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Technical Sheet

Cell Line Characterization

The characterization of cell banks is essential to ensure the quality of cell-derived biopharmaceutical products. Cell banks must be extensively characterized to assess the cell source with regard to its identity and the presence of other cell lines, adventitious and endogenous agents or molecular contaminants. However, 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. The testing described below will most likely be part of the characterization of your cell line but Charles River can also tailor a testing program to suit your individual needs.

Microbial Cell Banks

Purity and Identity Testing

The ICH regulatory guidelines advise that a purity test be performed to determine the presence of contaminating organisms in cell banks. Purity testing of microbial cell banks includes screening on multiple types of agar plates in a variety of incubation conditions for a wide range of possible contaminants. It is also important to ensure that the microbial cell bank is free of bacteriophage. Contaminating bacteriophage are detected through induction with mitomycin C or exposure to UV irradiation. If the cell bank was made with any materials derived from plants it is also recommended that an assay for Spiroplasma detection is performed.

For accurate species identification, genotypic and proteotypic identification methods are available. Strain typing can also be performed upon request.

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 and is performed by plating serial dilutions of the preserved cells on agar plates.

Analysis of Plasmid Stability

For microbial cell banks, the stability of the microbial expression system is necessary to confirm the expression of the desired product. Plasmid 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.

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Mammalian, Insect, Avian and other Cell Line Characterization

Identity Testing

Isoenzyme analysis is used to confirm the identity of the species of a 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, STR analysis and karyology. These methods may be required for newly established cell lines and for vaccine production.

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.

Mollicutes

Contamination by mollicutes, including Mycoplasma, Acholeplasma and Spiroplasma species, is a common problem associated with cell cultures and is not easily identifiable. According to regulations, 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. Non-agar cultivable Mycoplasma species are detected by a DNA fluorochrome stain (Hoechst stain) of Mycoplasma-free Vero indicator cell cultures that have been incubated together with the test article. Charles River also has rapid, sensitive and specific NAT-based detection assays for a wide range of mollicutes including Mycoplasma, Spiroplasma and Acholeplasma species. The assays for detection of Mycoplasma and Acholeplasma species have been demonstrated comparable 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 to detect viable organisms.

Genetic 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, along with restriction mapping and copy number determination, are some of the indicators of genetic stability. Our Biologics Testing Solutions group performs stability studies under ICH guidelines 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* adventitious agent 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 nonhuman primate cell line that is susceptible to a broad range of viruses affecting humans. We can provide the appropriate guidance for the indicator cell lines to be used. *In vivo* adventitious agent 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 during *in vitro* testing. 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). In our 9CFR compliant assay, cell lysates are incubated together with bovine cells and examined for bovine viruses based on cytopathic effects and using fluorescent antibody staining techniques. In addition, we offer specific PCR testing for bovine viruses.

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. Charles River offers real-time PCR testing methods that 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). In our 9CFR-compliant assay, cell lysates are incubated together with porcine cells and examined for cytopathic effects or reactivity using fluorescent antibody staining assays. In addition, we offer specific PCR testing for porcine viruses.

Other Virus-Specific Assays

Charles River has many other virus-specific assays to include in testing panels depending on the type of cells that you are working with. This includes avian leukosis virus testing for those working with avian cell lines. Our team of experts will be able to recommend the virus panel that is best suited for your cell line.

Retrovirus

The production of retroviruses by cell cultures may be the result of endogenous retroviral genome expression (e.g., rodent and avian cells) and/or laboratory contamination. Cell culture methods where plaque or focus formation is seen in detector cells where a replication-competent retrovirus is acting as a helper virus are commonly used to detect retroviruses. 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. The XC plaque assay is able to detect ecotropic murine retroviruses that are capable of infecting only murine cells.

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 can be used as a reliable means for the detection of retrovirus in these cases. The detection of the reverse transcriptase enzyme 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.

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