RAPID, SIMPLE, ACCURATE MYCOPLASMA DETECTION

FOR SIMPLIFIED NEAR-LINE OPERATION

SHENTEK

COMPLIANCE

WHY RAPID MYCOPLASMA TESTING MATTERS FOR CGT

Given the short manufacturing and administration windows of many CGT products, mycoplasma testing must deliver reliable results within a timeframe compatible with product release. Rapid, robust, and fit-for-purpose testing is therefore essential to minimize QC delays and support timely, data-driven batch-release decisions.

KEY PERFORMANCE CONSIDERATIONS (PH. EUR. 2.6.7 & USP ‹63›/‹77›)

- **Sensitivity:** ≤10 CFU/mL or ‹100 genome copies/mL, as applicable.
- **Specificity:** Broad coverage of relevant mycoplasma species.
- **Method Suitability:** Demonstrated performance without significant matrix interference.
- **Controls:** Appropriate controls to ensure test validity and reliable results.
- **Rapid TAT:** Short turnaround time to support timely CGT batch release.

METHOD

FULLY AUTOMATED SAMPLE-TO-RESULT WORKFLOW FOR RAPID, RELIABLE RESULTS

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.

*HUBBLE™ FAST QC

Method	Automated nucleic acid extraction + real-time PCR
Detection Limit	≤10 cfu/mL or 100 cp/mL
Format	Single-use cassette
Time to result	~3 hours
Hands-on time	‹5 min for simple samples
Sample input	0.1-1.0 mL
Throughput	Up to 4 independent assays per run
Detection coverage	180+ species of Mycoplasma, Spiroplasma, Acholeplasma
Software/compliance	21 CFR PART 11 / USP / EP / JP

VALIDATION

DEMONSTRATING SENSITIVITY, ACCURACY & ROBUSTNESS

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

* Strains sourced from TTR of 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

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

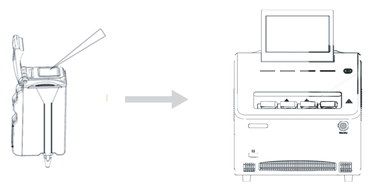
CONCLUSION

*HUBBLE™:ACCELERATING QC RELEASE FOR CGT PRODUCTS

The *Hubble™ System integrates nucleic acid extraction and real-time PCR into a single, automated workflow, delivering sample-to-result in 3.5 hours with less than 5 minutes of hands-on time, without requiring specialized molecular testing expertise.

Built on a "Lab-in-a-Cassette" concept, *Hubble™ enables same-day mycoplasma detection, supports audit-ready QC, simplifies sample processing, and minimizes contamination risks—ultimately accelerating CGT QC and batch release.

SUITABLE FOR IN-PROCESS CONTROLS & RELEASE TESTING



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