Regulatory Aspects of Mycoplasma Testing for Cell Therapy Products

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FDA-Approvable Release Tests for Detecting Mycoplasma Contamination

Until quite recently, there were only two mycoplasma testing paradigms that were approvable by the U.S. Food and Drug Administration (FDA) as lot release tests for all biologics, including cell therapies[1]. Both involved detection of live mycoplasma in culture. The primary method applicable to products such as recombinant proteins, monoclonal antibodies and cell therapies is described in the FDA Points to Consider (PTC), 1993[2]. This document has recently been updated, and in the view of many regulatory affairs specialists, was superseded in 2010 by the United States Pharmacopeia (USP) chapter <63> Mycoplasma Tests[3]. As a result of the latter guidance, this particular method may be considered compendial, meaning that the assay need not be validated by the laboratory performing the test as long as the described methodology is strictly adhered to and is found to be suitable for the specific sample matrix being tested (more on this later). This monograph will refer to the PTC/USP methods collectively as USP, as the USP method is more stringent in terms of the requirements for assessing suitability of mycoplasma growth media and for conducting inhibition (mycoplasmastasis) testing. The USP method involves the inoculation of test sample into liquid media and agar, as well as a mammalian cell line, to detect fastidious (i.e., non-cultivable) mycoplasma that may not be detectable in the growth media.

Applicable to traditional live virus-based vaccines and viral stocks, the testing paradigm described in 21 CFR 610.30 Test for Mycoplasma is another growth-based (culture) method. The primary differences between this method, referred to here as the CFR method, and the USP method are in the media and atmospheric conditions used and that the mammalian cell line portion of the assay is not called for in the CFR method. In fact, the types of test samples that are evaluated in the CFR method (i.e., live virus containing vaccines and viral stocks) are in many instances incompatible with the mammalian cell portion of the USP assay, as the viruses may be capable of growing in and causing cytopathic effect and/or lysis of the host cells.

Both the USP and CFR methods take 28 days to complete and consume 9-15 mL of test sample. It is important to note that modifications to these culture-based methods are considered by the FDA on a product-by-product, process-by-process basis. As is the case with any compendial method, changes that are made to the procedures must be justified. If the changes are considered significant, the modified compendial test may be subject to requirement for full validation.

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[1] Cell therapies include more than minimally manipulated cells (stem cells and somatic cells), regenerative medicine, and tissue engineered products.

[2] Center for Biologics Evaluation and Research. US Food and Drug Administration. Points to consider in the characterization of cell lines used to produce biologicals, 1993. http://www.fda.gov/downloads/biologicsbloodvaccines/safetyavailability/ucm162863.pdf. Accessed 6/20/2014.

[3] General Chapter 63, "Mycoplasma Tests". USP 33-NF 28 Reissue. US Pharmacopeial Convention, Rockville, MD, pp. 88-91.

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When the USP test is used, each type of test sample to be assayed must be qualified for use to guard against false negative results. This suitability or inhibition testing, commonly referred to as mycoplasmastasis testing, is required to ensure that nothing inherent in the test sample interferes with (inhibits) growth of mycoplasmas under the described culture conditions. While not specifically called for in the CFR test description, it is scientifically correct to include such mycoplasmastasis testing when using this method. Mycoplasmastasis is evaluated through spike-recovery experiments that are described in the USP chapter, along with the acceptance criteria used to interpret the results.

Benefits of Moving Away from Traditional Culture-based Mycoplasma Tests

Despite having been in use for many years, the culture-based mycoplasma tests were not developed with today's next-generation products in mind. In the case of many modern biologics, including stem cell and regenerative medicine products, the culture-based tests cannot be completed rapidly enough to be of use in assuring patient safety. As a consequence of the limited shelf-life of many cell therapy products, time on test is a major consideration in selecting an appropriate mycoplasma detection test. It is possible to treat patients with such products (i.e., before mycoplasma results are obtained on day 28 of the culture-based test), but it is not the optimal situation as freedom from mycoplasma is part of the safety profile of these products. In these cases, fast access to test results is of utmost importance. Turn-around time is not the only inconvenient aspect of the compendial test: the culture methods also require a relatively great volume of test sample. For therapies that are single-dose or limited-dose products, it might not be practical to allocate the volume required for culture-based testing. For example, expanded cord blood units rely on a minimum transplantable cell number to confer the optimal therapeutic benefit, which could be compromised if a disproportionately large percentage of the manufacturing run is dedicated to mycoplasma testing. Having results reported out sooner while reducing the overall amount of product needed for testing could have a positive impact on the process and profitability.

Working PCR-based Mycoplasma Testing into Your Processes

Until quite recently, PCR-based methodologies for mycoplasma testing have not been approved for lot release of biologics bulk harvest materials, more than minimally manipulated cell therapies or viral vaccines. Despite becoming more familiar to regulators and some recent acceptances, PCR-based mycoplasma testing is still considered on a case-by-case basis as universal approval for PCR testing has not been granted. In the current environment, it is possible to take advantage of the efficiency gains offered by PCR-based mycoplasma testing. Rapid turn-around makes PCR-based mycoplasma testing perfect for spot-checking research materials. The quick time to results and low product requirement also makes PCR-based mycoplasma testing ideal for assessing in-process samples and test samples intended for IND-enabling studies. PCR can also be used to assess Phase I and Phase II clinical material for the presence of mycoplasma before distribution. That being said, it is always a good idea to consult your regulatory team and alert the FDA before applying alternative mycoplasma testing methodologies for release of early-stage clinical material.

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PCR-based Mycoplasma Testing for Lot Release of Cell Therapies

Since only culture-based mycoplasma detection methods have been approved by the FDA for lot release of biologics, alternative mycoplasma testing platforms, including modifications to the compendial tests and total replacement with nucleic acid testing, are being considered by the FDA on a product-by-product, process-by-process basis. Demonstrating appropriateness of an alternative mycoplasma testing platform involves comparing several key performance attributes to the culture-based compendial method. These include but are not limited to sample suitability, breadth of detection of mollicute species, specificity for mollicutes and sensitivity. Although no formal FDA or PTC documents currently exist to structure such comparability studies for FDA review, insight into study design can be drawn from guidance published by the European Pharmacopoeia in chapter 2.6.7 Mycoplasmas[4]. In addition to the important assay performance criteria described in that chapter, the FDA may expect to see a risk assessment addressing a variety of factors pertaining to the specific manufacturing process and product. These factors include the types of raw materials being used in the manufacture, the testing of such materials, the in-process testing conducted, the downstream purification processing used (if any), the track record for mycoplasma contamination of similar production processes, the patient profile and the dosage regimen, and other considerations. As mentioned above, manufacturers contemplating the replacement of the culture methods with a PCR-based method should be prepared to work with the FDA early in the process and should be prepared to justify the use of the methods both in terms of assay performance and patient risk. We anticipate a growing number of cell therapy in addition to more traditional biologic products will take advantage of PCR-based mycoplasma testing for release of clinical trial material and licensed product in the coming years.

LABS Provides PCR-based Mycoplasma Testing

LABS offers the MycoSEQ™ Mycoplasma Detection Kit from Life Technologies as a service for those who require research, informational use only, in-process and Phase I/II clinical trial mycoplasma testing. MycoSEQ™ has the following advantages over other PCR-based mycoplasma test kits that LABS has evaluated:

- Real-time PCR
- Shortest time-on-test
- Highest throughput
- Can accommodate a wide range of test sample volumes
- Greatest breadth of mycoplasma species detected (> 90 species)
- Tightly controlled manufacturing / quality assurance
- Most comprehensive evaluation of sample suitability and PCR inhibition
- Orthogonal readouts that include cycle threshold, amplicon melt temperature and amplicon quantity
- Does not cross-react with DNA from common production hosts or closely related bacteria

[4] European Pharmacopoeia, 7th Ed., Section 2.6.7: Mycoplasmas. 01/2008:20607. Corrected 6.1.

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As with all tests offered at LABS, MycoSEQ™ was thoroughly validated in-house before it was offered to the community. Rapid turnaround and accommodating sample volumes ranging from as little as 0.1 mL to 15 mL (or more) represent considerable improvements over the culture-based mycoplasma testing methods. Consistent with culture-based methods, samples must be determined to be suitable for testing on the MycoSEQ™ platform. As a measure of suitability, samples from at least three different test sample lots must be evaluated and found to be free of PCR inhibition before recurring testing can begin.

Summary

- Culture-based mycoplasma testing is currently the method most commonly approved by the FDA for lot release testing for biologics, cell therapies and viral vaccines.
- PCR-based mycoplasma testing has not been universally approved for release of clinical material or marketed product.
- PCR-based mycoplasma testing can be used in your process.
  - Method should be validated before use.
  - Sample type must not interfere with the assay.
- LABS validated the MycoSEQ™ Mycoplasma Detection Kit from Life Technologies.
  - The test can currently be used to assess research samples, in-process samples and Phase I/II clinical samples for the presence of mycoplasma.
  - Suitability testing to replace culture-based methods with a PCR-based mycoplasma detection method for release of Phase III clinical material and marketed product can be performed at LABS in support of discussions with the FDA.

Contact The LABS Team for more information about our fast and reliable mycoplasma detection testing services.

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About the Authors

Stacey LaBombard, Matthew Brenton, Aaron Schieving and Russell C. Marians, Ph.D. are Associates of LABS, Inc. Dr. Dionne is the Laboratory Director of LABS-Philadelphia and Dr. Bauer is the Medical Director of LABS, Inc. and also serves as the Laboratory Director for the Centennial, CO facility. Dr. Marians is the Manager of Molecular and Cell Biology at LABS, Inc. and can be reached with any questions or comments by email Russell_Marians@LABS-Inc.org.

About LABS

Our mission is to ensure safe and effective transplantation by providing the highest quality laboratory testing services and information right when it is needed. LABS, Inc. is a comprehensive, highly accredited clinical reference testing laboratory serving the transplantation community. Our customers include Organ Procurement Organizations, Reproductive Health and Transplant Centers, Tissue, Eye and Blood Banks, and Biomedical and Regenerative Medicine product manufacturers. The testing services provided by LABS have been inspected by multiple regulatory and accrediting agencies for more than 30 years to ensure our services aid in providing quality outcomes to our valuable customers. Please refer to our website for a complete list of LABS accrediting bodies. When quality is key and timing is everything, LABS provides accurate results and information when needed. Our goal is to understand your operational needs and provide a level of responsiveness that makes a meaningful difference to your business. Contact us if you would like to speak to a customer support representative to learn more about LABS.

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