



Cell Line Characterization

Characterization and safety testing of cell banks are essential to ensure the quality of your cell-derived biopharmaceutical products. Cell banks must be extensively characterized to assess the cell source with regard to its identity, to the presence of other cell lines, adventitious agents, endogenous agents, and molecular contaminants. Charles River offers the specialized expertise and rigorous biochemical, molecular, and virological tests that ensure the identity, purity, stability, and safety of your cell banks.

The test design for the characterization of mammalian, microbial, and other cell lines is species-specific and can vary depending on the history of the cell line and the type of media components that may be present. Charles River Biopharmaceutical Services (BPS) can advise you in the design and execution of a complete testing program for your cell line that is customized to meet your exact specifications and is compliant with international regulatory expectations.

Mammalian and Other Cell Banks

IDENTITY TESTING

Isoenzyme analysis is used to confirm the identity of the species of the cell line. The electrophoretic mobility and the banding pattern, which is species-specific, of different intracellular enzymes are determined using agarose gels. Alternative methods for identity testing include DNA fingerprinting and karyology. These methods may be required for cell banks used for newly established cell lines and for vaccine production.

Mammalian and Non-Traditional Cell Line Characterization

- Purity
- Identity
- Stability
- Virological safety

Microbial Cell Line Characterization

- Purity
- Identity
- Viability
- Stability



PURITY TESTING

Sterility

Cell banks and bulk harvest material are tested for the presence of bacterial and fungal contaminants using a direct inoculation method with two different media. The International Conference on Harmonisation (ICH) recommends that at least 1% or a minimum of two vials from the cell bank be tested. A bacteriostasis/fungistasis test is normally performed prior to testing to determine any inhibitory effects of the test material on microbial growth.

Mycoplasma

According to the European Pharmacopoeia, USP, US FDA Points to Consider, and Japanese Pharmacopoeia, two methods should be used to detect the broadest possible variety of mycoplasma species. Cultivable mycoplasma species are tested for by incubation on agar plates and in broth media. It should be determined if any inhibitors for this cultivation test are present in the test article. Non-agar cultivable mycoplasma species are detected by a DNA fluorochrome stain on mycoplasma-free Vero indicator cell cultures that have been incubated together with the test article. An alternative validated method such as PCR can also be used.

Charles River has validated a rapid, sensitive, and specific NAT-based detection assay for a wide range of mollicutes including Mycoplasma and Acholeplasma species, utilizing RT-PCR technology with sequence-specific primers and dual-labeled fluorescent probes developed at Charles River, demonstrating comparability to traditional methods based on the guidelines of the European Pharmacopoeia 5.8 (July, 2007) and 6.1 (January, 2008), section 2.6.7, with samples assayed directly for the presence of mycoplasma nucleic acid or following a growth enrichment step for viable organisms.

STABILITY TESTING

According to ICH guidelines, evaluation of cell substrate stability during cultivation for production should be examined at a minimum of two time points. Genomic and transcript sequencing are some of the indicators of genetic stability. Our BPS group performs stability studies under ICH guidelines, where applicable, for biopharmaceutical and pharmaceutical products at all stages of the registration process, including cell banking. We can provide guidance on the appropriate testing program to meet regulatory requirements.

VIROLOGICAL SAFETY TESTING

Adventitious Viruses

The *in vitro* assay uses a variety of indicator cell lines that are selected on the basis of the history and species of the production cell line. According to ICH and EU guidelines, the choice of cells is governed by the species of origin of the cell bank to be tested but should include a human and/or nonhuman primate cell line that is susceptible to human viruses. We can provide the appropriate guidance for the indicator cell lines to be used.

In vivo assays utilizing various animal systems are performed to determine if viruses are present in the test item that do not cause cytopathic or other noticeable effects on *in vitro* tests. Lysates from the cells are injected into various animal species. The animal systems commonly used are embryonated chicken eggs and adult and suckling mice. Guinea pigs may also be used when required.

Bovine Viruses

A bovine virus test is used when the cells have been or may have been exposed to bovine raw material (such as FCS or BSA). Cell lysates are incubated together with bovine cells and examined for bovine viruses based on cytopathic effects and using fluorescent antibody staining techniques.

Human and Simian Viruses

According to ICH guidelines, if the cell line used for production is of human or nonhuman primate origin, additional tests for human viruses, such as those causing immunodeficiency diseases and hepatitis, should be performed unless otherwise justified. PCR testing methods may be appropriate for the detection of sequences of these viruses.

Porcine Viruses

A porcine virus test is used when the cells have been or may have been exposed to porcine raw material (such as trypsin). Cell lysates are incubated together with porcine cells and examined for cytopathic effects or reactivity using fluorescent antibody staining assays.

Retrovirus

A number of cell lines used to produce biopharmaceuticals, such as rodent and avian cells, are known to contain endogenous retroviruses, so it must be determined if these retroviruses are infectious. Not all of these viruses cause cytopathic effects with indicator cell lines, so other methods must also be used.

Methods based on cell cultures are commonly used that exhibit plaque or focus formation with detector cells where a replication-competent retrovirus is acting as a helper virus. The S+L- focus assay is able to detect xenotropic and amphotropic murine retroviruses that are capable of infecting both murine and non-murine cells. Infected cells form foci that can be detected microscopically. The XC plaque assay is able to detect ecotropic murine retroviruses that are capable of infecting only murine cells. Retroviral infection causes formation of large syncytia-resembling plaques that can be visualized.

The production of retroviruses by cell cultures may be the result of endogenous retroviral genome expression and/or laboratory contamination. Most endogenous and exogenous retroviruses do not produce morphologic transformation or cytopathogenesis in cell culture, thus the production of these viruses in cell cultures is generally not detected. The presence of the enzyme reverse transcriptase (RT, RNA-dependent DNA polymerase) can be used as a reliable means for the detection of retrovirus. Testing for adventitious retroviruses can be accomplished using a polymerase chain reaction (PCR)-based reverse transcriptase assay (PBRT), which is a modified RT-PCR assay.

Transmission electron microscopy (TEM) is used to determine the viral load by visualizing and quantifying viral particles in biological fluids or within cells. Furthermore, it is a useful tool for characterizing viral-like particles based on their size and morphological characteristics.

Specific Rodent Viruses

Mouse, hamster, and rat antibody production (MAP, HAP, and RAP, respectively) tests are indirect methods for detecting virus contaminants by test article inoculation in mice, hamsters, and rats. Serum from these animals is then tested for the presence of antibodies reactive with a panel of viruses specific for each animal system. Immunofluorescence and ELISA techniques are employed for these tests. Testing of CHO cell lines for the presence of minute virus of mice (MVM) by PCR is also recommended.

Microbial Cell Banks

PURITY AND IDENTITY TESTING

A critical aspect of cell development and banking is determining that the Master Cell Bank (MCB) and Working Cell Bank (WCB) are biologically pure. The ICH regulatory guidelines advise that a purity test be performed to determine the presence of existing contaminating organisms. Purity testing includes screening on multiple growth media for a wide-ranging isolation of contaminating organisms. Identification testing is also performed using comparative sequence analysis of selected genes, including ribosomal RNA genes, for accurate species identification.

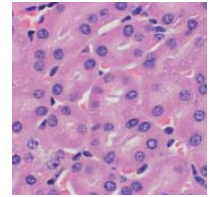
It is also important to ensure that the microbial cell bank is free of contaminating bacteriophage. The bacteriophage test determines the presence of bacterial virus by induction with Mitomycin C or exposure to UV irradiation.

VIABILITY TESTING

Evidence for cell bank stability under defined conditions is an important criterion for downstream production. Testing for cell viability demonstrates whether preserved cells have the ability to survive a preservation process.

ANALYSIS OF PLASMID STABILITY

For cell banks, the stability of the microbial expression system is necessary to confirm the expression of the desired product. Plasmid, genomic, and transcript sequencing are indicators of genetic stability. The purpose of analyzing the expression construct is to establish that the correct coding sequence of the product has been incorporated into the host cell and is maintained during culture to the end of production. Several tests are used to assess the integrity of the desired product, including RNA and DNA sequence analysis, retention of selectable markers, retention of recombinant construct, copy number determination, and restriction map analysis.



Mammalian and Non-Traditional Cell Line Characterization

- Identity testing
 - Isoenzyme analysis
 - DNA and RNA sequencing
 - DNA fingerprinting by Southern hybridization
 - Karyology
- Purity testing
 - Bacteriostasis/fungistasis test
 - Mycoplasma (cultivable and non-cultivable)
 - Mycoplasma by PCR
 - *In vitro* assay
 - *In vivo* assay
- Stability testing
 - Copy number determination
 - DNA and RNA sequencing
 - Restriction map analysis
- Virological safety testing
 - XC plaque assay
 - S+L- focus assay
 - PCR-based reverse transcriptase (PBRT) assay
 - Reverse transcriptase assay
 - Transmission electron microscopy (TEM)
 - Mouse, rat and hamster antibody production test assays
 - PCR targeting specific viruses
 - Bovine and porcine virus testing

Microbial Cell Line Characterization

- Purity and identification testing
- Bacteriophage testing
- Viability testing
- Analysis of plasmid stability
 - DNA and RNA sequencing
 - Retention of selectable markers
 - Retention of recombinant construct
 - Copy number determination
 - Restriction map analysis

