in order to evaluate cryopreservation efficiency. Furthermore, trypan blue staining is usually performed along with cell culture proliferation. Several methods for the authentication of cell lines have been developed for the detection of inter- and intra-species cross-contamination (Reid, 2011). Isoenzyme profiling is the method suggested by the European Pharmacopoeia to detect interspecies cross-contamination; this analysis uses polymorphic enzymes that can be visualised as electrophoretic variants, giving rise to a specific pattern for each species (Nims *et al.*, 1998). This assay, if applied routinely in cell culture management, can greatly improve the detection of cellular cross-contamination. Furthermore, karyotyping can be used alone or to complement isoenzyme analysis, in order to reveal interspecies genetic differences to distinguish between cell lines with characteristic karyotypes (Ono *et al.*, 2007). Additionally, profiling of short tandem repeat (STR, also called genomic microsatellite) polymorphisms has been adopted for forensic work and by major repositories for the detection of intra-species differences in human cell lines and tissues (O'Brian, 2001; Reid and Mintzer, 2012).

STANDARD MATERIALS

■ 1.2 Microbiological quality controls

The most significant risk in cell culture laboratories is contamination by several microorganisms that include bacteria, fungi, yeasts, mycoplasmas, and endogenous and exogenous viruses. Contaminating microorganisms can be present in the sample tissue or organ from which the cells were derived. They can also be transferred with animal reagents or unintentionally introduced into the manufacturing process by inadequate laboratory practices.

Bacteria, yeasts and fungi contamination

Currently, specific tests for the detection of bacteria, yeasts and fungi are used as part of routine and regular quality control screening procedures for biological samples. These tests are usually performed in aseptic conditions to avoid interfering contaminations. Bacterial contamination in cell cultures is frequently evident to the naked eye, showing as sudden increasing turbidity and colour change of the culture medium, due to pH variation. Daily observation of cultures ensures early detection of contaminants and helps to prevent contamination of other cultures. Nevertheless, to detect low levels of contamination, samples from the cell cultures or supernatants should be inoculated either in liquid Tryptic Soy Broth (TSB) for the detection of aerobes, facultative anaerobes and fungi, Fluid Thioglycollate Medium (FTM) for the detection of aerobes and anaerobes, or onto solid growth media (Trypticase-Soya agar, Blood agar, Sabouraud's Dextrose agar and Malt Extract agar). These inoculated media are incubated for different amounts of time and at different temperatures (generally 25°C or 37°C), depending on the optimal conditions required for pathogen growth and depending on the testing standards used.

Although these conventional microbiological techniques are in routine, widespread use as standard sterility tests, they are based on inoculation of broth cultures that may not support the growth of all contaminating microorganisms. Alternative molecular methods, such as identification by PCR and DNA sequencing of ribosomal RNA may be used.

Mycoplasma **contamination**

Mycoplasmas have long been recognised as common contaminants of cells in continuous culture, but their presence could go unnoticed for months and even years. In fact, even though many *Mycoplasma* species produce severe cytopathic effects, others may cause very little evident morphological modification of the cultured cells (Drexler and Uphoff, 2002). As mycoplasma competes with the cells for the nutrients in the culture medium, typical signs of contamination consist of a reduction in the cell proliferation rate and changes in cellular physiology including gene expression, metabolism and phenotype (Nübling *et al.*, 2015). Even though these multiple effects do not affect the various cells in the same manner and to the same extent, mycoplasma contamination significantly impacts all cell cultures in terms of quality and safety, and may affect the scientific results of cell culture-based research, as well as the quality of biologics manufactured by cell culture in the biopharmaceutical industry (Armstrong *et al.*, 2010; Knezevic *et al.*, 2010; Laborde *et al.*, 2010). *Mycoplasma*-infected cell lines are themselves the most important source for further spreading of the contamination. Consequently, it is essential for all cell stocks and all new cultures entering a laboratory to be routinely tested for the presence of mycoplasmas. The most frequent contaminants of the bovine group of mycoplasmas are *Mycoplasma arginini* and *Acholeplasma laidlawii*: these species have a relatively wide host range as they have been isolated from cattle, sheep, and goats, and from a variety of other mammals, as well as from birds and insects (Drexler and Uphoff, 2002). In the past, these cell culture contaminants derived from bovine sera that were not routinely screened for mycoplasmas. *Mycoplasma orale* is the most frequent mycoplasma of human origin and it can be transmitted by the personnel if good laboratory practices are not followed. *Mycoplasma hyorhinis*, a common inhabitant of the nasal cavity of swine, also accounts for a high proportion of the contaminations (Drexler and Uphoff, 2002). Nowadays, several methods are available for the detection of mycoplasmas, including isolation on selective

STANDARD MATERIALS

microbiological growth media, direct or indirect fluorescent staining, ELISA, immunostaining, and PCR-based techniques (Pisal *et al.*, 2016). In the past, culture in agar was considered the gold standard assay, but some "difficult" species of *Mycoplasma*, which require specialised culture conditions, can be missed.

The tests established for these organisms include: broth/agar culture, assays for mycoplasma-characteristic enzyme activities, and DNA staining. Currently, mycoplasmas are tested for in all cell cultures stored in the BVR by indirect staining with a fluorescent dye such as Hoechst 33258 which binds DNA. Among the wide variety of techniques that have been developed to detect *Mycoplasma* contamination of cell cultures, Uphoff and Drexler (2013) recommended PCR analysis, as it is considered the most reliable and useful detection method. Most primers use highly conserved sequences, attempting to detect a broad range of *Mycoplasma* species. On the contrary, direct staining of cultures is not recommended, as it often yields unclear results and will only reliably detect heavily contaminated cultures (Young *et al.*, 2010). Furthermore, the presence of *Mycoplasma* infection could be evaluated by several biochemical tests that detect mycoplasmal toxicity or enzymes.

Many of these methods are used in several commercial kits and are specifically able to detect viable organisms. By measuring the level of ATP in a sample, both before and after the addition of the substrate, a ratio can be obtained that is indicative of the presence or absence of mycoplasmas. This is measured indirectly with a luminometer, recording biolumination catalysed by the reaction of the ATP and luciferase.

Viral contamination

The risk of viral contamination is a common feature to all biologicals, whose production involves the use of reagents of animal or human origin. Viral contamination of cell cultures may arise from the source material (cell banks of animal origin, human or animal tissues that may contain endogenous viruses) or as adventitious (exogenous) agents introduced by laboratory handling or during the production process (Merten, 2002). The animal-derived materials used for the growth of the cells, such as animal sera, or for detaching cells, such as porcine trypsin, are of particular concern as many different animal viruses can potentially be present (Chen *et al.*, 2008). Their presence could influence the biology of cells in a significant way, as amongst other effects they may modify the transcription factor networks and change the susceptibility of these cells to infection by other viruses. Viral contamination can be evaluated by a panel of tests to detect pathogens, other endogenous viruses (such as retroviruses) and adventitious viruses. Usually, this panel of tests includes: electronic microscopy investigation for the observation of endogenous viruses, reverse transcriptase (RT) detection (as a general test for retroviruses), and indirect techniques such as immunofluorescence. Further tests can also be performed to find specific suspected agents, depending on the animal species of the sample and on the origin of the biological products used in the cultures.

In addition, the presence of suspected infectious adventitious viruses is investigated by *in vitro* direct methods: cell samples are co-cultured with susceptible cell lines (indicator cells) capable of detecting a wide range of viruses. As reported by Schiff (2005), a minimum of three cell lines that include a human diploid cell line (MRC-5), a monkey kidney cell line (Vero), and a cell type of the same species and tissue of origin are usually selected. After inoculation, cultures are incubated at 37°C in 5% CO2 for 5-7 days and observed daily for the potential cytopathic effect of several viruses. Virus detection by testing the inoculated cell culture for haemadsorption and/or haemagglutination at the end of the examination period is necessary for viruses with no observable cytopathic effect. At present, cell cultures can also be tested for a panel of potential adventitious viruses by PCR or real-time PCR analysis.

STANDARD MATERIALS

2. Bacteria

The majority of bacterial strains stored in the Biobank of IZSLER are isolated during laboratory diagnostic activities on field-biological samples originating from farm animals, pets, and wildlife or isolated from feed and food of animal origin. Microorganism identification, including bacterial classification and pathogen detection, is essential for the correct diagnosis of diseases, the possible treatment of the infection, and the epidemiological investigation of outbreaks associated with microbial infections. Bacterial strains are identified either by phenotypic or genotypic tests. Phenotypic testing consists of a preliminary analysis to check the taxonomic identity of the isolated strain that often involves one or more phenotypic techniques, including the study of the biochemical profiles and metabolic properties of a microorganism by testing its growth requirements and enzymatic activities. Phenotypic identification methods are suitable for microorganisms with well-established growth parameters, and physiological and biochemical profiles. The biochemical tests are performed in specific growth media, compounds or growth conditions to stimulate an observable or measurable biochemical response of the microorganism, thereby enabling its identification and characterisation. Several commercial kits for biochemical tests like the API or the Vitek systems are currently available for rapid identification of microorganisms. Furthermore, the analysis of morphological traits can be performed to obtain an initial identification of a microorganism by routine techniques such as culture tests in specific culture media, and subsequent microscopic observation. Morphological properties include: shape, size, surface characteristics and pigmentation, cell wall characteristics (Gram-staining), sporulation characteristics, mechanisms of motility, and other cellular characteristics. Genotypic tests, mainly based on PCR, are carried out to detect genes of virulence. Moreover, 16S rRNA gene sequence analysis can be used to confirm the bacterial species (Iraola *et al.*, 2016). The development of molecular methods has greatly improved the ability to rapidly detect, identify and classify bacteria and also establish the taxonomic relationship among closely related genera and species. Identification, using molecular methods, relies on the comparison of the nucleic acid sequences (DNA, RNA) of a microorganism with documented data on known organisms. These methods are considered sensitive enough to allow detection of low concentrations of viable or non-viable microorganisms in both pure cultures and complex samples. Real-time PCR has proven useful for distinguishing specific sequences from a complex mixture of DNA. More recently, genome studies have been performed to characterise organisms. In the future, further information gained from complete profiling investigations of the transcriptome, proteome and metabolome may be available. Several modern technologies such as microarray analysis are increasingly used for the characterisation of microorganisms, with particular reference to some genotypic characteristics, including virulence determinants and antimicrobial resistance patterns (El-Adawy *et al.*, 2016). Finally, each isolated and propagated strain is subjected to quality controls for viability and purity by culture and microscopic investigation before biobanking. Each batch is tested again for viability after preservation and then, at regular intervals, for viability and compliance with the expected features

3. Mycoplasmas

The *Mycoplasma* genus includes several human and animal pathogens. Mycoplasmas are the smallest (0.2-0.8 μm in diameter) and simplest prokaryotes that lack a cell wall; the flexibility of their cell membrane allows them to pass through commonly used anti-bacteriological filters with diameters of 0.45 μm. These small bacteria depend on their hosts for many nutrients, due to their limited biosynthetic capabilities. The majority of the strains stored in the biobank of IZSLER are isolated from different animal species and selected among the pathogens of veterinary interest. Molecular assays are performed directly on the tissue or diagnostic sample. If the culture fails and *Mycoplasma* infection is suspected, the identification of the strain can be done by sequencing the PCR product. Once isolated from the field samples, these strains are classified for their morphology and growth behaviour, and purified by cloning. They are iso-

STANDARD MATERIALS

lated in liquid and/or on solid media and identified by biochemical and genetic methods. Colony cloning of the isolates is performed in order to evaluate their viability before the storage and to obtain the necessary amount of culture, to prepare the necessary number of lyophilised vials. Biochemical and/or molecular tests, including sequencing, are adopted as standardised methods. Furthermore, all strains provided for biobanking deposit are tested for viability and purity by sub-culturing, after time intervals planned for the different strains.

4. Viruses

Viruses are obligate intracellular parasites that require living systems for their replication. Viral culturing is an amplification method that increases the amount of the pathogen, facilitating detection and characterisation. Culture methods allow the detection of many different viruses, including some that are not suspected when the culture is established, and can provide an isolate of viable virus that can be further characterised and stored for future studies. Both reference and field viruses isolated from several animal species are stored in the biobank of IZSLER. Several methods can be used to detect and identify viruses for diagnostic purposes and are mainly based on serology tests and end-point or real-time PCR. These assays amplify specific viral genome sequences known to be characteristic of a virus with a nucleotide sequence available in database collections. In addition, two types of electron microscopy methods are available for viral particle detection: direct or immuno-electron microscopy. With direct methods, negative staining is most often used and the specimens may be used directly or the virus particles may be concentrated before negative staining, to increase the sensitivity of the detection level. Immuno-electron microscopy methods may be particularly useful for viral identification and classification if the number of viral particles present is small. Furthermore, cell-associated viruses can be isolated from several types of samples and grown in adherent or suspension cell cultures or chorio-allantoic membranes of embryonated hen's eggs. The main principle for isolating viruses is to choose the most suitable cell line and subsequently carry out several amplification passages to increase the virus titre, in order to produce the "master samples" and the "working samples". These batches are tested for potential microbiological contamination by microbiology, virology, serology and molecular biology methods. Contamination may take place at various steps of the manufacturing process, including the starting sample, the amplification procedure itself (through biological reagents and media), or during inadequate laboratory handling. Currently, contamination by extraneous viruses is verified through several assays based on molecular biology techniques. Bacterial contamination is verified through the inoculation of non-selective culture media, and the absence of *Mycoplasma spp.* in the final stock is assessed by real-time PCR, as also reported for cell cultures.

5. Storage of biological storage

Proper storage of the biobank resources includes the use of cryovials and labelling systems that will withstand the storage conditions: vessels and labels are selected for extended storage periods. Depending on specimen features, the intended use, and the estimated length of storage, bio-specimens are usually stored at −80°C, at −196°C (liquid nitrogen) or at –189°C (vapour phase nitrogen). Otherwise, microorganisms and viruses can undergo a lyophilisation process that ensures the viability of freeze-dried samples for extended periods at 4°C or at –20°C. Temperature represents a potential risk in the storage process and is one of the main points that must be controlled to maintain sample integrity. In fact, in addition to safety and security of the buildings to protect against fire, unauthorised access, and other usual hazards, monitoring temperature within mechanical freezers and refrigerators is an essential quality assurance measure. For these reasons, all freezers of the infrastructure are fitted with real-time temperature monitoring and alarm systems. The corresponding temperature logging information is automatically transferred through an electronic interface to the biorepository management system and is linked to the samples stored in the corresponding freezer. If a measured value is no longer between the upper or lower limits, an alarm is sent to the staff

STANDARD MATERIALS

members. Any outage of electrical power is compensated by an independent system such as a generator with locally controlled production. To preserve biological resources in the case of a disaster or unexpected events, part of the samples stored at the BVR are intended as backup samples and are located in a separate infrastructure for safety and security reasons. This additional “mirror banking” ensures that if samples are compromised for whatever reason, replicate aliquots of good integrity will still be available.

CONCLUSION

Biobanking is an emerging multidisciplinary and dynamic activity that involves the collection and preservation of several types of samples, but the potential value of a biobank depends on the quality of samples and on the maintenance of their integrity. Biobanks with well characterised specimens will be essential for future research and development efforts for retrospective studies, epidemiological investigations, and for providing reference materials used in assay standardisation, validation, and proficiency testing programmes. Although there has been significant progress in this field, several issues remain to be addressed at the global level concerning the whole process of biobanking, such as the lack of a harmonised approach to standard procedures for processing samples and saving the related data. Currently, there is significant variability regarding the collection, processing and storage of the majority of biological materials available for research and diagnosis, and regarding their associated data. To overcome this problem, the existing procedures provided in the guidelines published by the international organisations mentioned above could be developed for the evaluation of quality assessment parameters. In this regard, one of the main projects that will be launched in the near future by the BVR and all the OIE Reference and Collaborating Centres is to standardise the quality controls of the stored reference materials.

References

Almeida JL, Cole KD, Plant AL. 2016. Standards for Cell Line Authentication and Beyond. *PLoS Biology* 14:1-9.

Armstrong SE, Mariano JA, Lundin DJ. 2010. The scope of mycoplasma contamination within the biopharmaceutical industry. *Biologicals* 38:211-213.

Balogun EO, Nok AJ, Kita K. 2016. Global warming and the possible globalization of vector-borne diseases: a call for increased awareness and action. *Tropical Medicine and Health* 44:38.

Capes-Davis A, Theodosopoulos G, Atkin I, Drexler HG, Kohara A, MacLeod RA, Masters JR, Nakamura Y, Reid YA, Reddel RR, Freshney RI. 2010. Check your cultures! A list of cross-contaminated or misidentified cell lines. *International Journal of Cancer* 127:1-8.

Capps B, Lederman Z. 2015. One Health and paradigms of public biobanking. *Journal of Medical Ethics 41:258-262*.

Carter A, Betsou F. 2011. Quality assurance in cancer biobanking. *Biopreservation and Biobanking* 9:157-163.

Chen D, Nims R, Dusing S, Miller P, Luo W, Quertinmont M, Parekh B, Poorbaugh J, Boose JA, Atkinson EM. 2008. Root cause investigation of a viral contamination incident occurred during master cell bank (MCB) testing and characterisation - A case study. *Biologicals* 36:393e402.

Drexler HG, Uphoff CC. 2002. Mycoplasma contamination of cell cultures: Incidence, sources, effects, detection, elimination, prevention. *Cytotechnology* 39:75-90.

El-Adawy H, Ahmed M, Hotzel H, Monecke S, Schulz J, Hartung J, Ehricht R, Neubauer H, Hafez HM. 2016. Characterization of Methicillin Resistant Staphylococcus aureus Isolated from Healthy Turkeys and Broilers Using DNA Microarrays. *Frontiers in Microbiology* 7:2019.

Hinchliffe S, 2015. More than one world, more than one health: Re-configuring interspecies health. *Social Science & Medicine* 129:28-35.

Hughes P, Marshall D, Reid Y, Parkes H, Gelber C. 2007. The costs of using unauthenticated, over-passaged cell lines: how much more data do we need? *Biotechniques* 43:575-586.

Iraola G, Pérez R, Betancor L, Marandino A, Morsella C, Méndez A, Paolicchi F, Piccirillo A, Tomás G, Velilla A, Calleros L. 2016. A novel real-time PCR assay for quantitative detection of Campylobacter fetus based on ribosomal sequences. *BMC Veterinary Research* 12:286.

STANDARD MATERIALS

Knezevic I, Stacey G, Petricciani J, Sheets R. 2010. Evaluation of cell substrates for the production of biologicals: revision of WHO recommendations. Report of the WHO Study Group on Cell Substrates for the Production of Biologicals, 22-23 April 2009, Bethesda, USA. *Biologicals* 38:162-169.

Kniss DA, Summerfield TL. 2014. Discovery of HeLa Cell Contamination in HES Cells: Call for Cell Line Authentication in Reproductive Biology Research. *Reproductive Sciences* 21:1015-1019.

Laborde S, Degrave A, Lehmann D, Jouette S, Rofel C, Muller T, Hertzog N, Rook M, Ribault S. 2010. Detection of Mollicutes in bioreactor samples by real-time transcription-mediated amplification. *Letters in Applied Microbiology* 50:633-638.

Merten O-W. 2002. Virus contaminations of cell cultures – A biotechnological view. *Cytotechnology* 39:91-116.

Nardone RM. 2007. Eradication of cross-contaminated cell lines: A call for action. *Cell Biology and Toxicology* 23:367-372.

Nims RW, Shoemaker AP, Bauernschub MA, Rec L J, Harbell JW. 1998. Sensitivity of isoenzyme analysis for the detection of interspecies cell line cross-contamination. *In Vitro Cellular & Developmental Biology* – Animal 341:35-39.

Nübling CM, Baylis SA, Hanschmann K-M, Montag-Lessing T, Chudy M, Kreb J, Ulrych U, Czurda S, Rosengarten R. 2015. World Health Organization International Standard To Harmonize Assays for Detection of Mycoplasma DNA. *Applied and Environmental Microbiology* 81:5694-5702.

O'Brian SJ, 2001. Cell culture forensics. *Proceedings of the National Academy of Sciences of the USA* 98:7656-7658.

OECD (2001). Biological Resource Centres: Underpinning the future of life sciences and biotechnology. http://www.oecd.org/sti/biotech/2487422.pdf.

OECD (2007). Best Practice Guidelines for Biological Resource Centres. http://www.oecd.org/sti/biotech/38777417.pdf.

OIE (World Organisation for Animal Health) Guide 3. International Reference Antibody Standards for Antibody Assays. *Quality Standard and Guidelines for Veterinary Laboratories: Infectious Diseases* 1-5.

Ono K, Satoh M, Yoshida T, OzawaY, KoharaA, TakeuchiM, Mizusawa H, Sawada H. 2007. Species identification of animal cells by nested PCR targeted to mitochondrial DNA. *In Vitro Cellular & Developmental Biology – Animal* 43:168-175.

Parodi B, Aresu O, Bini D, Lorenzini R, Schena F, Visconti P, Cesaro M, Ferrera D, Andreotti V, Ruzzon T. 2002. Species identification and confirmation of human and animal cell lines: a PCR-based method. *Biotechniques* 322:432-434, 436, 438-440.

Pisal RV, Hrebíková H, Chvátalová J, Kunke D, Filip S, Mokrý J. 2016. Detection of Mycoplasma Contamination Directly from Culture Supernatant Using Polymerase Chain Reaction. *Folia Biologica (Praha)* 62:203-206.

Rabinowitz P, Conti L. 2013. Links among human health, animal health, and ecosystem health. *Annual Review of Public Health* 34:1-16.

Reid Y, Mintzer J. 2012. The Current State of Cell Contamination and Authentication—And What It Means for Biobanks. *Biopreservation and Biobanking* 10:236-238.

Reid YA. 2011. Characterization and authentication of cancer cell lines: An overview. *Methods in Molecular Biology* 731:35-43.

Schiff LJ. 2005. Review: production, characterization, and testing of banked mammalian cell substrates used to produce biological products. *In Vitro Cellular & Developmental Biology – Animal* 41:65-70.

Scotch M, Odofin L, Rabinowitz P. 2009. Linkages between animal and human health sentinel data. *BMC Veterinary Research* 5:15.

Uphoff CC, Drexler HG. 2013. Detection of mycoplasma contaminations. *Methods in Molecular Biology* 946:1-13.

World Health Organization. 2010. Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks. http://www.who.int/biologicals/Cell_Substrates_clean_version_18_April.pdf

Young L, Sung J, Stacey G, Masters JR. 2010. Detection of Mycoplasma in cell cultures. *Nature Protocols* 5:929-934.

Zielhuis GA. 2012. *Biobanking for epidemiology. Public health* 126:214-216.