Flip Mycoplasma
Performance Characteristics Summary

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1 OVERVIEW

Flip Mycoplasma is a test developed by Fuse Diagnostics Ltd to detect Mycoplasma contamination of cell culture with unrivalled speed, accuracy and simplicity. The test is provided in a ready-to-use assay format with ambient storage and directly detects Mycoplasma without sample processing for maximum simplicity in the test workflow. The user simply mixes a cell culture supernatant sample with the provided sample buffer and incubates for 10 minutes in any 0.2 mL heat block or PCR machine before flipping the test to trigger a clear visual result on a test strip after a further two minutes. The test has a limit of detection (LoD) for Mycoplasma species of just 25 colony forming units (CFU) per mL of cell culture supernatant sample. Inclusivity of the test has been confirmed with the six Mycoplasma species responsible for >95% of cell culture contaminations: *M. arginini*, *M. orale*, *M. fermentans*, *M. hominis*, *M. hyorhinis* and *A. laidlawii*. The LoD is consistent ranging from 25-125 CFU/mL depending on the Mycoplasma species. The assay targets the 16S ribosomal RNA gene for robust specificity and exhibits no cross reactivity or interference from closely related bacteria that may be present in cell culture samples, including *E. coli*, *S. epidermidis* and *S. aureus*. Flip Mycoplasma has been tested and found to be compatibility with a wide range of alternative media, additives and cell lines. This report described the results of the comprehensive analytical studies that have been performed to verify the performance of the Flip Mycoplasma test.

2 ANALYTICAL SENSITIVITY

2.1 Limit of Detection

Limit of Detection (LoD) testing was performed with quantified inactivated Mycoplasma standards (Minerva Biolabs GmbH) in Sample Buffer containing 10% (v/v) cell culture supernatant prepared by pooling samples obtained from a range of different cell lines and media types after 5 days of growth. For each Mycoplasma species, the preliminary LoD was established by testing each dilution in triplicate. Confirmatory testing was performed by testing 20 individual replicates at the preliminary LoD concentration. The confirmed LoD is the lowest concentration at which ≥95% of replicates are positive. The LoD was confirmed to be 25 CFU/mL for *M. orale* (NCTC 10112; ATCC 23714) and 50 CFU/mL for *M. hyorhinis* (NCTC 10130; ATCC 17981) (Table 1).

Table 1 – LoD for *M. orale* and *M. hyorhinis*

Concentration (CFU / mL)	*M. orale* (% detected)	*M. hyorhinis* (% detected)
200	100% (3/3)	100% (3/3)
100	100% (3/3)	100% (3/3)
50	100% (3/3)	95.7% (22/23)
25	100% (23/23)	82.6% (19/23)
12.5	73.9% (17/23)	33.3% (1/3)
6.25	0% (0/3)	n/a

2.2 Inclusivity

Six Mycoplasma species are responsible for the vast majority (>95%) of incidences of cell culture contamination (Ref: 1-3): M. arginini, M. orale, M. fermentans, M. hominis, M. hyorhinis and A. laidlawii. In order to demonstrate the ability of the Flip Mycoplasma test to detect all six of these species, further testing has been performed comprising both wet testing and in silico sequence analysis as summarised below.

LoD testing performed as described above was expanded to three additional Mycoplasma species, M. arginini, M. fermentans and A. laidlawii using quantified inactivated standards (Minerva Biolabs GmbH). The LoD was confirmed to be 25 CFU/mL for M. arginini, 125 CFU/mL for M. fermentans and 50 CFU/mL for A. laidlawii (Table 2).

Table 2 – Inclusivity for *M. arginini*, *M. fermentans* and *A. laidlawii*

Species	*Strain*	Limit of Detection (CFU/mL)
M. arginini	NCTC 10129; ATCC 23838	25
M. fermentans	NCTC 10117; ATCC 19989	125
A. laidlawii	NCTC 10116; ATCC 23206	50

For *M. hominis*, sequence analysis revealed a perfect match between the NCBI reference genome (strain LBD-4; ATCC 27545) and the strain of *M. orale* (NCTC 10112; ATCC 23714) included in the LoD wet testing covering the entire Flip Mycoplasma assay target sequence region including the assay primer and probe sequences. Inclusivity for *M. hominis* is therefore also assumed.

A further *in silico* inclusivity study was performed (May 2025) to determine the conservation of Flip Mycoplasma target region in published sequences deposited in the NCBI database for the six inclusivity species. A total of 209 sequences were analysed and >99% of sequences were found to have a perfect match to the strains tested in the LoD and inclusivity wet testing described above. Inclusivity across all strains of each of the key six Mycoplasma species has therefore been demonstrated.

The Flip Mycoplasma test targets a highly conserved region of the 16S rRNA gene and is therefore able to detect many other Mycoplasma species which are less frequent contaminants of cell culture. Based upon *in silico* sequence analysis Fuse has identified a total of at least 111 different Mycoplasma species with inclusivity for the Flip Mycoplasma test.

3 ANALYTICAL SPECIFICITY

3.1 Cross-Reactivity / Microbial Interference

Wet testing was performed to evaluate Flip Mycoplasma performance in the presence of different bacteria that may contaminate cell culture supernatant samples. Each organism was individually tested in the presence of negative cell culture supernatant pool (NCSP) at the concentration indicated. Three tests with negative samples and three tests with Mycoplasma (*M. orale*) positive samples using a quantified inactivated standards (Minerva Biolabs GmbH) at 3x LoD (75 CFU/mL). The expected results were achieved in all cases. None of the bacteria tested caused cross-reactivity or interference to Flip Mycoplasma performance. The results are displayed in Table 3.

Table 3 – Cross Reactivity and Microbial Interference

Organism	Strain	Concentration (CFU/mL)	NCSP Negative / Tested	*M. orale* Positive / Tested (3x LoD)
Enterococcus faecalis	ATCC 29212	2E7	3/3 (100%)	3/3 (100%)
Escherichia coli	ATCC 8739	2E7	3/3 (100%)	3/3 (100%)
Klebsiella pneumoniae	ATCC 10031	2E7	3/3 (100%)	3/3 (100%)
Salmonella enterica	ATCC 13311	2E7	3/3 (100%)	3/3 (100%)
Staphylococcus aureus	ATCC 6538	2E7	3/3 (100%)	3/3 (100%)
Staphylococcus epidermidis	ATCC 12228	2E7	3/3 (100%)	3/3 (100%)

An *in silico* study was performed to assess for potential cross-reactivity of the Flip Mycoplasma assay region encompasses the primer and probe sequences with other microorganisms that are reasonably likely to be encountered in cell culture supernatant specimens.

No significant homologies were found in the microorganisms analysed (listed in Table 4) that would predict false results when combining the Flip Mycoplasma primer and probe sequences.

Table 4 – *in silico* Cross Reactivity and Microbial Interference

List of Organisms Analysed	
Bacillus cereus	*Lactobacillus acidophilus*
Candida albicans	*Pseudomonas aeruginosa*
Clostridium sporogenes	*Salmonella enterica*
Enterococcus faecalis	*Staphylococcus aureus*
Escherichia coli	*Staphylococcus epidermidis*
Klebsiella pneumoniae subsp. Pneumoniae	*Streptococcus pyogenes*
Listeria monocytogenes	

3.2 Interfering Substances

A study was performed to evaluate the effect of potentially interfering substances which could be present in cell culture supernatant, on the performance of Flip Mycoplasma. Each substance listed in Table 5 was tested individually at the concentration indicated on three negative samples and three positive samples prepared using a quantified inactivated *M. orale* standard (Minerva Biolabs GmbH) at 3x LoD. The expected results were achieved in all cases. None of the eight substances tested were found to interfere with Flip Mycoplasma performance.

Table 5 – Summary of Interfering Substances Tested

Substance	Concentration Tested	NCSP Negative / Tested	*M. orale* Positive / Tested (3x LoD)
DMEM, high glucose, pyruvate	10% (v/v)	3/3 (100%)	3/3 (100%)
Ham's F-10 Nutrient Mix, HEPES	10% (v/v)	3/3 (100%)	3/3 (100%)
Ham's F-12 Nutrient Mix	10% (v/v)	3/3 (100%)	3/3 (100%)
Medium 199, Hanks' Balanced Salts	10% (v/v)	3/3 (100%)	3/3 (100%)
MEM (Minimal Essential Medium)	10% (v/v)	3/3 (100%)	3/3 (100%)
RPMI 1640 Medium	10% (v/v)	3/3 (100%)	3/3 (100%)
RPMI 1640 Medium, HEPES	10% (v/v)	3/3 (100%)	3/3 (100%)
Fetal Bovine Serum	10% (v/v)	3/3 (100%)	3/3 (100%)

During the development of the Flip Mycoplasma test, a wide range of cell culture supernatant samples using different media, cell types (human and mouse) and additives and recovered after 5 days of growth have been evaluated. The test has been found to function effectively in all samples tested to date revealing excellent robustness to components that may be present in cell culture supernatant samples.

References

1. Hu, M., Buck, C., Jacobs, D. et al. Application of PCR for detection and identification of mycoplasma contamination in virus stocks. In Vitro Cell Dev Biol - Animal 31, 710–715 (1995). https://doi.org/10.1007/BF02634093.
2. Uphoff CC, Drexler HG. Detection of mycoplasma contaminations. Methods Mol Biol. 2005;290:13-23. doi: 10.1385/1-59259-838-2:013. PMID: 15361652.
3. Nikfarjam L, Farzaneh P. Prevention and detection of Mycoplasma contamination in cell culture. Cell J. 2012 Winter;13(4):203-12. Epub 2011 Dec 22. PMID: 23508237; PMCID: PMC3584481.