## Comparability Study between the MycoSEQ™ Plus Mycoplasma Detection Kit and Compendial USP<63> Test

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Introduction

These studies were performed using Clarified CHO Bioreactor Bulk Harvests as representative test articles, meaning that cells were removed by centrifugation and filtration prior to test. The test article

There is high demand for rapid, automated alternative microbial detection methods to streamline workflows and reduce turnaround times. For mycoplasma testing, the compendial USP<63> test consists of a culture method, in broth and on agar, as well as an indicator cell culture method, and requires multiple analysts and up to 28-days to complete.<sup>1</sup>

The MycoSEQ™ Plus Mycoplasma Detection Kit², from Thermo Fisher Scientific, is a probe-based qPCR assay that offers improved workflow efficiency through simplified sample preparation and built-in assay controls, resulting in shorter turnaround times compared to compendial culture-based methods.

Bionique Testing Laboratories has performed studies to assess comparability between the MycoSEQ Plus lot-release method<sup>3</sup>, for supernatant, spent media, or waste stream samples, and our Harmonized USP<63> Mycoplasma Test.

## Objectives

These studies aimed to:

- Assess the ability of the MycoSEQ Plus method to successfully recover and detect viable mycoplasma at ≤10 CFU/mL from the representative test article
- Confirm mycoplasma detection from the same representative test article at 10 CFU/mL following the compendial USP<63> test
- Evaluate equivalency between the MycoSEQ Plus and compendial testing methods through the comparison of qualitative results

was evaluated as a non-spiked sample and independently spiked with 7 strains of viable mycoplasma using both the MycoSEQ Plus and compendial methods.

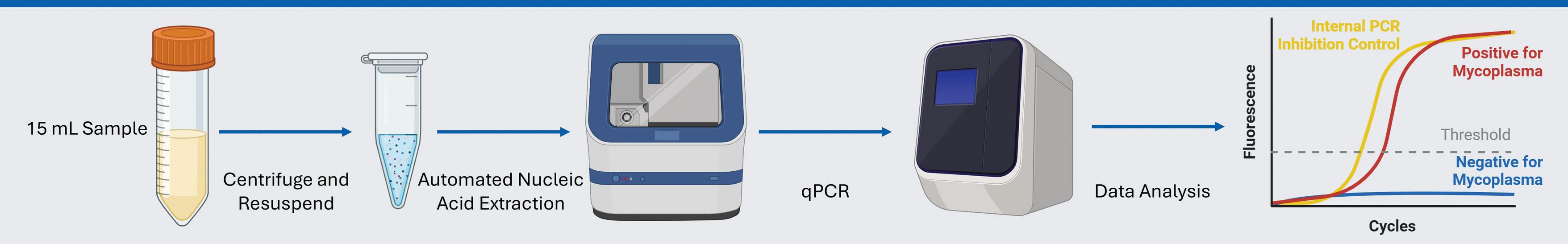
Study Design

The MycoSEQ Plus method was evaluated over the course of two days per species. For this method, 15 mL sample volumes were prepared from two batches of the test article that were pooled together due to volume constraints. On the first day, samples were prepared, and an initial evaluation was completed to confirm the DNA content aligned with the standard curve expectations for the given mycoplasma species. All remaining samples were stored at 5±3°C. The next day these samples were adjusted, if necessary, and evaluated following the same MycoSEQ Plus method.

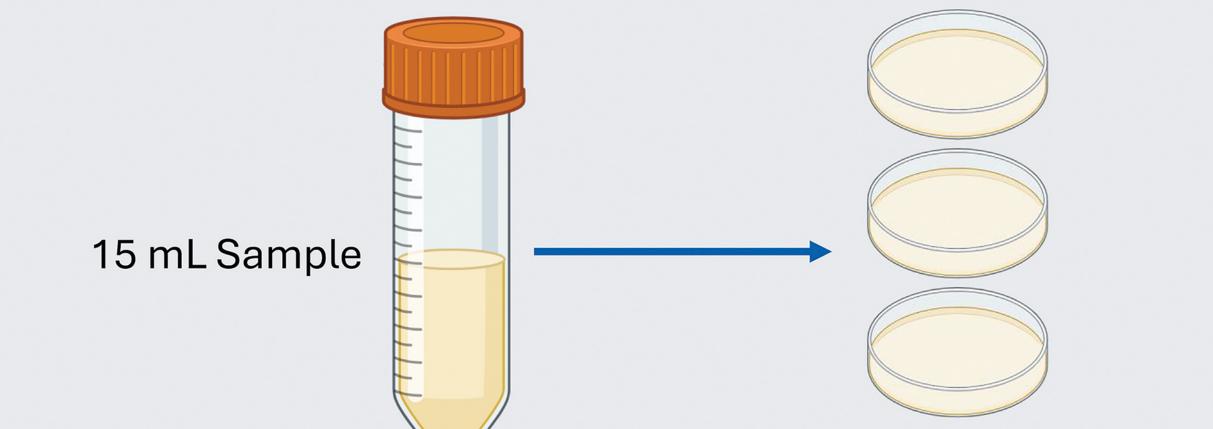
For the compendial USP<63> method, 15 mL samples were prepared with one of the test article batches used for the MycoSEQ Plus method and spiked using the same lots of mycoplasma. All samples were frozen at -80±15°C to facilitate shipping to our compendial methods lab for evaluation with the Harmonized USP <63> Mycoplasma Test.

The qualitative results for the non-spiked and mycoplasma-spiked samples have been directly compared to assess equivalency between the MycoSEQ Plus and compendial USP<63> methods.

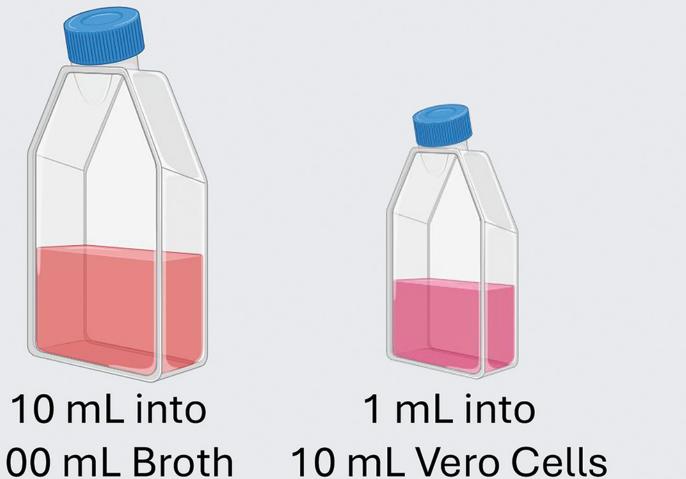
## MycoSEQ Plus Method

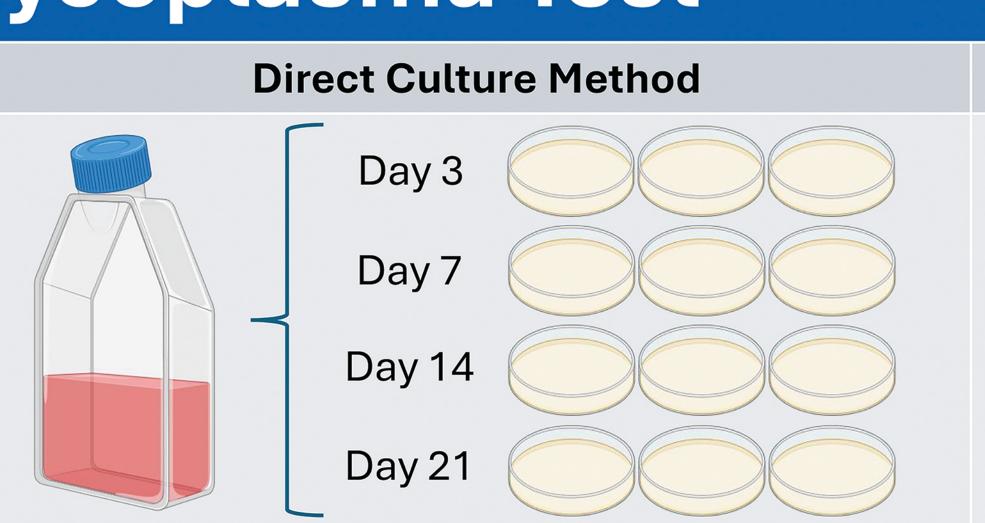


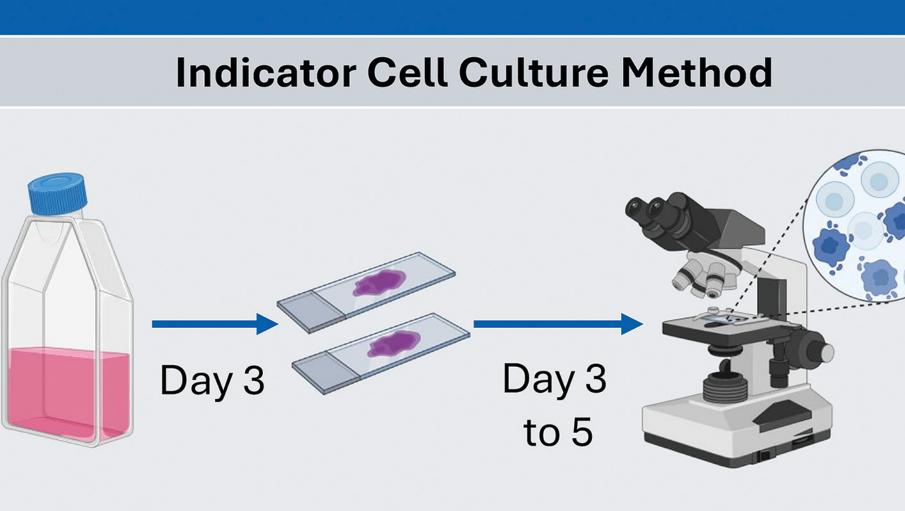
## Harmonized USP<63> Mycoplasma Test



0.1 mL each







# Discussion

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While the compendial test relies on mycoplasma detection through viable organisms, nucleic acid-based methods detect DNA/RNA. The need to reduce the final concentration of both M. pneumoniae and M. fermentans emphasizes the importance of using accurately characterized mycoplasma stocks with low GC:CFU ratios.

Initial MycoSEQ Plus evaluations for 10 CFU/mL of M. pneumoniae generated significantly stronger signals (lower Ct values) than expected for this concentration. This could be attributed to the post-freeze GC:CFU ratio of the stock being 101. Prior to the second evaluation, the test articles spiked with M. pneumoniae were diluted 1:10 to lower the final genomic copy concentration, simulating a lower GC:CFU ratio as required to obtain reliable results in nucleic acid-based applications.4 M. pneumoniae was ultimately evaluated at a theoretical spike of 1 CFU/mL with a GC:CFU ratio of 101, rather than 10 CFU/mL with the same ratio.

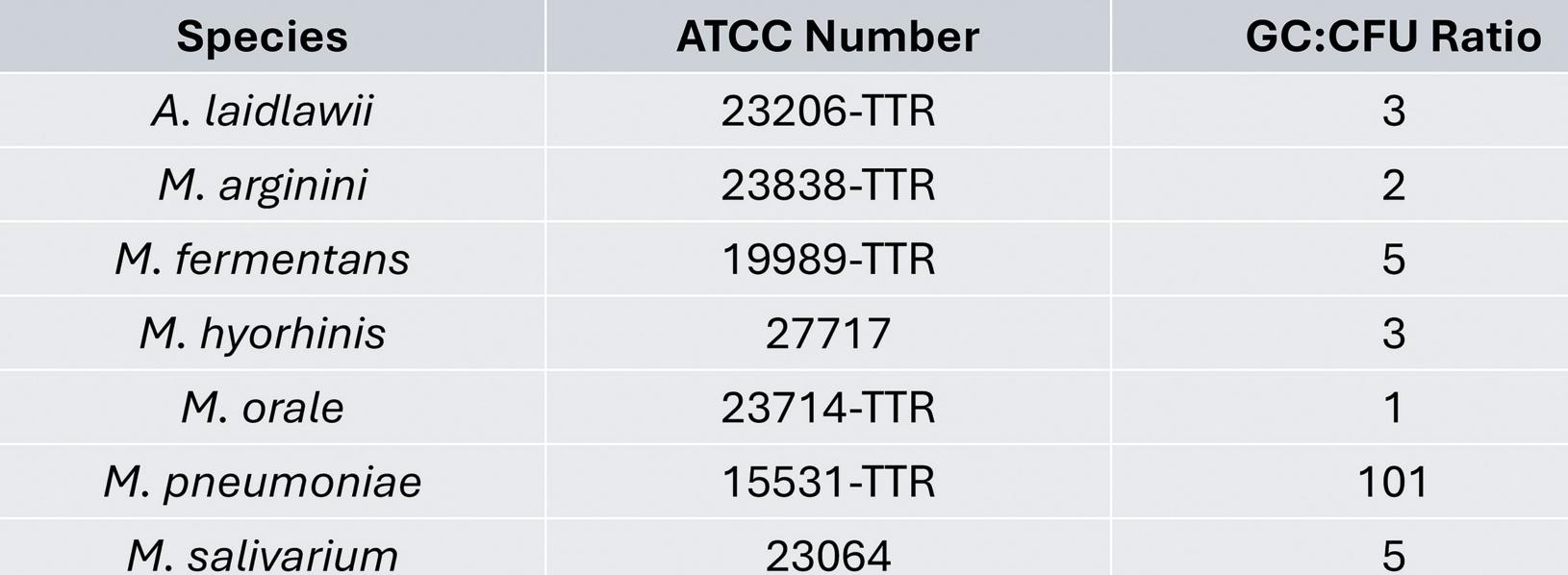
Similarly, the evaluation of *M. fermentans* was repeated at 2 to 3 CFU/mL after initial results yielded Ct values that reflected a higher GC:CFU ratio than the reported ratio of 5.

## Conclusions

For these Clarified CHO Bioreactor Bulk Harvests, both the MycoSEQ Plus and USP<63> methods achieved complete detection of each mycoplasma species evaluated, with no evidence of assay interference. As such, the data support product-specific equivalency between the methods when testing these Clarified CHO Bioreactor Bulk Harvests.

The MycoSEQ Plus method was also shown to be capable of obtaining same-day results, compared to the 28 days required for the compendial method. Furthermore, this MycoSEQ Plus method demonstrated improved efficiency compared to traditional qPCR platforms by simplifying sample preparation and removing the need to run external PCR inhibition controls.

Mycoplasma Species		
Species	ATCC Number	GC:CFU Ratio
A. laidlawii	23206-TTR	3
M. arginini	23838-TTR	2
M. fermentans	19989-TTR	5
M. hvorhinis	27717	3



The mycoplasma stocks used in these studies were sourced from ATCC, except for M. hyorhinis and M. salivarium. Bionique supplied the M. hyorhinis stock as ATCC did not have availability of the desired BTS-7 strain. *M. salivarium* was also provided by Bionique after ATCC's stock produced qPCR results that did not align with the reported genomic content. The reported GC:CFU ratios for each stock were determined based on post-freeze titers.

### Harmonized USP<63> Results Equivalency MycoSEQ Plus Results Indicator **Equivalent/Not Equivalent** Sample Description Sample Description Results (Reactions) Cell Culture Culture Non-Spiked Test Article 4/4 Negative Non-Spiked Test Article Equivalent Negative Test Article + 10 CFU/mL A. laidlawii Test Article + 10 CFU/mL A. laidlawii Equivalent 24/24 Positive Positive Equivalent Test Article + 10 CFU/mL M. arginini Test Article + 10 CFU/mL M. arginini 24/24 Positive Positive Equivalent Test Article + 2-3 CFU/mL *M. fermentans* A 24/24 Positive Test Article + 10 CFU/mL M. fermentans Positive Test Article + 10 CFU/mL M. hyorhinis 24/24 Positive Test Article + 10 CFU/mL M. hyorhinis Equivalent Positive Equivalent Test Article + 10 CFU/mL M. orale 24/24 Positive Test Article + 10 CFU/mL M. orale Positive Test Article + 1 CFU/mL M. pneumoniae B 24/24 Positive Test Article + 10 CFU/mL M. pneumoniae Equivalent Positive Test Article + 10 CFU/mL M. salivarium 24/24 Positive Test Article + 10 CFU/mL M. salivarium Positive Equivalent

A Samples containing M. fermentans were prepared a second time with ¼ the original spiking concentration after successful detection at 10 CFU/mL. <sup>B</sup> Samples containing *M. pneumoniae* were diluted 1:10 following the first evaluation.

<sup>C</sup> A positive result is reported if evidence of mycoplasma growth is detected in any portion of the Harmonized USP<63> Mycoplasma Test. For M. orale and M. salivarium, samples were deemed positive based on detection by the direct culture portion of the assay only, and not the indicator cell culture portion.

## References & Acknowledgements

- General Chapter: USP. Mycoplasma Tests <63>. In: USP-NF. Rockville, MD: USP; 2010. DOI: https://doi.org/10.31003/USPNF\_M3687\_01\_01
- Thermo Fisher Scientific Inc. (2023). Product bulletin | MycoSEQ Plus Mycoplasma Detection Kit. Thermo Fisher Scientific Inc. (2024). MycoSEQ Plus Mycoplasma Detection Kit User Guide. Publication Number
- MAN0028695, Revision B00. Dabrazhynetskaya, A., Volokhov, D. V., David, S. W., Ikonomi, P., Brewer, A., Chang, A., & Chizhikov, V. (2011). Preparation of reference strains for validation and comparison of mycoplasma testing methods. Journal of

Applied Microbiology, 111(4). https://doi.org/10.1111/j.1365-2672.2011.05108.x MycoSEQ Plus is a trademark of Thermo Fisher Scientific Inc. (Waltham, MA, USA).

Images were Created with BioRender.com.

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