# Evaluation of MycoSEQ Plus Method for Rapid Mycoplasma Contamination Detection

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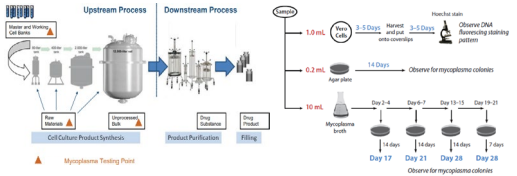
## MYCOPLASMA CONTAMINATION DETECTION

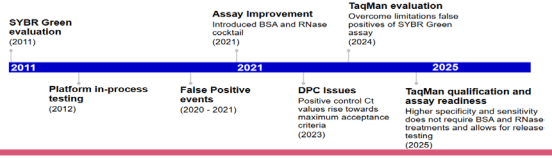
**Testing for mycoplasma contamination in cell banks and bioreactor cell cultures is a regulatory requirement for production of biological products. The compendial tests for mycoplasma detection require 14-28 days incubation, imposing a limitation on batch release timelines and rapid containment in the event of a contamination. The alternative rapid PCR-based mycoplasma detection kit, MycoSEQ Plus, provides results within a few hours and meets regulatory guidelines regarding sensitivity (10 CFU/mL or the genomic equivalent of 10 GC/mL) and specificity as outlined in the European Pharmacopoeia (E.P. 2.6.7, 2007), US Pharmacopoeia (US63), and Japanese Pharmacopoeia.**

**Mycoplasma Testing Points and Approaches**

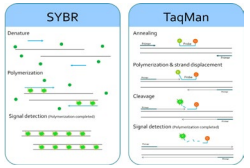



| | Culture Methods | PCR | Key Differences |
|---|---|---|---|
| Test Method | Agar, Broth and Cell Culture | Real-Time PCR | PCR does not require cultivation, therefore limited impact of biocidal or bacteriostatic compounds. |
| Specificity | High | High | PCR uses primers specific to mycoplasma and eliminating the need for multiple culture methods. |
| Sensitivity | 10-100 CFU/mL | 10 CFU/mL | Equivalent sensitivity. |
| Sample Requirements | 15 mL | 15 mL | Equivalent sample requirement. |
| Timeline | ~28 days | Hours | Significantly reduced timeline. |
| Species Detected | Viable Mycoplasma | > 200 species | PCR can detect Mycoplasma contamination regardless of viability. |

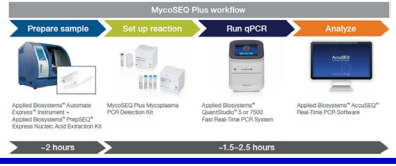
## ARD MYCOSEQ ASSAY HISTORY




## MYCOSEQ ASSAY CHEMISTRY




| | SYBR Green | TaqMan |
|---|---|---|
| Detection (Fluorescence) | SYBR Green dye that binds to double stranded DNA | Uses probes specific to target genes |
| Specificity | Low | High |
| Sensitivity | Variable (Can bind to non-target amplicons) | 1-10 Genomic copies |
| Analysis | Fluorescence cycle threshold + melting curve | Fluorescence cycle threshold |
| Application | Low specificity and sensitivity restricts use to routine in-process testing | High specificity and sensitivity meets regulatory criteria for release testing |




## MYCOSEQ PLUS QUALIFICATION PLAN

| Parameter | USP <1223> |
|---|---|
| Specificity | Ability to detect organism with no interference from sample |
| Limit of Detection (LOD) | Lowest number of organisms that can be detected |
| Robustness | A measure of method's capacity to remain unaffected by small but deliberate variations in method |
| Ruggedness | Challenge with different analysts, reagent lots, instruments, etc. |

### Specificity

| Mycoplasma Strains | Non-Mycoplasma Strains | Cell Culture Matrix (Assess Inhibition) |
|---|---|---|
| **Required Mycoplasma strains by regulatory in CFU and GC titer** | **Closely related bacteria to Mycoplasma required by regulatory** | **12 different products from different modalities** |
| Acholeplasma laidlawii<br>Mycoplasma arginini<br>Mycoplasma fermentans<br>Mycoplasma gallisepticum<br>Mycoplasma hominis<br>Mycoplasma hyorhinis<br>Mycoplasma orale<br>Mycoplasma pneumoniae<br>Mycoplasma salivarium<br>Mycoplasma synoviae | Clostridium sporogenes<br>Lactobacillus casei<br>Lactobacillus brevis<br>Streptococcus pyogenes<br>Streptococcus salivarius<br>Clostridium acetobutylicum<br>Bacillus subtilis<br>Pseudomonas aeruginosa<br>Micrococcus luteus<br>Stenotrophomonas maltophilia<br>Enterobacter cloacae | |

### LOD

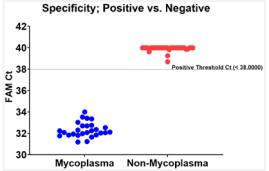
| Inactivated Mycoplasma Stock | Genomic DNA Stock | # of Replicates |
|---|---|---|
| 5 CFU/mL | 5 GC/mL | 5 |
| 10 CFU/mL | 10 GC/mL | 5 and 24 |
| 20 CFU/mL | 20 GC/mL | 5 |

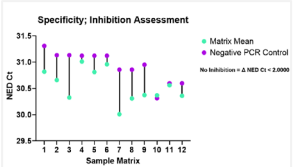
### Robustness

| Variations | Testing |
|---|---|
| Sample Matrix Interface | 12 Products |
| Hold Time Storage of extracted genome | 3 Time Points |
| Sample Volume | 2 Volumes |
| Alternate Sample Type | 3 Types |
| Sample Hold Time | 2 Storage Conditions and 2 Time Points each |

## QUALIFICATION RESULTS

### Specificity




### Defining LOD

| Positive Mycoplasma Species | Concentration Tested | # of Replicates Per Species | Replicates Positive | % Positive |
|---|---|---|---|---|
| 9 | 5 CFU/mL | 5 | 45/45 | 100% |
| | 10 CFU/mL | 5 | 45/45 | 100% |
| | 20 CFU/mL | 5 | 45/45 | 100% |
| 10 | 5 GC/mL | 5 | 48/50 | 96% |
| | 10 GC/mL | 5 | 50/50 | 100% |
| | 20 GC/mL | 5 | 50/50 | 100% |

### Confirming the LOD

| # of Mycoplasma Species | Concentration Tested | Replicates Positive | % Positive |
|---|---|---|---|
| 9 | 10 CFU/mL | 24/24 | 100% |
| 10 | 10 GC/mL | 24/24 | 100% |

### Robustness

| Experiment | Details | Results |
|---|---|---|
| Altering sample matrix type | 12 products tested | No interference |
| Testing storage duration of extracted genomes | 3 time points (1, 4 and 8 weeks) at -20°C | Confirmed robustness |
| Varying sample volume during processing | 1 mL and 15 mL of CHO cell volumes tested | |
| Substituting different sample types for processing | Supernatant, pooled supernatant and pellet | |
| Comparing sample hold times using fresh and frozen cells | Spiked samples frozen at -20°C tested same day and at 21 days | |
| | Spiked samples held at 4-8°C tested at 7 days and 21 days | |

## CONCLUSION

The assay can be used as a platform assay in CHO products. The MycoSEQ plus assay generates results in a few hours with a LOD of 10CFU or GC/mL, meeting regulatory criteria. Our testing demonstrated no interactions with other bacteria or sample matrices. We have concluded that this assay is suitable as a platform method for mycoplasma detection for in-process and release testing of bioproduction samples.