A REPRINT FROM

GEN Genetic Engineering & Biotechnology News

Point of View

GENengnews.com • August 2024

The Time Is Now for New Biosafety Testing Services

Thermo Fisher Scientific highlights the need to test for mycoplasmas and replication-competent viruses

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The rapid growth of the biologics safety testing market has led to the need for more testing capabilities in manufacturing and clinical research. Industry guidelines require biopharmaceutical and biotechnology companies to test their vaccines and other biologics, including cell and gene therapy (CGT) products, to assure that the raw materials and unprocessed bulk materials used to manufacture final products are free of adventitious agents (that is, microorganisms inadvertently introduced during production) and safe for patients.

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Human CGT products could dramatically change the treatment landscape of the rare genetic diseases that affect millions of patients. However, because CGT is a new technology, additional risks from contaminants have emerged, particularly from mycoplasmas and replication-competent viruses (RCVs).

Recognizing technical challenges

Biosafety methods are available for detection of these adventitious agents, but technical challenges still exist that can lead to unnecessary delays, including:

- Demonstration of equivalencies between compendial and emerging (for example, PCR-based) methods for mycoplasma detection.
- Validation complexity in implementation of good manufacturing practices (GMPs) for the detection of RCVs.
- Potential false-positive test results for both mycoplasma and RCV PCR methods.
- Length of time required for mycoplasma compendial culture methods or rounds of culture for RCVs.

Mycoplasmas are the smallest free-living bacteria—about 0.1 µm in diameter. Because of their broad distribution in nature, resistance to many antibiotics, and ability to pass through the smallest filters, mycoplasmas have become the most common contaminants of cell-derived biologics. Mycoplasma analysis conducted by commercial testing laboratories in 2010 revealed a 0.4–6.7% level of contamination in cell cultures used to produce biologics.[1]

Keeping abreast of regulatory guidelines

Four guidelines were issued that same year by the United States Pharmacopeia (USP), European Pharmacopeia (EP), Japanese Pharmacopeia (JP), and U.S. Food and Drug Administration (FDA) to establish mycoplasma safeguards at specific steps during manufacturing of cell-derived biologics to address safety concerns.[2–6]

RCVs in manufactured vectors and transduced cell products may cause cancer and clonal cell expansion in patients. New guidelines published recently for CGT products address risks associated with RCVs, the testing of CGT products, and collection of data about patients, before and after treatment, for investigational new drug and biologics license application submissions.[7,8]

Changing the treatment landscape

As indicated above, two key competencies—mycoplasma testing and RCV testing—are required for biopharmaceutical and biotechnology companies to meet

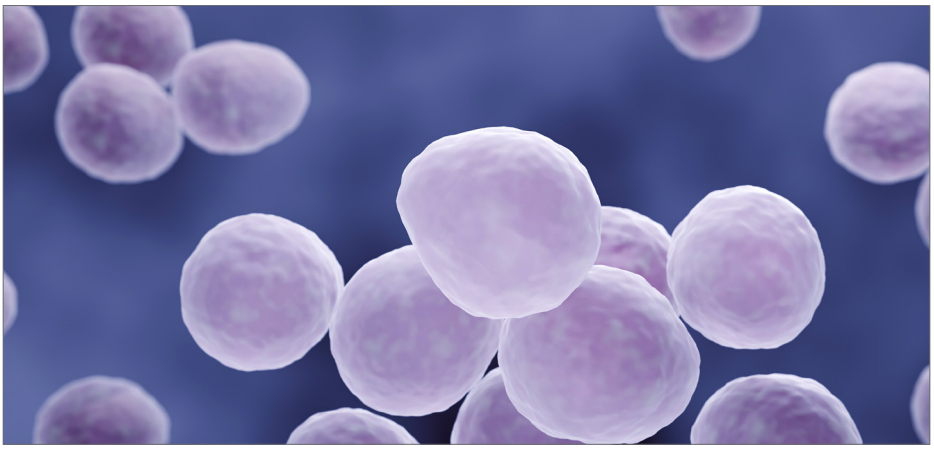
regulatory agency requirements for biosafety testing of biological products and their materials used for GMP production.

Mycoplasma testing. Cell lines used for manufacturing biologics should be tested for the presence of mycoplasmas as described in the U.S. Code of Federal Regulations 21 CFR.610.18.[3] In addition to master and working cell banks and cells at the end of production, unprocessed bulk, any control cells, virus seeds, and final products should be tested for mycoplasmas. That testing should include all compendial procedures prescribed in USP <63> and EP2.6.7[4–5]:

- Broth and agar culture tests.
- Indicator cell culture test.
- Inhibitory substances (mycoplasmastasis) test.

All these procedures are required as per regulation and are necessary for each production lot of biological products licensed in the United States, unless the FDA grants a waiver in accordance with 21 CFR 610.9[9] for the use of an alternative mycoplasma testing method(s) as described in the EP2.6.7.[5] Incubation times may be reduced with higher sample inoculation volumes, but larger delivery gains are achieved with nucleic acid–based testing (for example, PCR).

RCV testing. When replication-defective and replication-selective vectors are used for manufacturing of biologics, both master cell and master viral banks should be shown to be free of RCVs as described in an update to "Points to Consider in Human Somatic Cell Therapy and Gene Therapy."[10] In addition, patients should be tested for the presence of RCV prior to and after treatment with CGT products.



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Although minimum culture regulations must be met, assay optimization for viral serotype can support reduced assay length. Reliable GMP replication-competent assays have been developed using the 7500 Fast Real-Time PCR System for detection RCVs, bringing assay length from over 40 days to just weeks.* These assays can be used to support manufacturing of CGT products.

Advances in science and patient therapies come with additional safety challenges. Testing for mycoplasma and RCV throughout the process of manufacturing biologics ensures adherence to regulatory requirements and allows for possible issues to be discovered before final product testing, contributing to the ultimate goal of quickly and safely getting life-saving treatments to patients. GEN

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*The MycoSEQ real-time PCR assay from Thermo Fisher Scientific can be used as an alternative to compendial mycoplasma methods. It is accepted by regulatory agencies for lot release testing of biological products, mostly for cell- and gene-based therapies, but also for in-line process sample analysis. The assay is developed on the Thermo Fisher Scientific 7500 Fast Real-Time PCR System and can be used with automated DNA extraction platforms.

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