Performance Evaluation of an Automated, Rapid Mycoplasma Detection System for CGT Products with Audit-Ready Compliance

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Background

Current pharmacopeial regulations (USP <63>, EP 2.6.7, JP XVIII) require mycoplasma testing prior to product release for products manufactured in the presence of eukaryotic cells. Conventional compendial methods typically require ≥28 days to generate results, creating significant challenges for time-sensitive applications such as autologous cell and gene therapies, where rapid patient treatment is critical. Although nucleic acid testing (NAT) methods provide a faster alternative, they often require dedicated laboratory infrastructure, highly skilled personnel, and complex result interpretation.

Introduction

The fully automated rapid mycoplasma detection system, DetectInnova, integrates automated extraction, amplification, and detection into a closed, single-use cassette platform, enabling the detection of contamination from nearly 200 mycoplasma species.

Optimized sample preparation workflows support both culture supernatants and cell suspension samples, enabling broad coverage of potential intracellular and extracellular mycoplasma targets. Multiple built-in quality control processes are incorporated into the cassette to ensure result reliability.

Operator-friendly cassette design reduces operational errors and contamination risk while improving data integrity. Independent parallel channels enable flexible and rapid sample processing, with results generated in less than 3 hours. Instrument software compliant with 21 CFR Part 11 performs fully automated data analysis and provides objective qualitative outputs.

Test Method

Figure 1. Automated workflow of the DetectInnova mycoplasma detection system.

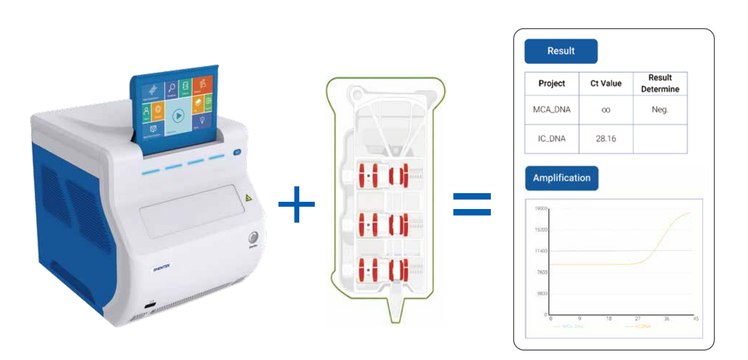


Figure 3. Schematic of the cassette with an integrated end-to-end workflow for rapid mycoplasma detection.

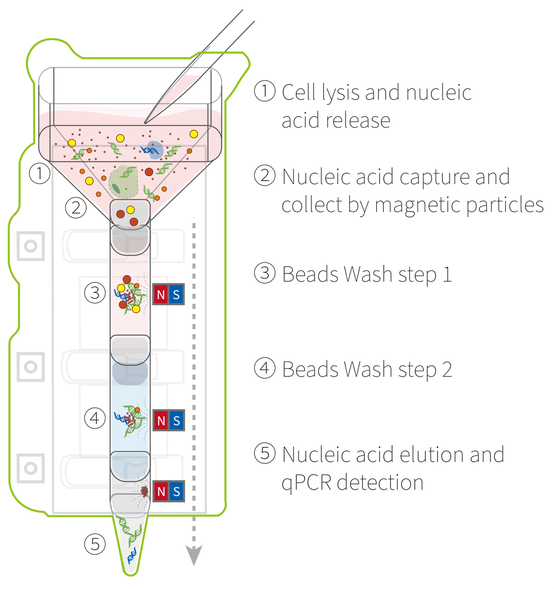
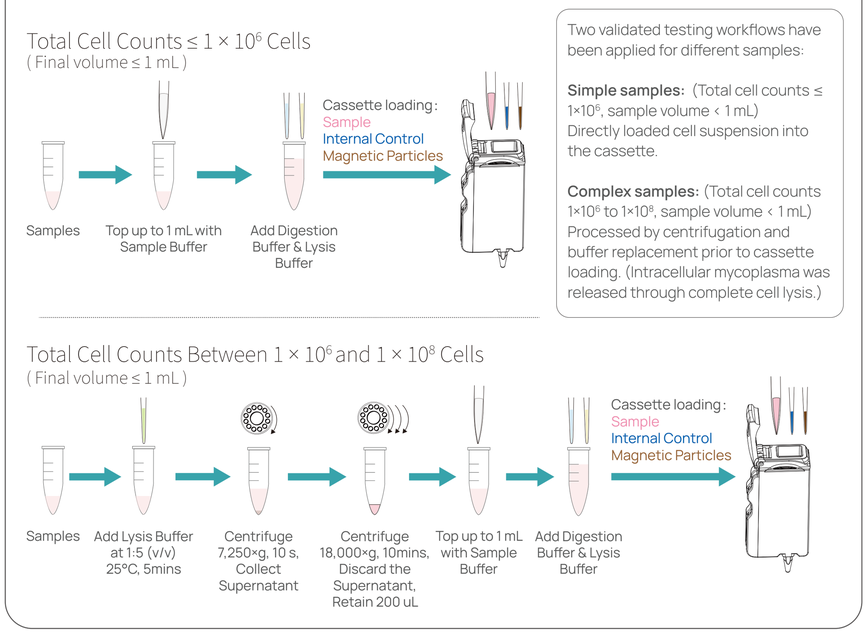


Figure 2. Direct loading of cell samples and pretreatment options for complex samples



Results

1. Detection Limit: at least 10 CFU/mL

LOD was determined for 11 compendial mycoplasmas in Phosphate Buffered Saline (PBS) and for 3 species in Chinese Hamster Ovary (CHO) cell suspension (1 x 10^6 & 1 x 10^7 CHO cells per mL) using 0.2 mL to 1 mL sample volumes.

Table 1 Sensitivity/Detection Limit at 10 & 100 CFU/mL For Different Mycoplasma Species

Strains	Detection Limit	
	10 CFU/mL	100 CFU/mL
**Mycoplasma orale*	24/24	24/24
Mycoplasma hyorhinis	24/24	24/24
**Acholeplasma laidlawii*	24/24	24/24
**Mycoplasma fermentans*	24/24	24/24
**Mycoplasma hominis*	24/24	24/24
**Mycoplasma pneumoniae*	24/24	24/24
**Mycoplasma salivarium*	24/24	24/24

* Strains sourced from ATCC

Note: 24/24=24 out of 24 test results positive

Table 2 Detection Limit at 10 CFU/mL in Different Matrices

Sample Matrices		Strains					
		Mycoplasma orale		*Mycoplasma hyorhinis*		*Mycoplasma pneumoniae*	
		10 CFU/mL	100 CFU/mL	10 CFU/mL	100 CFU/mL	10 CFU/mL	100 CFU/mL
5% human albumin		24/24	24/24	24/24	24/24	24/24	24/24
CHO-S	10^6 cells/mL	24/24	24/24	24/24	24/24	24/24	24/24
	10^7 cells/mL	24/24	24/24	24/24	24/24	24/24	24/24
DMEM (20%FBS, 10%DMSO)		24/24	24/24	24/24	24/24	24/24	24/24
ExpiCHO™ (10%DMSO)		24/24	24/24	24/24	24/24	24/24	24/24

Note: 24/24=24 out of 24 test results positive

2. Specificity:

2.1 Strain coverage:

Through intensive bioinformatics analysis of the amplification targets, the detection system allowsgenomic DNA of nearly 200 species, including *Mycoplasma, Mycoplasmoides, Mesomycoplasma, Metamycoplasma, Mycoplasmopsis, Ureaplasma, Acholeplasma,* and *Spiroplasma*, among others.

2.2 Strain specificity:

No cross-reactivity with *Clostridium, Lactobacillus, Streptococcus*, and different cell lines.

Table 3 Results of Cross Reactivity

Cross Reactant No.	Resource	Cross Reactivity
1	CHO	N/A
2	Vero	N/A
3	293T	N/A
4	HEK 293	N/A
5	MDCK	N/A
6	NS0	N/A
7	Sf9	N/A
8	Hi5	N/A
9	*E. coli*	N/A
10	*Pichia pastoris*	N/A
11	*Saccharomyces cerevisiae*	N/A
12	*Hans enula polymorpha*	N/A
13	*Clostridium acetobutylicum*	N/A
14	*Clostridium perfringens*	N/A
15	*Clostridium sporogenes*	N/A
16	*Streptococcus pneumoniae*	N/A
17	*Streptococcus agalactiae*	N/A
18	*Streptococcus mutans*	N/A

3. Robustness: Detection limit at 10 CFU/mL in complex matrices

Table 4 Matrix Robustness Results

Strains	Matrix	Test Conc. (CFU/mL)	Detection rate(%)	Acceptance Criteria	Conclusion
Mycoplasma orale	CHO-S (10^7 cells/mL)	10	95.8	Detection rate at least over 95%.	Pass
Mycoplasma hyorhinis		10	100		Pass
Mycoplasma pneumoniae		10	100		Pass
Mycoplasma fermentans		10	100		Pass

4. Case Study

Sample Types	Sample Details	Methods	Sample Volume	M. orale/ M. hyorhinis/ M. pneumoniae (10 CFU)
Culture Media	DMEM	Assay	1 mL	24/24
	DMEM(10% FBS)			24/24
	DMEM(20% FBS+10% DMSO)			24/24
	Cryostor CS10			24/24
	PBS			24/24
	ExpiCHO			24/24
Cell therapy product	Jurkat Cell line 10^6-10^7	Pre-treatment + Assay	1 mL	24/24
	Jurkat Cell line 10^8 (5% DMSO+5% HBS)			24/24
	TILs 10^6-10^7			24/24
	CD19 CAR-T 2.0 × 10^6 (axi-cel)		200 µL-1 mL	24/24
	CD19 CAR-T 2.0 × 10^6 (relma-cel)			24/24
	CD19 CAR-T 2.0 × 10^6 (CNCT19)			24/24
	BCMA CAR-T 10^7(zevor-cel)			24/24
	BCMA CAR-T 10^7 (CARVYKTI)			24/24
	BCMA CAR-T 10^7 (FUCASO)			24/24
	IIT samples (10^6-5x10^7 cells)			24/24
	Fresh CAR-T (10^5-10^6 cells)	Assay	100 µL	24/24

Conclusion

Using a closed and fully-automated “lab in a cassette” system that integrates nucleic acid purification and nested real-time PCR with less than three hour of run time and requires minimal training and skill to operate. It has a equivalent Limit of Detection of 10 cfu/ml, as general qPCR testing in a lab. It is qualified to facilitate screening throughout various stages of the cell and gene therapy manufacturing process. Capable of handling a broad range of sample types, a fully automated testing solution provides an easy way for rapid mycoplasma testing.

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