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

SHENTEK

Background

Rapid Mycoplasma Test Methods Essential for CGT Products

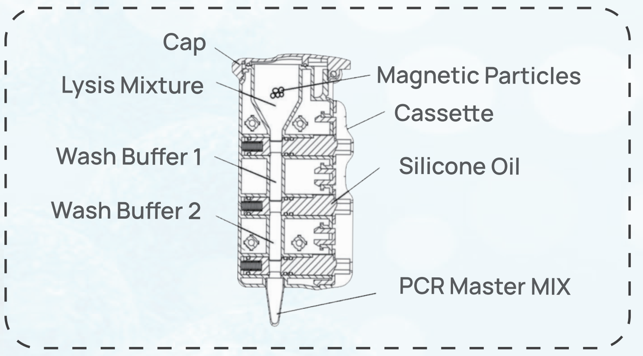
Cell and gene therapy (CGT) products present unique quality control challenges, including:

- Short shelf life and time-critical product release
- Limited and valuable sample volumes
- High sensitivity of living cells to microbial contamination
- Strong regulatory emphasis on risk-based quality control strategies

The Hubble™ System provides an automated, rapid, risk-based mycoplasma detection solution designed to meet these challenges while supporting GMP-compliant, audit-ready data integrity.

Methods

Automated End-to-End Workflow Compliant with GMP Data Integrity Expectations



	BEFORE	Hubble™
Testing Location	Sent out to external labs	Done in your own lab / on-site
Turnaround Time	1 week	3 hours
Operetion Requirement	Requires a specialist	Easy for anyone to use
Key Features	Higher cost & long wait	Lower cost & quick results

BIG CHANGE!

The Hubble™ System integrates nucleic acid extraction and real-time PCR assay, allowing a turnaround time of 3.5 hours from sample to result. It only requires less than 5 minutes of hands-on time, without specific DNA extraction and PCR expertise to operate.

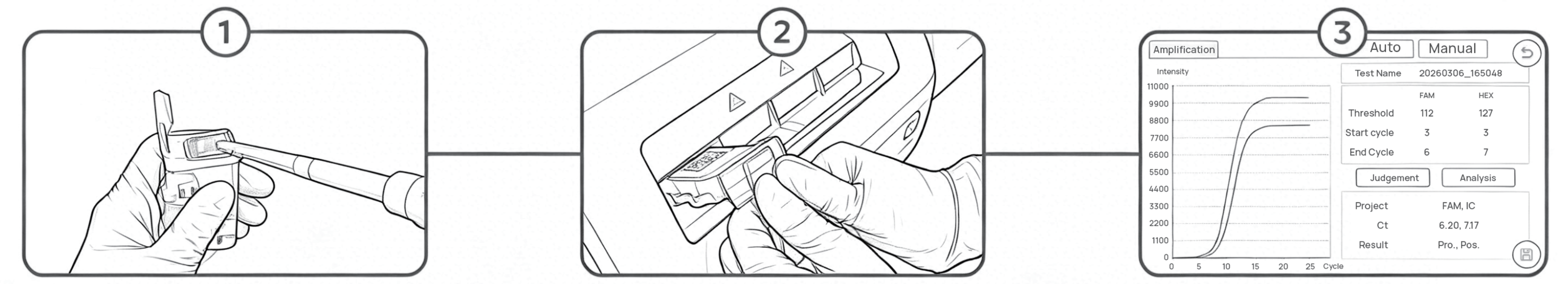
The Hubble™ System demonstrated rapid, same-day detection of mycoplasma contamination across CGT samples.

High sensitivity and broad species inclusivity were confirmed through validation studies.

Internal controls consistently identified invalid or inconclusive samples, supporting audit-ready QC data.

Minimal sample volume requirements preserved valuable cell therapy material without compromising test performance.

The closed, single-use system prevented operator-induced contamination and maintained workflow integrity.



Low Sample Volume

- Sample input range: 0.1-1.0 mL
- Designed to preserve valuable cell therapy material
- Applicable across all stages of CGT development and manufacturing

Closed System Ensuring Contamination Control

- Single-use, sealed microfluidic cassette
- Integrated nucleic acid extraction, amplification, and detection
- Minimal operator intervention to reduce contamination risk

Build-In Internal Control

- Internal Control processed with each sample
- Monitors extraction efficiency and PCR inhibition
- Flags invalid or inconclusive results to ensure reliable QC data

Sensitivity and Inclusivity

- Detection limit: ≤ 10 CFU/mL
- Broad detection across ~200 species of Mycoplasma, Acholeplasma, Spiroplasma, Entomoplasma, and Mesoplasma
- Method validation performed according to USP ‹63›, EP 2.6.7, JP XVIII.

Regulatory Positioning

- Provides qualitative (Yes/No) results
- Intended as a compendial-alternative or complementary molecular method
- Use requires product and matrix-specific verification
- Supports risk-based QC strategies in line with regulatory expectations for CGT products

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 (1.0E+06 & 1.0E+07 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 synoviae	24/24	24/24
Mycoplasma arginine	24/24	24/24
Mycoplasma gallisepticam	24/24	24/24
Spiroplasma citri	24/24	24/24
**Mycoplasma orale*	24/24	24/24
Mycoplasma hyorhinis	24/24	24/24

Strains	Detection Limit	
	10 CFU/mL	100 CFU/mL
**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

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

* Strains sourced from ATCC

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 135 species of Mycoplasma, 38 species of Spiroplasma and 8 species of Acholeplasma.

2.2 Strain specificity:

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

Table 3 Results of Cross Reactivity

NO.	Cross Reactants	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

NO.	Cross Reactants	Cross Reactivity
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

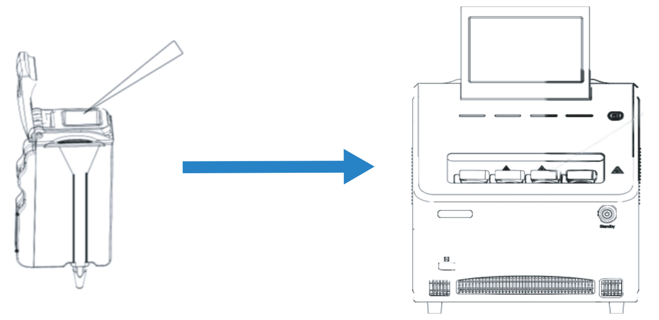
Sample Types	Sample Details	Methods	Sample Volume	*M. orale/ M. hyorhinis/ M. pneumoniae* (10 CFU)
Culture Media	DMEM	Assay	1 mL	24/24
	DMEM(10% FBS)			24/24
	DMEM(20% FBS+10% DMSO)			24/24
	Cryostor CS10			24/24
	PBS			24/24
	ExpiCHO			24/24
Cell therapy product	Jurkat Cell line 10^6-10^7	Pre-treatment + 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

Fast QC Solution in the "Lab in a Cassette" System

Using a closed and fully-automated "lab in a cassette" system that integrates nucleic acid purification and nested real-time PCR with about three hours of run time and requires minimal training and skill to operate. Its combination of low sample volume requirements, closed automated workflow, high sensitivity, broad inclusivity, and build-in internal controls supports risk-based, audit-ready quality control in line with regulatory expectations. This approach enables CGT manufacturers to make timely, informed release decisions while maintaining GMP compliance.

SUITABLE FOR IN-PROCESS CONTROLS & RELEASE TESTING



1 mL direct assay
Validated LOD of ≤ 10 CFU/mL

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