

# Validation of a PCR coupled to a microarray method for detection of mycoplasma in vaccines

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## Abstract

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The revised section of the European, United States, and Japan Pharmacopeias on mycoplasma testing provided guidance for the set up and validation of a nucleic acid amplification technique (NAT) as an alternative method to agar culture and indicator cell culture compendial methods. The CytolInspect™ method, based on Polymerase Chain Reaction (PCR) coupled to microarray analysis, has been selected for detection and identification of mycoplasma in vaccines. To replace compendial methods, the alternative method must demonstrate equivalence in both limit of detection (LOD) and specificity compared with compendial methods. Here, we summarize the validation of the CytolInspect™ method according to current pharmacopeia requirements. Validation of the robustness, sensitivity (at least 10 colony forming units/ml) and specificity of the CytolInspect™ method are demonstrated. Likewise, a comparability study was performed to compare the LOD for CytolInspect™ compared with the previously validated LOD for compendial culture tests.

## Summary

Sanofi Pasteur in Marcy l'Etoile is using the CytolInspect™ of Greiner Bio-One GmbH in routine testing of vaccines “for rapid, specific and sensitive detection and identification of mycoplasma species” and we are pleased to give you an overview of Sanofi Pasteur’s recently published paper describing the successful validation strategy and data using the CytolInspect™ for mycoplasma testing.

For access to the full text version of the interesting and informative paper “**Validation of a PCR coupled to a microarray method for detection of mycoplasma in vaccines**”, **Abachin E et.al, *Biologicals* (2017)** please follow the link:

<https://www.ncbi.nlm.nih.gov/pubmed/28951118>

**In the paper Sanofi Pasteur outlines the importance of mycoplasma testing and presents an overview of regulatory requirements in biopharmaceutical production, which we summarized as follows:**

Mycoplasmas (class of *Mollicutes*) frequently occur as contaminants in cell-derived biological products, that is why international regulatory agencies (e.g. European Pharmacopoeia, Japanese Pharmacopoeia, United States Pharmacopeias) require the demonstration of mycoplasma absence at various stages in the manufacturing process. In order to overcome disadvantages of routinely used compendial methods – agar culture and indicator cell culture methods – including the long-time testing (28 days), the narrow detection range and the impossibility of mycoplasma identification, Pharmacopeias worldwide have introduced nucleic acid amplification technique (NAT)-based methods for potential replacement of compendial methods after suitable validation to demonstrate equivalence in limit of detection (LOD) and specificity compared to compendial methods.

With the aim to implement a rapid and robust mycoplasma testing method, Sanofi Pasteur has assessed different NAT-based methods within their development. We are proud that Sanofi Pasteur has selected the CytolInspect™ because of several main advantages of the test, including the broad range of detectable and identifiable species for increasing the agility in root-cause analysis and the high specificity and the robustness of the assay.

**As Sanofi Pasteur intended to replace both compendial methods by the CytolInspect™, they comprehensively demonstrated the equivalence or better performance of the CytolInspect™ compared to compendial methods in their validation study:**

Sanofi Pasteur’s validation strategy mainly based on European Pharmacopoeia (chapter 2.6.7) and Japanese Pharmacopoeia (chapter G3) and included a full validation on a generic matrix for determination of specificity, robustness and performance comparison with compendial culture-based methods, a specific matrix verification for demonstrating the absence of interference and a comparability study for replacing both compendial methods.

**In the section below, we summarized the used material and methods, which are specified in the original article in detail:**

For ensuring a reliable comparison of compendial methods with NAT-based technologies, a well-characterized reference material is considered as an important prerequisite. Sanofi Pasteur participated in collaborative studies of the FDA and WHO for mycoplasma strain characterization and the respective importance for a meaningful comparability of conventional test methods and NAT-based assays.

**For further information on the CytolInspect™ please contact us ([support.dx@gbo.com](mailto:support.dx@gbo.com)) or visit our website:**

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[www.gbo.com/diagnostics](http://www.gbo.com/diagnostics)

To determine sensitivity, specificity and robustness of the CytolInspect™, Sanofi Pasteur used mycoplasma strains of ATCC and EDQM, harvested in the exponential phase, aliquoted and titrated. Purity and identity as well as the GC/CFU ratio were characterized prior freezing. For specificity testing, phylogenetically related bacteria were tested and a commonly used cell culture medium for vaccine manufacturing was chosen as generic matrix.

Subsequent of DNA extraction, the detection and identification of mycoplasmas was performed using the CytolInspect™ test kit containing a comprehensive control system for monitoring each step of the analysis (extraction, PCR and hybridization). The CytolInspect™ is based on PCR- and microarray-technology, allowing the detection of all mycoplasmas via one universal probe while simultaneously identifying 41 of the most important mycoplasma species (species found in >99% of reported contamination events).

**Sanofi Pasteur's generic validation according European Pharmacopoeia and Japanese Pharmacopoeia consisted of the testing on following parameters and we would like to give you a short overview of the respective results in the section below:**

- Robustness study including the variation of several parameters with regard to temperatures and reagents was carried out with *M. orale* spiked generic matrix (10cfu/ml) in independent test runs:
  - ➔ The outcome of the robustness study was stated in the paper: *"For the tested parameters, the CytolInspect™ method was robust since no deviation from the expected assay results was observed."*
- Specificity testing assessed in context of internal cross-reactivity (*M. orale* 10cfu/ml spiked generic matrix) and cross-reactivity with related bacteria (close relation to mycoplasmas) in independently performed test runs and by various analysts using different lots of generic matrix:
  - ➔ Expected results have been obtained by Sanofi Pasteur's validation, *"indicating the specificity of the method."*
- The LOD corresponds to the minimum cfu number per volume, that is detected in 95% of test runs. Sanofi Pasteur determined the LOD in independent test runs performed by different analysts in various dilutions and multiple replicates.
  - ➔ In the paper Sanofi Pasteur described that *"all mycoplasma species tested were detected with limits of detection between <10 and <1 cfu/ml. The test fulfills the requirement of the international compendia pharmacopoeias ..."*
- Several parameters including limit of detection, specificity and robustness have been part of the comparability testing of the CytolInspect™ with the agar culture and indicator cell culture methods. An at least equivalent or even better performance of the CytolInspect™ was the outcome of the comparison study:
  - ➔ *"The CytolInspect™ method detects a broad range of mycoplasma and is specific to mycoplasma detection and identification of the 41 most important mycoplasma species, including non-cultivable mycoplasma" that is why "...this method, in term of breadth of specificity can replace both compendial methods."*
  - ➔ *"...CytolInspect™ method has shown a LOD of <10 cfu/ml for all mycoplasma species mentioned in the Ph. Eur. including S. citri. The LOD with the CytolInspect™ method is better than or as sensitive as compendial methods."*

**In the last sections of Sanofi Pasteur's paper, the authors discussed and concluded the results of their validation study. We resumed the outcome of the discussed topics as follows:**

- Sanofi Pasteur's validation design is fully complaint with Japanese Pharmacopoeia (chapter G3) and European Pharmacopoeia (chapter 2.6.7) and is aligned with recommendations of USP <1223> and PDA technical report No 50.
- CytolInspect™ is *"capable of detecting a wide variety of mycoplasma species"* and *"was shown to detect an additional relevant species, S. citri, which was not detected by either the culture method or in the indicator cell culture method ..."*. Temperature and volume variations are not influencing the CytolInspect™ results, thus the assay *"is suitable to be utilized on a routine basis."*
- The CytolInspect™ was Sanofi Pasteur's method of choice to overcome long time testing and sensitivity issues of compendial methods related to *"inconsistency in media preparation and quality of media components."* In the publication the rapid identification of mycoplasmas for fast definition of corrective actions due to facilitated root-cause determination was highlighted, beside the increase of representativeness by the ability of processing 10ml samples (considered as representative sample volume).

Sanofi Pasteur has presented their successful validation and submission strategy as well as the return of experience using the CytolInspect™ to release vaccines on several conferences including the Pharmalab 2017 in Düsseldorf and the SMI 2019 in London. Their innovative and global approach for mycoplasma testing is seen as a model for high industrial performance and securing quality and safety.

**For further information on the CytolInspect™ please contact us ([support.dx@gbo.com](mailto:support.dx@gbo.com)) or visit our website:**