VIRUS TESTING FOR BIOLOGICAL PRODUCTS: Partnering With a Contract Lab

INTRODUCTION

Many drugs on the market and in development are characterized as biologicals. Biological products are those derived from living organisms, such as bacterial, yeast, or mammalian cells, animals, and humans. In the manufacture of biological products, there is risk of virus contamination at many levels, from the starting materials to introduction during processing. Virus testing is required when there is any risk of contamination. Guidance documents from the regulatory agencies describe the requirements for such testing. They are the references for what testing should be considered, depending on the product and the system used for its manufacture. Some virus testing considerations are provided below for mammalian cell culture based systems. For biologics produced in other systems, some of these will apply as well, with additional considerations likely. By working with a contract laboratory, the best testing strategy can be executed to assure product safety.

SOURCES OF CONTAMINATION

The manufacture of a biological product can be a very complex process and the potential for virus contamination can occur at many levels. For example, there are many biological products that are prepared from mammalian cell lines. Often, these cell lines have been genetically engineered to express a particular protein or possess a certain trait. They must be generated, characterized, and banked. The cells are expanded for use in a bioreactor and the bulk material harvested from this culture. The biological product is then purified from the bulk harvest to produce the final drug product.

Using the above as an example of a biological product's manufacturing process, one can see many opportunities for virus contamination. Therefore, testing for viruses is necessary to ensure the safety of these products prior to use in humans. For a cell culture based system, the contamination may be introduced from the raw materials used in the culture of the cells. The source cells may be infected without any outward signs of contamination. During the manufacturing process, low-level virus not detected in the raw material screen or the tests of the cell banks may be amplified. Further, during the manufacture and the processing steps to prepare the final biological product, there may be accidental operator introduction of viruses into the system as the product is prepared.

Animal-derived raw materials

When mammalian cell cultures are used for biological products, bovine serum is a raw material that poses a risk for virus contamination. Fetal bovine serum (FBS) has often been used in the culture of cells. As FBS and other bovine serum types are derived directly from cows, there is concern that serum lots could be contaminated when sourced from cows unknowingly infected with virus. It is critical that FBS is tested for the presence of a number of different bovine viruses. The Code of Federal Regulations, Animals and Animal Products, Title 9 (9CFR), provides regulations for the testing of serum for bovine viruses. There are seven specific viruses in the regulations, but testing for others can be incorporated into the test when desired. A qualified contract testing supplier can incorporate the additional viruses into the test, as required.

European guidances include the need for additional testing of bovine serum, lending particular concern to potential contamination by pestiviruses (specifically, Bovine viral diarrhea virus [BVDV]). If the intent is to market the product in Europe, the requirement for this additional testing must be considered for the virus testing strategy.
While the industry trend has been toward preparing cell banks and producing biological products in serum-free conditions, the need for bovine virus testing is not necessarily precluded. It is possible that the cells, even though banked in serum-free conditions, have a history where FBS was used prior to banking. Testing for bovine viruses should be incorporated into the overall virus testing strategy (see below for cell bank characterization).

Further, serum-free media formulations are not necessarily animal-product free. Many growth factors that are present in serum-free media formulations are growth factors that are derived from bovine sources. The presence of these agents in the media will therefore make tests for bovine viruses important for both the raw materials and the cultured cells.

Another animal-derived raw material often used in cell cultures is trypsin. Many adherent cell cultures are passaged using trypsin, commonly derived from porcine pancreas. Testing of trypsin for PPV is a requirement also described in the 9CFR. As there are moves to work in serum-free conditions, there are also trends towards using trypsin that is not animal-derived. However, as previously mentioned, the history of the cell lines may indicate that there had been prior exposure to porcine trypsin, therefore necessitating that this issue be addressed in the virus testing strategy.

Depending on the nature of the biological product and its production process, there may be other raw materials of concern. A tailored testing program for these may be required. It is important to get as much information on the raw materials that are being used to help plan the required testing. A contract testing supplier will be able to provide support to help determine the best approach.

**Cell bank characterization (virus safety)**

Cell lines used for the production of biologics must be characterized. There are many regulatory guidance documents available that provide the appropriate testing approaches. Regulatory documents for the testing of cell lines include the Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals, from the FDA, and the Guidance on Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin, from the International Conference on Harmonisation (ICH). It is important to be aware that, depending on the nature of the biological product, other regulatory documents also address the required characterization for the cells and biological product. To assist in determining which regulations apply, a contract testing supplier can help identify the appropriate documents for determining the best testing strategy.

The characterization of the cells is important to ensure that the products are not contaminated with adventitious agents. Virus infection may not be apparent as the cell banks were prepared, or during the expansion and seeding of the bioreactor for the manufacturing process. Virus may be endogenous to the cells, such as that found in murine cells. It is well known that murine cells can contain endogenous retrovirus sequences and infectious retrovirus can be recovered from these cells. There could also be a latent virus infection that only under certain conditions could express itself, such as that seen in herpes virus infections. There could be an active inapparent virus infection not producing any observable signs in the culture that would indicate a virus infection. An example of a virus that is able to persist in cell culture without causing observable signs of infection is BVDV (non-cytopathic strains).

Based on the origin of the cells, and the materials to which they were exposed, virus testing needs to be performed. A virus test to detect adventitious agents uses the human cell line, MRC-5, the monkey cell line, Vero, and a cell type of the same species and tissue type as the biological product. The cells are inoculated with the test sample and observed for cytopathic effect and hemadsorption. This test is a general test for detecting viruses that could ultimately infect humans. Other cell lines may be included to broaden the potential to detect viruses that may be present. Which cell lines to include in the test needs to be considered as part of the testing strategy.

As many cell lines have been exposed to FBS and trypsin in their histories, the contamination by bovine and porcine viruses should be included in the testing strategy as well. The bovine and porcine virus tests, performed in reference to the methods described in the 9CFR, also offer a general test to detect a broad range of viruses, while incorporating immunofluorescent antibody tests to detect the specific viruses prescribed in the 9CFR. The tests will detect virus contamination of the cells due to use of contaminated raw materials such as FBS, porcine trypsin, or media supplements of a bovine or other animal origin.
The above tests are examples of cell-based tests that can detect a broad range of viruses. Other cell-based tests may be needed. Additional types of virus tests are also required to fully characterize the cells and test the biological product. For example, the testing strategy should include in vivo studies looking for evidence of virus infection, electron microscopy studies of cells and of materials harvested from the bioreactor, and molecular-based assays to detect specific viruses. The regulatory documents provide guidance, and working with a contract testing supplier, these studies can be incorporated into the testing strategy.

**Virus concerns during the manufacturing process**

Amplification of viruses not detected in the screening of the raw materials or the testing of the cell banks could occur during the manufacturing process. This necessitates virus testing of the bulk product. Further, there could be introduction of viruses at this stage by the use of virus contaminated raw materials, or accidental exposure due to a breach in the process. The virus testing strategy employed for the cell banks will also apply to samples harvested from the bioreactor system. Some of the same considerations for the cell bank testing come into play for the bulk harvest: such as the raw materials the cells were exposed to, the nature of the cell line, and the nature of the product.

**OTHER BIOLOGIC PRODUCTS AND CONSIDERATIONS**

The above provides information on approaching a virus testing strategy for therapeutic drugs prepared from cell lines. Other types of biological products include vaccines, and therapeutic drugs made from animals. In designing a testing strategy for these types of products, additional and/or alternative testing strategies must be considered.

Many vaccines are prepared in cell lines; therefore the cell bank characterization described above is required. As a vaccine is subsequently prepared by inoculation of the cells with a virus, the virus stocks, too, need to be fully characterized before use in the manufacturing process. The testing for virus contaminants in a vaccine stock can be challenging, but there are methods to accomplish this task. For animal-derived therapeutics, animal husbandry and health surveillance issues need to be incorporated into the testing strategy. Various regulatory documents provide guidance on how to proceed with testing. A contract testing supplier can assist to ensure they are followed.

While the production of biologics carries an inherent risk of virus contamination, the manufacturing process to prepare the final product often contains steps that will clear potential virus contaminants. When this is the case, the process must undergo validation and regulatory documents provide a guide for performing this work. Viral clearance validation studies are often conducted by outside contractors, who can provide guidance on performing the studies.

**WORKING WITH A CONTRACT TESTING SUPPLIER**

The regulatory framework for biological product testing can be complex, especially when there is uncertainty on how to proceed. While some manufacturers may have in-house testing capability, often much of the work must be outsourced. Contract testing companies have the expertise to provide the required testing.

In selecting a contract testing supplier, it is important to review their capabilities and how they can help with your specific product. Not all products are alike. There may be a general testing strategy for therapeutic products made in a particular cell line; however, this strategy might not be the appropriate testing strategy for your therapeutic product made in a similar cell line.

**PLANNING AND INITIATING THE TESTING**

Planning for virus testing needs to begin early in the development of a biological product. Whether the product is made in a mammalian cell line, in an animal model, or other biologic system, it is important to have solid documentation of the source and history of the materials involved. If there is any missing information, it is better to be aware of it early in the development process.
While the best advice is to begin the virus testing as soon as possible in the development process, this may not always be feasible. It is important to note, however, that many of the virus tests will extend well over 4 weeks. This must be considered when establishing deadlines. While there is little that can be done to shorten the tests, a contract testing supplier can help you manage turnaround times to meet your deadlines.

SUMMARY

This paper is an introduction to the virus testing that is required for biological products. When working with a contract testing supplier, each product must be evaluated individually and given the customized treatment it needs. Your contract testing supplier should be a partner in the process to ensure that the virus safety requirements are met, and the testing strategy is tailored for you and your product.

REFERENCES

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