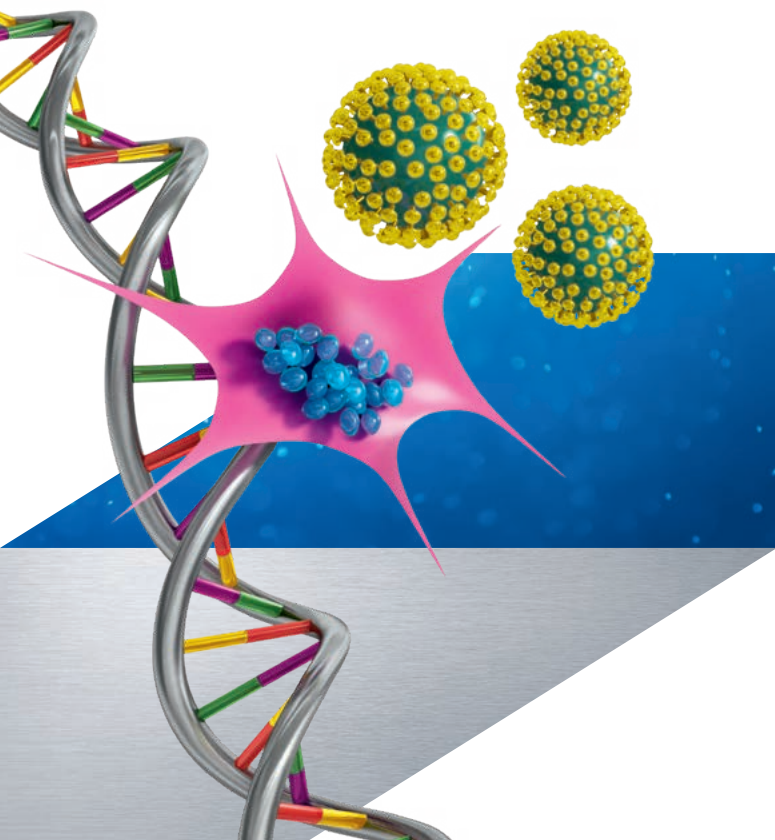


Mycoplasma Conference Summary

Preparing for an imminent transformation in how we treat diseases

*2nd International Mycoplasma qPCR Testing User Day -
PharmaLab Pre-Conference Event in Düsseldorf on
November 11th 2019*



Introduction

As medicines evolve towards biologics, gene and cell therapies, experts view nucleic acid amplification techniques (NAT) as suitable methods to ensure product safety in the face of new modalities, complex matrices, and tight production timelines. Mycoplasma testing is no exception.

- What are some **practical** considerations for implementing NAT-based methods?
- Where are **concrete** recommendations necessary?
- How can guidelines remain **relevant** for new medicinal products?

These questions were discussed at the PharmaLab Pre-Conference Workshop on Mycoplasma qPCR Testing on November 11, 2019.

New medicinal products bring new challenges for mycoplasma testing

Novel medicinal products like gene and cell therapies are revolutionizing healthcare, shifting the focus of treatment development from small molecules to so-called biologics, cells, and even tissues. The shift, however, poses new challenges for quality and safety assurance strategies. This is especially true in testing for mycoplasmas, a ubiquitous and practically invisible bacterial contamination of cell cultures.

These challenges were a common thread in presentations at the 2019 PharmaLab Pre-Conference Workshop on Mycoplasma qPCR Testing. The gathering of academic and industry experts was befittingly kicked off with a talk by Jan-Oliver Karo of the Paul-Ehrlich-Institut on trends and expectations for mycoplasma testing given the anticipated revision of European Pharmacopoeia chapter 2.6.7. The lively exchange between presentations was a testimony to the active involvement of a research community exploring solutions.

Preparing for the new and the yet-to-come

Turnaround time is a critical issue for mycoplasma testing of biopharmaceuticals because delays in manufacturing can be expensive and restrict the availability of much-needed medications. Now, with the advent of advanced therapy medicinal products (ATMP) based on genes, cells, and tissues, turnaround is even more poignant. Many of these products have a short shelf life and are destined for patients with high medical need. Time is of the essence.

The demand for faster detection of mycoplasmas has moved nucleic acid amplification techniques (NAT) into the spotlight. Compared to the 'classical' compendial methods that are culture-based, NAT detect the presence of mycoplasma DNA via a polymerase chain reaction (PCR), which slashes time-to-results from days to hours.

Turnaround time, however, is only the tip of the iceberg. Other considerations are also critical in implementing NAT-based methods to ensure product safety. "A major challenge," points out Karo, "is that ATMP are widely diverse, for example, in terms of the origin of starting materials and final biological matrix." This diversity will also increase with time as further innovation expands the spectrum of approaches and applications for ATMP.

- What stages in a process are appropriate testing points?
- Which sample types are most suitable for the test?
- How to construct a streamlined validation design that meets regulatory requirements?

These are essential questions to ask and answer with the revision of guidelines.

Intracellular infection is the focus of detection

Experience has shown that not all time points in the production of a biopharmaceutical or ATMP are adequate for mycoplasma testing. Furthermore, culture supernatant or other cell-free matrices can be insufficient for testing because mycoplasmas typically attach to or even invade cells. Clearly, sample selection is a critical parameter for a validated testing strategy. "It is therefore recommended that cell-free matrices be avoided or thoroughly justified," stated Karo. In fact, most commercial test developers are moving toward cellular matrices and looking at ways to overcome technical hurdles caused by high cell counts.

From a validation standpoint, it is imperative to work with complex samples or "worst-case products," as Christiana Schnitzler called them in her presentation. She is leading a team at Boehringer Ingelheim through the generic validation of Roche's MycoTOOL Mycoplasma Real-Time PCR Kit. The kit was designed to work with native samples, and Schnitzler is examining if the assay is robust to PCR inhibitors, to DNA extraction variation, and to reagent and sample handling. Ultimately, her goal is to demonstrate that the sensitivity of the assay is at least on par with 'classical' compendial methods. The team's data on the limit of detection are still being collected, but preliminary results indicate that the regulatory requirement for sensitivity (≤ 10 CFU/mL) can be achieved for the ten tested mycoplasma species.

A new role for compendial methods

Schnitzler's implementation approach couples the assay validation with comparability studies to determine if the PCR method is as sensitive, specific, and robust as the 'classical' compendial Culture and Indicator Cell Culture Methods. At this point, NAT-based methods can substitute the more laborious, sample-intensive, and lengthy gold standard methods only if this equivalency can be demonstrated.

Yet, a strong implementation strategy would be one that uses NAT as a first line of detection and culture-based compendial methods for results verification. Roche Pharma, for one, has been using its MycoTOOL Mycoplasma Real-Time PCR solution on its manufacturing floors for years and leverages the culture-based methods for verification purposes. "Our experience so far has been that this strategy is welcome by regulatory bodies and results in successful submissions," declared Alexander Bartes from Roche Pharma.

The right constellation of technologies for a fast yet comprehensive testing strategy can also leverage existing facilities. Schnitzler, for example, opted for another method to verify study results. "We are in the fortunate situation," she described, "of having a sequencing lab next door. We confirmed all species identifications through sequencing."

From validation to workflow integration, details matter

Commercial mycoplasma tests based on NAT offer the convenience of an optimized assay architecture with broad species coverage and high sensitivity, plus the option of high-throughput testing and automation. Several presenters shared assessments of tests on the market and lessons learned that could make implementing NAT-based methods easier for others.

For example, Andrej Steyer from the Institute of Microbiology and Immunology of the University of Ljubljana Faculty of Medicine highlighted the need for well-characterized validation standards with a low genome copy to CFU ratio (GC:CFU). Steyer compared an SYBR Green mycoplasma test and the probe-based MycoTOOL Mycoplasma Real-Time PCR Kit. Having opted to focus on the probe-based kit because it exhibited slightly better sensitivity and less variability in CT values,

Steyer examined its performance on two different cyclers and with automated DNA extraction. A ten-fold difference in GC:CFU ratio between the two mycoplasma reference standards used in the study re-emerged in the resulting PCR and sequencing data. Steyer advised to “bear in mind the quality of mycoplasma reference standards and the impact that these might have.”

The robustness of a commercial solution to adaptations that enable its integration into existing automated lab setups was the focus of another presenter. Christie English from Mycoplasma Experience Ltd. described input volume adjustments her team made to allow automated DNA extraction for the MycoTOOL Mycoplasma Real-Time PCR Kit on the MagNA pure Compact instrument (the kit was developed on the larger MagNA Pure 96). The adaptations were successful, and a comparison with the mycoplasma testing solution already used in the lab revealed complementary benefits of the two solutions. “In fact, we are now using both kits,” concluded English.

At a glance

- Nucleic acid amplification techniques (NAT) detect the presence of mycoplasmas in a few hours, a welcome improvement in turnaround time that can prevent manufacturing disruption and supply shortages of biopharmaceutical products and advanced therapy medicinal products (ATMP).
- Implementing NAT-based methods for mycoplasma testing requires comprehensive validation and demonstration that performance is at least equivalent to the ‘classical’ compendial methods.
- Experts at this year’s PharmaLab Pre-Conference Workshop on Mycoplasma qPCR Testing discussed the anticipated revision of European Pharmacopoeia chapter 2.6.7. They made suggestions to create „one mycoplasma control concept“ that also covers the diversity of novel medicinal products in development.
- Experiences of the presenters and the audience highlighted several consensus points, including the importance of using cellular matrices for testing and the potential of an implementation strategy that positions NAT as a fast first line of detection, followed by results verification via culture-based compendial methods.

Balancing requirement and foresight

The topics discussed at the workshop accentuated the need for more detailed guidance in validating and implementing NAT-based methods. However, it will be essential to ensure that concrete recommendations do not distract from creating guidelines that remain relevant for future products.

A case in point was an exchange about the mycoplasma reference strains required as test organisms for validating NAT-based methods and as positive controls in routine testing. The species coverage of existing PCR-based mycoplasma tests is quite comprehensive. For example, the MycoTOOL Mycoplasma Real-Time PCR Kit detects roughly 150 culturable and non-culturable strains. This broad coverage can be leveraged for generic validation. Among others, Prof. Dr. Renate Rosengarten of the Institute of Microbiology at the University of Veterinary Medicine in Vienna and Director of Mycosafe Consulting recommended expanding the list of mycoplasma species required for testing with NAT-based methods. Karo agreed with the suggestion and underscored that a corresponding discussion is within the scope of guideline revisions. In this context, he pointed out that validation strategies must capture the specific range of mycoplasma species that can potentially contaminate a product. “We endorse the use of validated NAT,” stated Karo. “However, in addition to a generic validation, we emphasize the need for a product-specific validation supported by a thorough risk assessment of the product.”

Ultimately, the open exchange witnessed at the pre-conference workshop will enrich the revision process of EP chapter 2.6.7. Expertise, lessons learned, and experiences of the community will help strike the right balance between clear expectations and broad scope for future ATMP. Inviting input from all workshop participants during the revision phase, Karo characterized the outcome for the updated regulatory document as “one mycoplasma control concept” that is aligned with the current state of knowledge and applicable to diverse products and processes.

A dialog to overcome hurdles

Together, the workshop presentations highlighted a range of relevant considerations for NAT validation – from the right matrix to test and the correct reference standards to use, to the feasibility of integrating a particular solution into an existing laboratory setup and routine. “In my experience,” pointed out Karo, “the issue boils down to the question ‘*how far do you want to stretch safety?*’ And most companies don’t seek advice, although it’s needed.” Luckily, the scientists evaluating the reality of NAT for mycoplasma testing are in dialog to overcome challenges and provide exactly that: advice that can usher in new **and safe** medicines.

About Roche CustomBiotech

Roche CustomBiotech is the business division through which Roche Pharma and Roche Diagnostics manufacture and supply high-quality raw materials for pharmaceutical and diagnostic enterprises.

Roche Pharma was one of the first Big Pharma to develop NAT methods for Mycoplasma detection and now uses the optimized solution to release marketed products and products in clinical development.

As of today, Roche Pharma has received FDA approval for 5 commercial products and more than 15 products in clinical development that were released through testing with the MycoTOOL Mycoplasma Real-Time PCR solution. The kit is commercially available.

Visit <http://go.roche.com/mycotool> for more information.

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