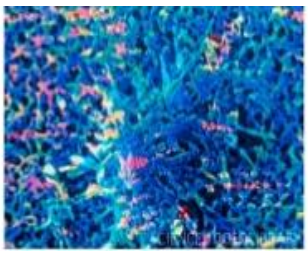
MYCOPLASMA PCR DETECTION KIT


iNtRON BIOTECHNOLOGY

MYCOPLASMA PCR TEST

E-MYCO VALiD PCR

E-MYCO VALiD qPCR

Mycoplasma PCR TEST according E.P Guideline:

e-Myco™ VALiD PCR
e-Myco™ VALiD qPCR

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BACKGROUND INFORMATION

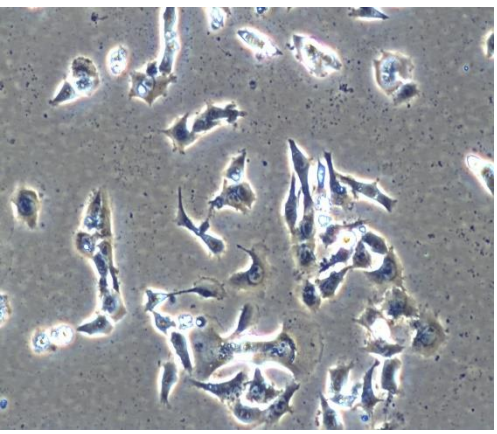
MYCOPLASMA'S IMPACT ON CELL CULTURE

Mycoplasmas compete with host cells for

- biosynthetic precursors nutrients
- can alter DNA, RNA and protein synthesis
- diminish amino acid and ATP levels
- introduce chromosomal alterations and mutation
- modify host-cell plasma membrane antigens

Common contaminants are:
Mycoplasma hyorhinis, *Mycoplasma arginini*, *Mycoplasma hominis*, *Mycoplasma synoviae*, *Mycoplasma orale*, *Mycoplasma fermentans*, *Acholephasma laidlawii*, *Mycoplasma pneumoniae*, *Mycoplasma gallisepticum* or the species *Ureaplasma urealyticum*

Analysis on contaminated cultured human cells has revealed the severe effects that mycoplasmas can have on the expression of hundreds of genes, including some that encode receptors, ion channels, growth factors and oncogenes. Moreover, mycoplasmas exert significant effects on cultured immune cells such as monocytes and macrophages.

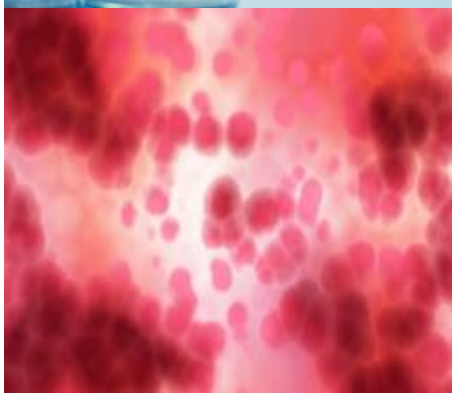


MAJOR SOURCES FOR MYCOPLASMA CONTAMINATION

Laboratory personnel infected with M. orale or M. fermentans. Furthermore, contamination can spread rapidly to other cell lines through dispersion of aerosol droplets.

- Contaminated cell culture medium reagents such as serum and trypsin
- Infected cells sent from another lab
- Human being

The most common mycoplasma cell culture contaminates are human, bovine or swine in origin, with human being the largest.
During the webinar, some interesting statistics were presented on how humans introduce mycoplasma that I wanted to share. First, 80.6% of lab techs are carriers and mycoplasma contamination can be spread with a single sneeze or even by talking.



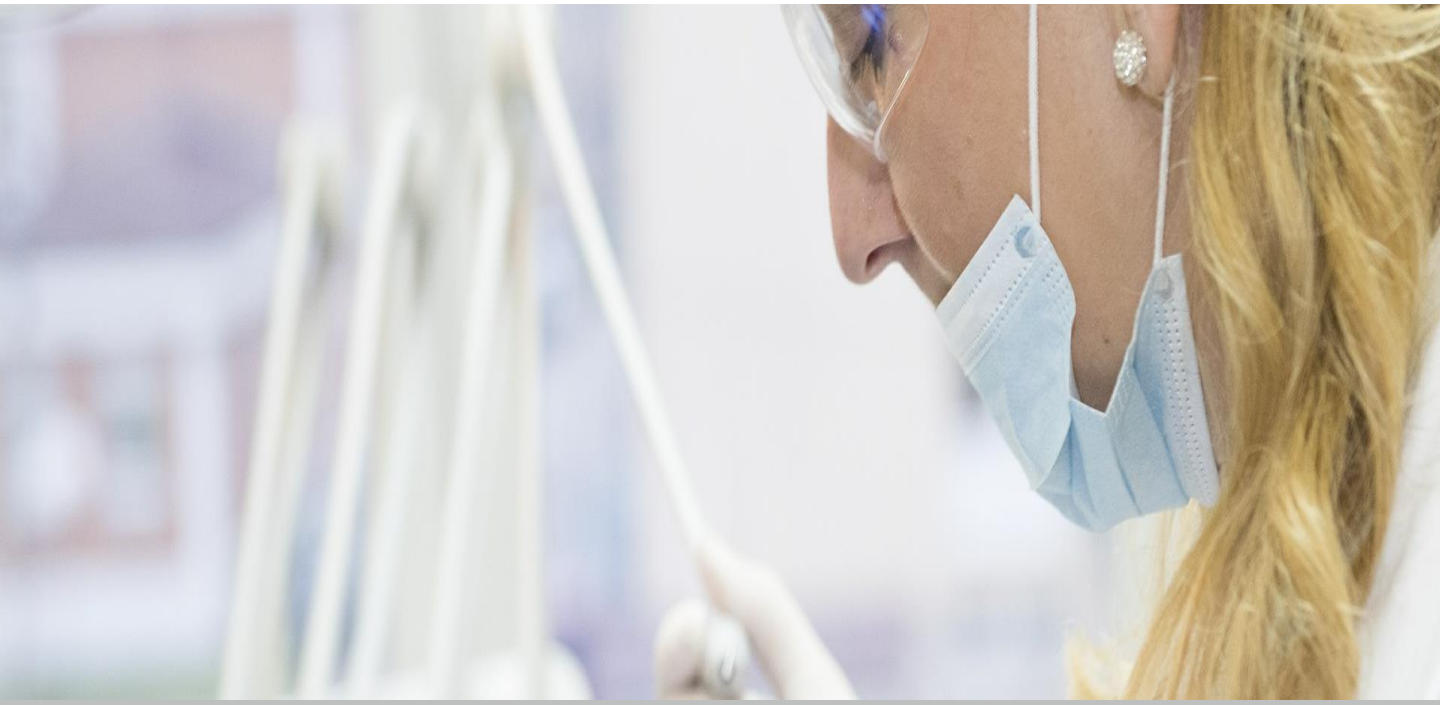
Because it often goes undetected, research continues to go on with contaminated cells and these cells are often shared with other labs, where contamination can spread. Due to the impact on the cells, experiments conducted with contaminated cells often can't be replicated with cells that aren't contaminated and vice versa. This inability to reproduce results can negate countless hours of work.

LiliF Diagnostics

MYCOPLASMA DETECTION

- WHY DETECTION IS NECESSARY
- WHERE TEST SHOULD BE CARRY OUT
- TESTING POINT IN LAB

REASON FOR DETECTING MYCOPLASMA



Mycoplasmas can have disastrous effects on eukaryotic cells, as they can alter every cellular function (proliferation, protein synthesis, susceptibility to viral infection, etc.).

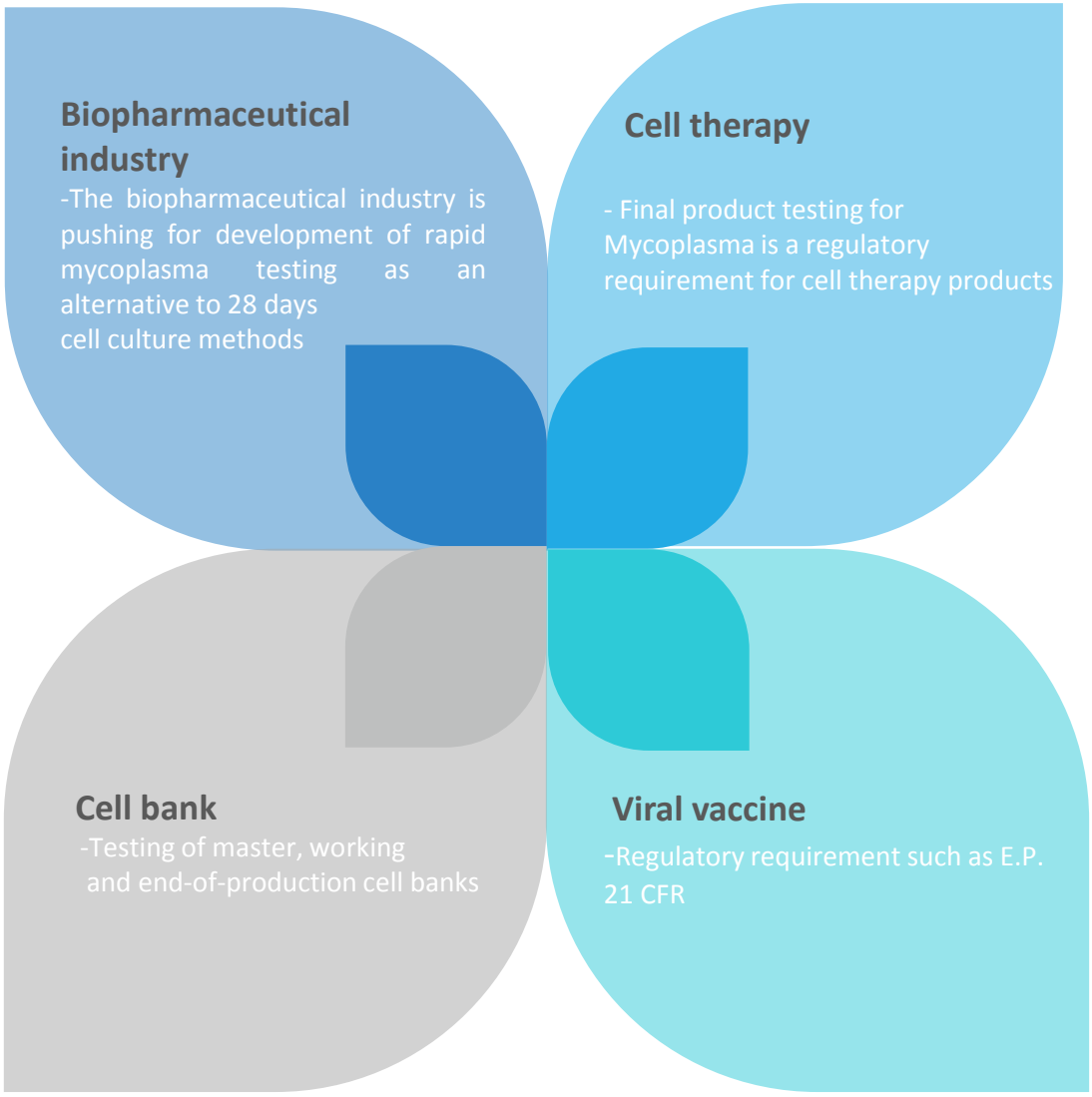
Mycoplasma in cell culture

Due to their small size and missing cell wall, it can pass through sterilizing filter and enter cell culture media. It is not visible, they often remain undetected.

Mycoplasma in Biopharmaceutical Manufacturing process

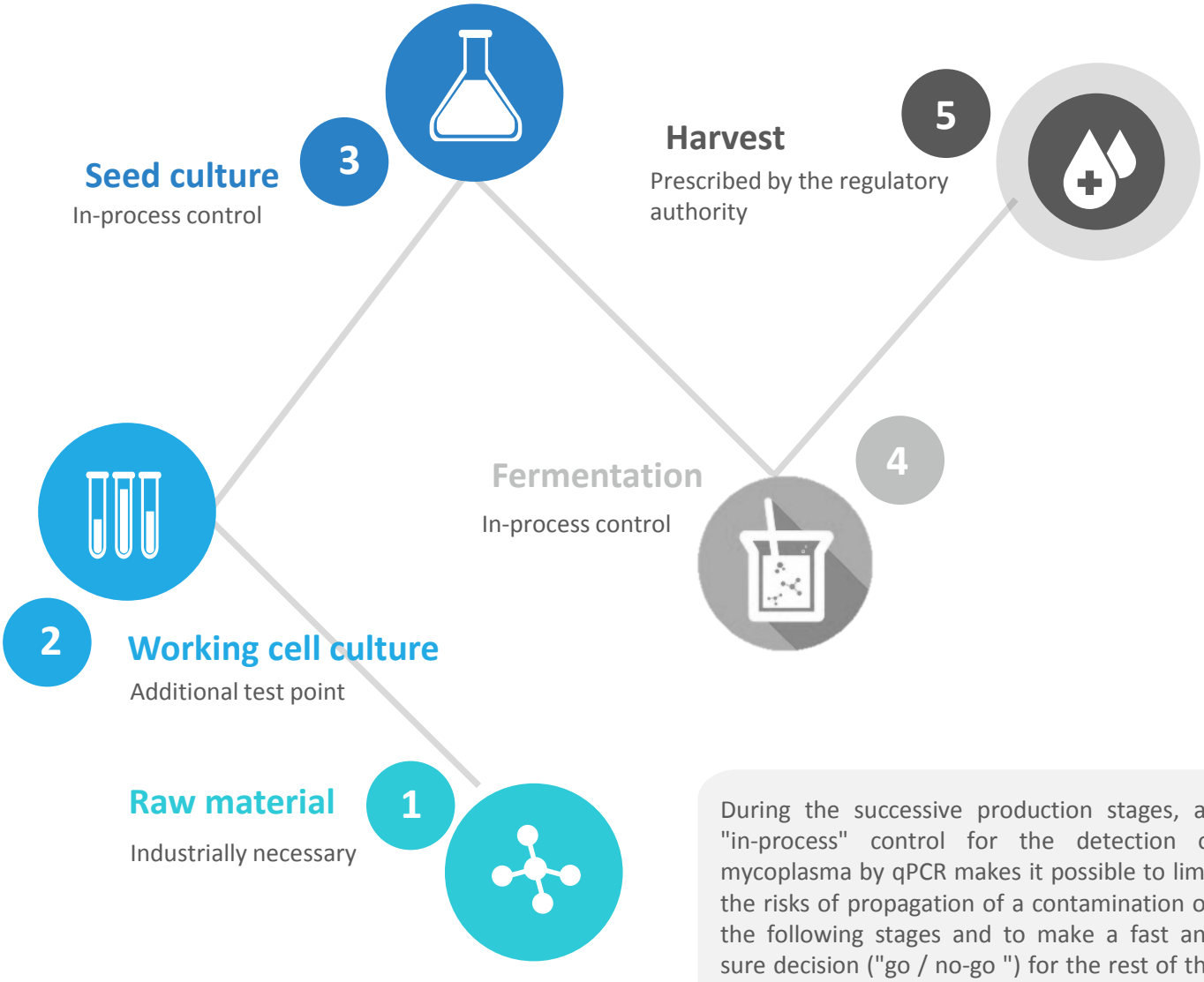
All production batches must be discarded and stop production.

CARRY OUT
MYCOPLASMA TEST ROUNTINELY



TESTING POINT FOR
MYCOPLASMA CONTAMINTAION

► **In the manufacturing process of biopharmaceuticals**



During the successive production stages, an "in-process" control for the detection of mycoplasma by qPCR makes it possible to limit the risks of propagation of a contamination on the following stages and to make a fast and sure decision ("go / no-go ") for the rest of the process.

REASON FOR USING PCR ACCORDING TO E.P GUIDELINE

qPCR is a direct, sensitive and rapid method which allows same-day results. In comparison, the standard regulatory method (a direct method involving culture in broth or on agar media in different conditions) requires a period of 28 days. This long delay of result rendering poses a problem for bio-therapeutic products with a short period of life such as cell therapy. This also poses another problem because some species of mycoplasma "grow" very badly or not at all on these specific environments.

The method of culture on 28 days may also be inappropriate for cytotoxic samples or for "in-process" samples requiring a fast and safe result. It is partly for these reasons that some regulatory authorities accept as an alternative the use of the real-time quantitative PCR detection test on a case-by-case basis. This acceptance requires a complete and documented validation according to specific guidelines (Ph.Eur.2.6.7).

To use this very rapid technique routinely, the European Pharmacopoeia (Ph. Eur. 2.6.7) requires that a detailed validation study be conducted to demonstrate equivalence with the regulatory method. The design of this qualification revolves around three criteria: the limit of detection (LOD: 10 CFU/ml), specificity and robustness.

iNtRON PRODUCTS

Mycoplasma detection method

e-Myco™ Valid PCR

e-Myco™ Valid qPCR

INTRODUCTION

CHARACTERISTICS

VALIDATION METHOD

COMPARISON

INTRODUCTION

Detection of mycoplasma using real time q-PCR/PCR can offer rapid turnaround to result. Validation reports have provided support for PCR based method as a suitable alternate to conventional test methods when appropriately validated.

iNtRON has developed PCR-based method for mycoplasma testing.
Classify as:

- e-Myco Valid PCR
- e-Myco Valid qPCR

Following E.P Guideline 2.6.7

CHARACTERISTICS

Simple

01

- Contains all the components
- for PCR reaction –PreMix &MasterMix type

Speed

02

- Takes only 90 minutes

Smart

03

- Detects 70 Mycoplasma species
- Eliminate carry-over contamination

Sensitivity

04

- 10CFU/ml

Reliable

05

- Validation analysis according to the E.P guideline

Competitive

06

- Reasonable prices

VALIDATION STUDY OF
e-Myco™ VALiD

Compliant with E.P. guideline

Sensitivity Test of e-Myco™ VALiD Mycoplasma PCR Detection Kit

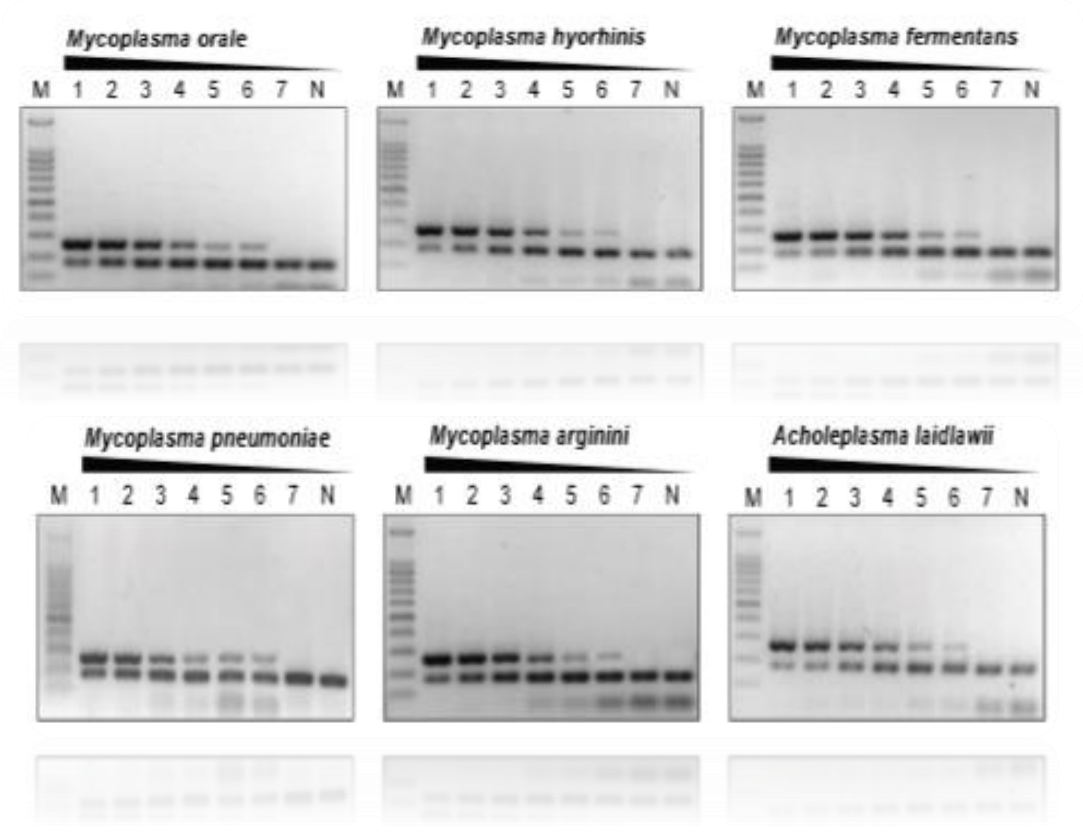


Fig.1. Analytical Sensitivity of e-Myco™ VALiD Mycoplasma PCR Detection Kit
Lane M, SiZer™-100 DNA Marker ; Lane 1, 1×10^6 CFU/ml of gDNA ; Lane 2, 1×10^5 CFU/ml of gDNA ; Lane 3, 1×10^4 CFU/ml of gDNA Lane 4, 1×10^3 CFU/ml of gDNA ; Lane 5, 1×10^2 CFU/ml of gDNA ; Lane 6, 10 CFU/ml of gDNA ; Lane 7, 1 CFU/ml of gDNA ; Lane N, Negative control.

- e-Myco™VALiD MycoplasmaPCR Detection Kit is a suitable for the efficient detection of Mycoplasma contamination in the culture with **high sensitivity, at least 10 CFU/ml**
- To identify the analytical sensitivity, the genomic DNA of six Mycoplasma spp. from cell cultured were purified.

VALIDATION STUDY OF
e-Myco™ VALiD

Compliant with E.P. guideline

Robustness test of e-Myco™ VALiD Mycoplasma PCR Detection Kit

No	Content	LOD (Limit of detection)
1	80% dNTPs solution (Below optimal concentration)	10^2 CFU/ml
2	90% dNTPs solution (Below optimal concentration)	10^1 CFU/ml
3	100% dNTPs solution (Optimal concentration)	10^1 CFU/ml
4	110% dNTPs solution (Above optimal concentration)	10^1 CFU/ml

- The results indicated that above 10CFU/ml of A.laidlawii was detected with each dNTPs concentration tested.
- Stronger signals were obtained with dNTPs solution at 90%, 100% or 110% than 80% dNTPs solution. The dNTPs solution has a clear influence on the sensitivity of the PCR reaction.

LOD(Limit of detection) determination of A.laidlawii in PCR method

Run	Sample Amount (CFU/ml)						
	10^6	10^5	10^4	10^3	10^2	10^1	10^0
1	8/8	8/8	8/8	8/8	8/8	8/8	2/8
2	8/8	8/8	8/8	8/8	8/8	8/8	0/8
3	8/8	8/8	8/8	8/8	8/8	8/8	1/8
Total	24/24	24/24	24/24	24/24	24/24	24/24	3/24

Note: 8/8 = 8 out of 8 test results positive.

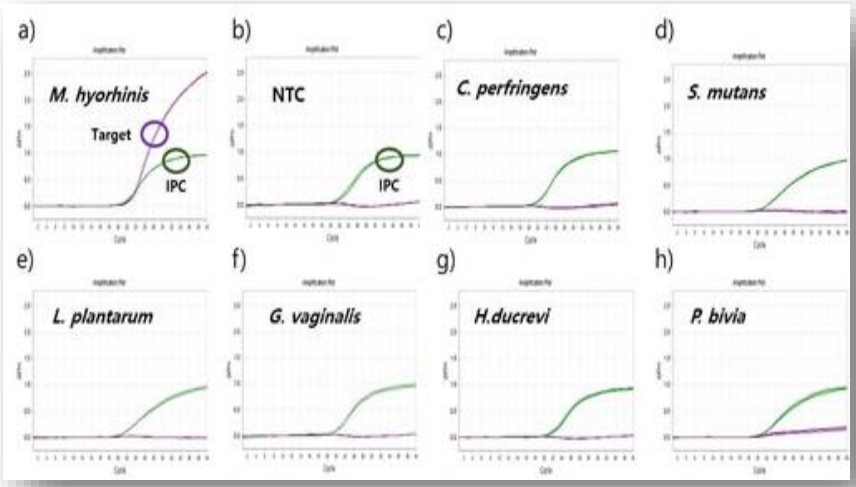
- With e-Myco™ VALiD Mycoplasma PCR Detection kit, mycoplasma could be detected with limit with 10CFU/ml.

VALIDATION STUDY OF
e-Myco™ VALiD qPCR

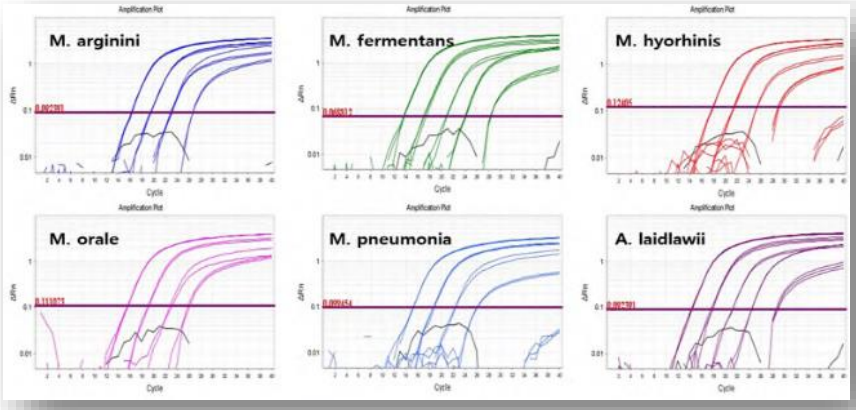
Compliant with E.P. guideline

Sensitivity Test of e-Myco™ VALiD Mycoplasma qPCR Detection Kit

- With **high sensitivity, at least 10 CFU/ml** and **high specificity**
- To identify the analytical specificity and sensitivity, the genomic DNA of 6 Mycoplasma spp. from cell cultured were purified.



Picture 1. The specificity test of of e-Myco VALiD-Q Mycoplasma Kit
Positive control (M.hyorhinis) and internal positive control were used for verifying specificity.
* IPC signal amplification was observed when tested with genomic DNA of 6 non-Mycoplasma samples and NTC (no template control) (picutre, b~h).



Picture 2. The sensitivity test of e-Myco™ VALiD-Q Mycoplasma Kit
The test proved the excellent sensitivity to detect all 6 Mycoplasma species with e-Myco™ VALiD qPCR Kit.
* Minimum detection sensitivity was identified as **10 CFU/ml** from 6 Mycoplasma species.

VALIDATION STUDY OF
e-Myco™ VALiD qPCR

Compliant with E.P. guideline

LOD test of e-Myco™ VALiD Mycoplasma qPCR Detection Kit

Table 11. LOD (Limit of detection) - *Acholeplasma laidlawii*

Run	CFU/ml (Acholeplasma laidlawii) 10^3			10^2			10^1			10^0			NTC			Limit of detection
1	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	10^0 CFU/ml
2	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	10^0 CFU/ml
3	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	10^1 CFU/ml
4	+	+	+	+	+	+	+	-	+	+	+	+	-	-	-	10^0 CFU/ml
5	+	+	+	+	+	+	+	+	+	-	+	+	-	-	-	10^1 CFU/ml
6	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	10^0 CFU/ml
7	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	10^0 CFU/ml
8	+	+	+	+	+	+	+	+	+	+	-	+	-	-	-	10^1 CFU/ml
Total	24/24			24/24			24/24			21/24			0/24			
Positive rate(%)	100%			100%			95.8%			87.5%			0%			10^1 CFU/ml

- Mycoplasma was detected at 10CFU/ml in 95% chance using e-Myco™ VALiD-Q Mycoplasma qPCR Detection Kit.

Robustness test of e-Myco™ VALiD Mycoplasma qPCR Detection Kit

Table 15. CT value of Mycoplasma detection in relation to users

CFU/ml	Experimenter A			Experimenter B			Experimenter C			mean Ct	STDEV	CV (%)
10^4	17.60	17.67	17.78	17.80	17.86	17.95	17.11	17.14	17.19	17.57	0.3320	1.89%
10^3	21.18	21.23	21.32	21.30	21.31	21.46	20.60	20.70	20.81	21.10	0.3112	1.47%
10^2	24.48	24.50	24.53	24.40	24.45	24.48	23.71	23.94	23.94	24.27	0.3159	1.30%
NTC	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	-	-	-

- The result of the test proves the excellent performance of e-Myco™ VALiD-Q Mycoplasma qPCR Detection Kit in any external conditions.

COMPARISON TO OTHER BRANDS

PRODUCT NAME	e-Myco VALiD PCR	e-Myco VALiD qPCR	MYCOTOOL	MycoSEQ™	Venor®GeM
COMPANY	iNtRON	iNtRON	Roche	Applied biosystems	Minerva biolabs
CAT.	25239	25245	05 200 709 001	11-91025	4482224
SIZE	48 Tube	50Test	1Kit (10 samples)	25 Test	100 Rxn.
METHOD	PCR	qPCR	PCR	qPCR	qPCR
FEATURES	70 Mycoplasma spp.	70 Mycoplasma spp.	Over 140 Mycoplasma spp.	15 Mycoplasma spp.	Over 90 Mycoplasma spp.
	10 CFU/mL	10 CFU/mL	1 CFU/mL	10 CFU/mL	< 10 CFU/mL
	EP 2.6.7 Compliance	EP 2.6.7 Compliance	FDA Approval EP 2.6.7 Compliance * Positive control, Internal control	EP 2.6.7 Compliance	EP 2.6.7 Compliance

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MYCOPLASMA PCR DETECTION KIT

MYCOPLASMA PCR TEST

E-MYCO VALiD PCR

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E-MYCO VALiD qPCR

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