BioReliance®
Pharma & Biopharma Manufacturing & Testing Services

White Paper

Avoid Surprises With Comprehensive Cell Line Characterization

Kathryn Martin Remington, Ph.D.

A robust strategy for viral safety assurance is an essential component of every biopharmaceutical manufacturing process and typically consists of three pillars: prevent, detect, and remove. Prevention includes measures to keep contaminants from entering the process, and includes careful selection and testing of raw materials. Detection involves testing in-process materials for contaminating adventitious agents, and removal encompasses different steps in downstream purification that inactivate or remove viral contaminants. This white paper focuses on the characterization of cell lines, a cornerstone of the "prevent" pillar, and is critical to stopping the entry of contaminants, including viruses, into the manufacturing process.

Conducting The Risk Assessment

The first step in ensuring viral safety is a safety risk assessment that encompasses the cells, other source materials, and the process for potential contamination (Table 1). The European Pharmacopoeia Chapter 5.1.7 serves as an excellent resource when developing a risk assessment[1].

It is also important to understand whether any animal-derived materials were used during the history of the cell line. If so, assays should be included in the testing program for potential viral contaminants, associated with the identified animal species.

Once the risk assessment is complete, guidance documents, regulations, and updates from regulatory authorities should be consulted to build out the specific safety strategy, which will serve as the road map for conducting safety testing.

Considerations for Risk Assessment

Table 1. Key factors from EP 5.1.7 to be considered during the risk assessment process.

• Species of origin
• Organ, tissue and fluid of origin
• Potential contaminants in view of origin of the raw material and history of donor(s)
• Potential contaminants from the manufacturing process
• Infectivity and pathogenicity of the potential contaminants for the recipients
• Route of administration of product
• Amount of material used to produce a dose of medicinal product
• Controls carried out on the donor(s), the raw material, during production and on the final product
• The manufacturing process and its capacity to remove/inactivate viruses
• Patient population being treated and disease indication

Cell Line Characterization

Comprehensive cell line characterization should be performed to verify purity, effectively avoid contamination, and ensure the genetic construct will be stable through out the production life of the cells[2-8].

Identity

Verification of the cell line identity is done using either genotyping or karyotyping. Genotypic approaches include the cytochrome c oxidase subunit 1 (CO1) gene barcode assay and short tandem repeat (STR) analysis to confirm species identity. The CO1 mitochondrial

MERCK

gene is free of introns, has haploid inheritance and very limited exposure to recombination, all of which help to keep it highly conserved. It has a greater range of phylogenetic signal than any other mitochondrial gene, allowing for clear species identification and is the method of choice for taxonomic identity and cell line identity confirmation at cell culture collections. For human cell lines, hypervariable regions comprising a variable number of short tandem repeats from microsatellite DNA are used in STR analysis to confirm identity and cell type. This is the method of choice for human cell lines.

Karyotyping examines chromosome numbers and markers to ensure the cell line is from the expected species. It is recommended for newly-established and diploid cell lines but is not necessary for well-characterized cell lines such as CHO and NS0. Karyology can also be used as an orthogonal method for authentication of the cell line's identity.

Sterility Testing

Sterility testing for bacteria and fungi uses different types of media and is incubated with samples for up to 14 days. Different conditions of incubation enable detection of aerobic, microaerophilic, and anaerobic organisms. For short shelf-life products, like cell therapies, a rapid method such as the BacT/Alert® detection system is needed and is accepted by regulatory authorities. This method detects organisms by measuring changes in carbon dioxide production.

In addition to bacteria and fungi, it is important to test for mycoplasmas in mammalian cells and spiroplasmas in insect cells. Figure 1 summarizes the three parts of the compendial, culture-based mycoplasma assay. To facilitate detection of mycoplasmas that don't grow well on agar or in broth, mammalian cells are inoculated, and following an incubation period, the cells are stained to elucidate mycoplasma DNA. Sample is also inoculated directly onto agar plates. The plates are incubated and then examined microscopically for fried egg-shaped colonies. Finally, test samples are also inoculated into broth and incubated for about three weeks, during which time, samples are withdrawn for subculture onto agar plates. The broth amplifies organisms that are then detected on agar plates. However, it is a lengthy assay. If a more rapid time-to-results is needed, a very sensitive, real-time PCR method is available (Table 2). The PCR assay can also be used for bulk harvest testing as well as for short shelf-life products.

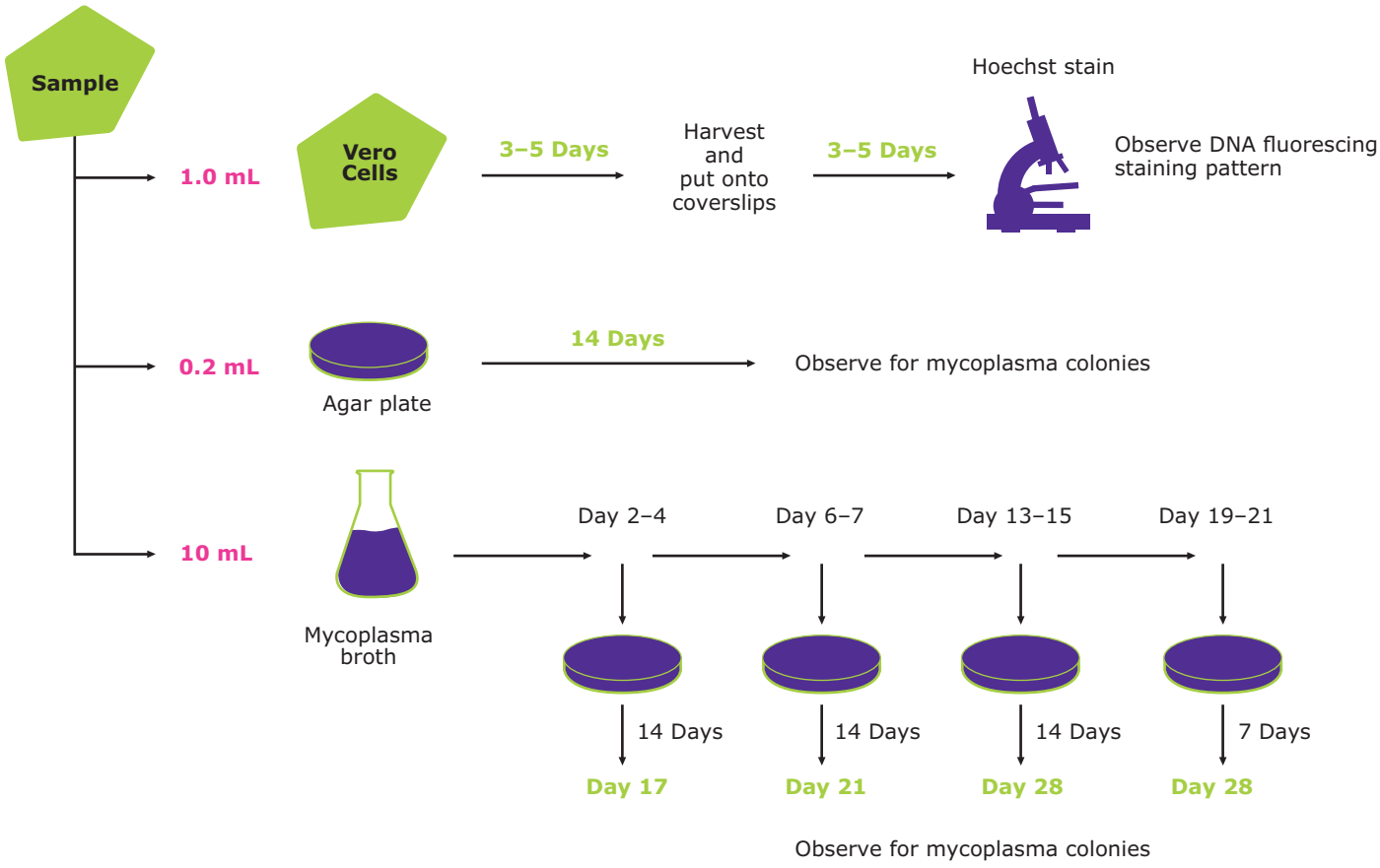

Figure 1. Culture-based assay for mycoplasma detection.

Table 2. Comparison of PCR and the conventional culture-based assay for mycoplasma detection.

Parameter	Pinnacle QPCR	Conventional Methods
Method	Real Time PCR	Agar & broth amplification; Indicator detection system
Endpoint	Fluorescence	Mycoplasma colony count
Duration	1 day	28 days
Sensitivity	10 cfu/mL	10–100 cfu/mL
Specificity	High	High
Validation	Yes	Yes
Range of Species	102 species*	Viable organisms capable of growth on the media and under incubation conditions used

* Validated against *Mycoplasma orale, Mycoplasma hyorhinis*, *Mycoplasma synoviae, Mycoplasma fermentans, Mycoplasma arginine, Mycoplasma pneumoniae, Acholeplasma laidlawii, Spiroplasma citri*.

Mycobacterium

A standard culture-based method for mycobacterium exists and both European and U.S. regulators recommend that all cells susceptible to infection by mycobacteria be tested. The culture-based infectivity assay requires approximately 56 days, while a PCR assay offers a rapid alternative. If cells are tested for mycobacterium by one of these assays, then guinea pigs are not required in the *in vivo* assay.

Viruses

To detect a wide range of possible viral contaminants, several different assays must be utilized. Broad specificity assays include *in vitro* and *in vivo* virus assays while species-specific assays detect contaminants associated with a particular species, such as rodent, bovine, porcine, and human viruses. PCR assays facilitate the detection of specific viruses that are known to infect a particular cell line. It is important to detect retroviruses, as they can insert themselves into a host cell genome. Assays for retroviruses include infectivity assays, PCR-based assays, morphological assays and biochemical or PCR assays for the detection of reverse transcriptase.

The *in vitro* adventitious virus assay is the foundation for cell line characterization. Samples are inoculated onto detector cell lines which include a human diploid line, a simian line and another cell line of the same species and tissue type as the cells from the bank. The inoculated cells are incubated for two weeks and several endpoints are used to determine if virus is present including virus-induced morphological changes in the cells (cytopathic effect; CPE), hemagglutination and hemadsorption. The assay is most sensitive if at this point, supernatant from the inoculated cells can be used to inoculate fresh cells. After an additional two weeks, cells are examined for evidence of viral infection using the same endpoint assays as were used previously. Similar assays are used to identify animal species-associated viruses using CPE and hemadsorption endpoints as well as fluorescent antibodies to a panel of viruses listed in 9CFR113.47. While broadly specific, highly sensitive (down to one infectious unit) and capable of detecting a wide range of viral contaminants, *in vitro* adventitious virus assays can only detect viruses that grow in the particular cell line and are detectable with the particular endpoint assay.

Another broad specificity assay for detecting virus is the *in vivo* assay. In this assay, suckling and adult mice, guinea pigs, and embryonated eggs are inoculated and observed for signs of infection. As noted earlier, if an *in vitro* mycobacteria assay is performed, guinea pigs can be eliminated.

Mouse, hamster and rat antibody production (MAP, HAP, RAP) assays are used to detect the presence of virus in rodent cells. The species of choice is inoculated with the test article and then after a period of time, serum is harvested from the animals and analyzed by enzyme-linked immunosorbant assay (ELISA) for a specific set of viruses listed in regulatory documents, including ICH Q5A (R1) (1997)[2].

To reduce the use of animals in virus testing, we developed the Blazar™ Platform, a molecular alternative to the rodent antibody production assays. The platform uses multiplex primers and degenerate PCR to detect more viruses, including variants, than can be detected using the typical antibody production assay. Overall, this assay can detect a wider variety of rodent viruses faster, and with higher sensitivity than the standard antibody production assay, without the need for animals.

Several assays can be used to detect retrovirus; the species of the cell line will determine the appropriate assays. One commonly used method for characterizing virus in cell banks is electron microscopy, which is broadly specific and can detect viruses, virus-like particles as well as other microbes. Another method is co-cultivation, in which a cell line known to be susceptible to infection with retroviruses is inoculated with a lysate of cells from the cell bank. A focus-forming assay or other endpoint assay is then used for retrovirus detection. Alternatively, a PCR-based assay can be used to detect reverse transcriptase, an enzyme found in retroviruses. Finally, virus-specific assays can be used to detect viruses known to be potential contaminants of a particular cell line.

Genetic Stability

Genetic stability is typically addressed later in drug development, often during phase three clinical studies. Cells from the master cell bank and from the end of production (EPC) are compared using a variety of molecular techniques to ensure the expression construct hasn't changed over the course of manufacturing in a way that might impact the quality and integrity of the product. Molecular studies will confirm copy number.

Conclusions

A robust risk assessment sets the stage for comprehensive cell line characterization by directing the type of testing that will be needed.

Table 3 summarizes testing required for master, working and end of production cell banks and highlights the fact that the testing for a master cell bank is more extensive than for a working cell bank. A working cell bank is typically only a few passages from the master bank and the potential for contamination of this bank is relatively low. Sterility, mycoplasma, identity and an *in vitro* assay is typically performed on the working cell bank. More extensive testing is then conducted on EPC or cells at the limit (CAL).

Cell Line Characterization

Table 3. Summary of cell line characterization tests.

Testing	Assays	MCB	WCB	CAL
Mircrobial Detection	CO1 Barcode Analysis	X	X	X
	Sterility	X	X	X
	Mycoplasma	X	X	X
	Mycobacteria	X	X	X
Virus Detection	*in vitro* virus assay	X	X	X
	in vivo virus assay	X		X
	Antibody production assay/Blazar™ rodent panel	D		
	TEM	X		X
	Retrovirus infectivity assay	D		D
	QPERT	D		D
	PCR for Specific viruses	X		X
	Bovine virus assay	B/P		B/P
	PCR for Bovine Parvoviruses	B/P		B/P
	Porcine virus assay/ PCR for PCV & HepE	B/P		B/P
	Karyotyping	D		D

D = Dependent on cell line
B/P = If bovine or porcine-derived materials have been used with cells

We have described a comprehensive approach to cell line characterization that is foundational to a robust manufacturing process and should consist of tests for identity, microbial and virus detection. As described in this white paper, initial investment in a detailed risk assessment will set the stage for the combination of assays required to fulfill regulatory requirements and ensure patient safety.

References

1. European Pharmacopoeia (Ph. Eur.): Chapter 5.1.7 on Viral Safety. 01/2008:50107.
2. ICH. 1999. Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin. International Conference for Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, Q5A(R1). Geneva, Switzerland. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/q5a-viral-safety-evaluation-biotechnology-products-derived-cell-lines-human-or-animal-origin. United States Food and Drug Administration. 1993.
3. Points to consider in the characterization of cell lines used to produce biologicals. United States Department of Health and Human Services, Food and Drug Administration, Center for Biologics Evaluation and Research. Rockville, MD. https://www.fda.gov/media/76255/download ICH. 1998.
4. Quality of Biotechnological Products: Derivation and Characterization of Cell Substrates Used for Production of Biotechnological/Biological Products. International Conference for Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, Q5D. Geneva, Switzerland. https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-5-d-derivation-characterisation-cell-substrates-used-production-biotechnological/biological-products-step-5_en.pdf
5. European Pharmacopoeia (Ph. Eur.): Chapter 5.2.3 on Cell Substrates for the Production of Vaccines for Human Use. 01/2005:50203.
6. European Pharmacopoeia (Ph. Eur.): Chapter 2.6.16 on Tests for Extraneous Agents in Viral Vaccines for Human Use. 01/2005:20616.
7. World Health Organization. 2013. Recommendations for the evaluation of animal cell cultures as substrates for the manufacture of biological medicinal products and for the characterization of cell banks. WHO Technical Report Series No. 978. https://www.who.int/biologicals/vaccines/TRS_978_Annex_3.pdf
8. United States Food and Drug Administration. 2010. Guidance for Industry: Characterization and Qualification of Cell Substrates and Other Biological Materials Used in the Production of Viral Vaccines for Infectious Disease Indications. United States Department of Health and Human Services, Food and Drug Administration, Center for Biologics Evaluation and Research. Rockville, MD. https://www.fda.gov/media/78428/download

Merck
West of Scotland Science Park
Glasgow G20 0XA
UK

For additional information, please visit **www.MerckMillipore.com/BioReliance**

Lit. No. MK_WP7777EN Ver. 1.0
05/2021