Evaluation of the mycoplasma detection capability of Microsart® ATMP Mycoplasma for qPCR

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1. Introduction

Mycoplasma are known as important contaminants of biological products derived from cell lines in the Biopharmaceutical Industry affecting every parameter of a cell culture system. Contaminated cultures can result in production loss and unsafe products. Mycoplasma are the smallest of the self-propagating organisms. Unlike viruses, mycoplasma can reproduce outside of living cells. Many species within the genera *Mycoplasma*, *Acholeplasma* and *Spiroplasma* thrive as parasites in humans, birds, plants and animal hosts. Some species can cause disease in humans. Such contaminations can arise from the contamination of the source cell lines themselves (cell substrates) or from adventitious introduction of mycoplasma particles during production. Based on this contamination risk guidelines and technical papers are published to give guidance on mycoplasma safety for the manufacturing of biological products as for instance the *European Pharmacopoeia*, chapter 2.6.7., “Mycoplasmas”.

The detection kit Microsart® ATMP Mycoplasma was designed especially for the detection of *Mollicutes* (*Mycoplasma*, *Acholeplasma*, *Spiroplasma*) contamination in ATMPs and cell cultures by using the cells itself, cell culture supernatant or a defined mixture. Special features are a handsome sample preparation procedure, an acceptable sample volume in respect of the expensive, unique and limited sample as well as short time-to-result.

The kit utilizes the polymerase chain reaction (PCR), which was established as the method of choice for high sensitivity. The kit includes a Primer/Probe/Nucleotide mix containing a FAM™ labelled probe specific for a broad range of different mycoplasma species. False negative results due to PCR inhibitors or improper DNA extraction are detected by the internal amplification control. The Internal Control DNA can be added to the sample prior to DNA extraction and analysis for verification of the complete process (DNA extraction and PCR reaction). The Internal Control DNA can also be added directly to the PCR master mix to act as a PCR control only. The amplification of the control reaction is detected at 610 nm (ROX™ channel) and the pathogen-specific sequence at 520 nm (FAM™ channel). The kit contains dUTP instead of dTTP, so the option is available to degrade amplicons from previous analysis by use of uracil-DNA glycosylase (UNG). Thus the occurrence of false-positive results can be minimized.

2. Objective

This non-GLP/GMP study is designed to evaluate the mycoplasma detection capability for the Mycoplasma Detection Kit Microsart® ATMP Mycoplasma for qPCR. Mycoplasmas and the protocol for validation are described in section 2.6.7 of the *European Pharmacopoeia*. This chapter includes guidelines and specifications for relevant parameters like specificity, detection limit and robustness in comparison to the traditional culture method. As for detection a nucleic acid amplification technique (NAT, PCR) is used, section 2.6.21 of the EP will also be considered. Validation should be conducted in accordance with ICH guideline Q2B. As the method employed is used for the purpose of obtaining a qualitative result only (positive/negative), it is not necessary to demonstrate compliance with all individual requirements of ICH Q2B. This opinion is based on the requirements of the European Pharmacopoeia 2.6.21. The validation plan does consider the core requirements of validation in accordance with ICH Q2B in the context of their applicability to the qualitative nature of the test employed.

3. Definitions and Abbreviations

ATMP	advanced therapy medical products
DMEM	Dulbecco´s modified Eagles medium
DNA	desoxyribonucleic acid
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH
ECACC	European Collection of Cell Cultures
EDQM	European Directorate for the Quality of Medicines & HealthCare
EP	European Pharmacopoeia
FCS	fetal bovine serum
g	g-force (unit for measurement of rotation speed of centrifugation)
GLP	good laboratory practice
GMP	good manufacturing practice
IC	internal amplification control
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
m	meter
N/A	not applicable
N/V	not valid
NCTC	National Collection of Type Cultures
nm	nanometer
NTC	no-template control
OD_{260}	optical density (at a wavelength of 260 nm)
PC	positive control
qPCR	quantitative polymerase chain reaction, “real-time” PCR
CFU/ml	colony-forming units per milliliter
pH	*potentia hydrogenii*
RPMI	Roswell Park Memorial Institute
s	second
Taq	*Thermus aquaticus*
Tris	tris(hydroxymethyl)-aminomethane

4. Responsibilities

The product development manager was responsible for drafting the test protocol in association with the management. Quality management was responsible for reviewing the test protocol to ensure its accuracy, completeness and validity.

Test initiation was initiated once all personal in charge had approved the protocol by signing and filing the protocol cover page and after production or receipt of the necessary material for testing.

Minerva Biolabs technicians executed the test protocol. The quality manager was responsible for the execution of the protocol.

The product development manager drafted the report and quality management reviewed and approved its validity. The report was closed by signing of the report cover page.

No deviations, including test failures or other unforeseen circumstances occurred during the execution of the test protocol. No further actions were required.

5. Test Material

The testing was conducted using the following test system, product solutions and material. Corresponding lot numbers were filed on the result reports.

5.1 Test System

The test system used for the detection of mycoplasma during this study was as follows:

Table 1. Test System Information

System type	Supplied by	Catalogue No.	Storage Conditions
Microsart® ATMP Mycoplasma	Sartorius Stedim Biotech	SMB951-3xxx	+2 – 8 °C

5.2 Sample Matrix

As sample matrix defined cell culture medium components (Table 2) were used for specificity testing. For robustness testing this medium containing approximately $1x10^6$ Vero-B4 cells/ml. The cell suspension was heat-treated for 20 min at 95 °C in portions of 13 ml to inactivate all containing DNases. The heat-treated suspension was stored below – 18 °C and intensively vortexed after thawing for immediate use.

Table 2. Matrix Formulation

Product Ingredient	Manufacturer/Source	Catalogue No.	Storage Conditions
Vero-B4	DSMZ	33	liquid nitrogen
DMEM medium	Biochrom AG	FG 0415	+2 – 8 °C
Foetal bovine serum	Biochrom AG	S0615	< -20 °C

5.3 Microorganisms and Eukaryotic Material

Microorganisms and eukaryotic material used for spiking or specificity testing during the study are described in the following tables:

Table 3. *Mollicutes* Description

Species	Family	Natural Host	Origin	No.	Quantification
EP 2.6.7. listed mycoplasma species used for spiking					
Acholeplasma laidlawii	*Acholeplasmataceae*	ubiquitous	NCTC	10116	See chapter 5.4
Mycoplasma fermentans	*Mycoplasmataceae*	human		10117	
Mycoplasma hyorhinis		mammal		10130	
Mycoplasma orale		human		10112	
Mycoplasma pneumoniae		human		10119	
Mycoplasma gallisepticum		bird		10115	
Mycoplasma synoviae		mammal		10124	
Mycoplasma arginini		mammal		10129	
Spiroplasma citri		plant		10164	

Table 4. Description of non-*Mollicutes* Bacterial Strains and Eukaryotic Materials

Species	Family	Natural Host	Origin	No.	Quantification
DNA from bacteria species used for specificity testing					
Clostridium acetobutylicum	*Clostridiaceae*	ubiquitous	Minerva Biolabs	51-0792	OD_{260}
Lactobacillus acidophilus	*Lactobacillaceae*	human		51-1723	
Streptococcus pneumoniae	*Streptococcaceae*	human		51-0566	
DNA from cell cultures and tissues used for specificity testing					
Species	Family	Natural Host	Origin	No.	Quantification
Vero-B4	kidney	African green monkey	DSMZ	ACC 33	OD_{260}
Per.C6	human embryonic retinoplasts	human	Cruecell	B127-006	
RK13	kidney	rabbit	ECACC	21715	
CHO-K1	ovary	hamster	DSMZ	ACC 110	
Murine Genomic DNA	blood	mouse	Bioline	Bio-35027	
Calf Thymus DNA	thymus	bovine	Invitrogen	15633019	

5.4 *Mycoplasma* Harvest

All mycoplasma listed in Table 3 were cultivated in 50 ml broth according to EP 2.6.7 in either Frey or Hayflick medium to mid log phase. The growth kinetic of the organisms under specific culture conditions was determined in former experiments to identify the best time point for harvesting.

The culture broth was divided into two portions: One portion was used for quantification of the mycoplasma. The broth was vortexed and treated for 5 min with ultrasonic to break up mycoplasma clusters prior titration. An aliquot remained untreated for vitality control. Two tenfold dilution series were prepared in culture broth. Of each dilution step two agar plates were inoculated with 20 µl each, incubated at 37 °C (30 °C for *Spiroplasma citri*) and checked frequently for colony formation by microscope. Frequent counting was stopped at constant colony numbers and titre calculated as CFU/ml culture broth. The preparation was valid if the CFU value for the untreated sample was below or equal to the CFU value of the treated sample. The second portion of the culture broth was filled in 1.5 ml reaction tubes at a volume of 500 µl/tube. All tubes were stored at -80 °C until use.

Table 5. Mycoplasma Cultivation Media

Medium	Manufacturer	Catalog no.
Frey liquid medium	Heipha Dr. Müller GmbH	397100
Frey agar	Heipha Dr. Müller GmbH	097e
Hayflick liquid medium	Heipha Dr. Müller GmbH	393100
Hayflick agar	Heipha Dr. Müller GmbH	093e

As the titration of the mycoplasma spike was of severe relevance for the subsequent spiking experiments the following materials was tested in parallel as orthogonal materials to confirm the determined titres of the mycoplasma harvest:

The targeted spike level for the study was ≥ 1 x 10^6 CFU per ml sample matrix as a starting material for dilution series.

Table 6. EDQM Reference Standards

Article no.	Article name	Estimated titre
Y0000692	*Mycoplasma fermentans* BRP, batch 1	$9.55x10^7$ CFU/ml with a range from $1.58x10^7$ to $5.75x10^8$ CFU/ml
Y0000691	*Mycoplasma orale* BRP, batch 1	$4.90x10^5$ CFU/ml with a range from $9.33x10^4$ to $2.57x10^6$ CFU/ml
Y0000689	*Mycoplasma synoviae* BRP, batch 1	$1.86x10^7$ CFU/ml with a range from $5.89x10^6$ to $5.8x10^7$ CFU/ml
Y0000690	*Mycoplasma hyorhinis* BRP, batch 1	$1.17x10^8$ CFU/ml with a range from $6.76x10^7$ to $2.34x10^8$ CFU/ml

5.5 Equipment

The following lab equipment was used at the test laboratory:

Table 7. Lab Equipment

Equipment	Equipment-ID	Manufacturer	Brand
qPCR cycler	R 04 0843, ES72	Corbett Research	RotorGene 6000
qPCR cycler	275001289, ES12	Applied BioSystems	ABI Prism 7500
qPCR cycler	Model 401513; Serial No DE00700786 ES 82 and ES129	Agilent Technologies	Mx3005P
qPCR cycler	Serial No. 1402717, ES11	Roche Diagnostics	LightCycler (LC) 1.3
qPCR cycler	R 04 0843, ES72	Corbett Research	RotorGene 6000
Pipetting robot	2010002.000.056 ES120, 2010154.002.039 ES127	Analytik Jena	FasTrans
Pipettes for Master Mix setup 0.5-10 µl 10–100 µl 100–1000 µl	 K0404590A, ES51 210267, ES60 315688, ES61	 Rainin Eppendorf Eppendorf	 L-10 Reference Reference
Pipettes for DNA/sample handling 10-100µl 100-1000µl	 207047,ES52 272304, ES53	 Eppendorf Eppendorf	 Reference Reference
PCR Hood	H02PC1N9861, ES29	Bioair	Aura PCR
Vortex	020314607, ES43	VWR	N/A
Centrifuge	5452YI748008, ES79	Eppendorf	MiniSpin

The following consumables were used at the test laboratory:

Table 8. Reagents, Materials and Critical Lab Ware

Article no.	Article name	Manufacturer / Supplier
72.690.001/ 72.699.001	Micro tubes, 1.5 ml/0.5 ml	Sarstedt
710970	PCR tubes	Biozym
04929292001	LC capillaries, 25 µl	Roche Diagnostics
56-1100	Microsart AMP Extraction	Sartorius Lab Products and Services
S1120-3810 S1120-1840 S1126-7810	0.1-10 µl filter tips ep type 10-100 µl bevelled filter tips 101-1000 µl filter tips	Starlab

6. Test Procedure

Based on the results of different proficiency tests (data available from Minerva Biolabs on request) DNA extraction prior testing is strictly required for highest confidence and sensitivity. The design and performance of pre-analytical procedures are part of this study in respect of the intended use but cannot reflect the diversity of the sample material in total. The performance of the kit within the entire analytical process has to be demonstrated by the user. The templates for the PCR analysis are prepared by direct extracting the sample and subsequent PCR analysis.

6.1 DNA Extraction

The Microsart AMP Extraction kit purifies genomic DNA from different sample matrices including cell culture samples. Mycoplasma are lysed by a combination of a detergent and chaotropic salt. The lysate is directly applied onto the spin columns. The DNA is selectively bound to the highly specified silica membrane. Two subsequent washes remove residual contaminants, like proteins, metabolites, dyes, detergent etc. The purified DNA is eluted in Tris buffer. The DNA is ready-to-use.

The Internal Control DNA of Microsart ® ATMP Mycoplasma can be used to monitor the extraction process. 2 µl of the Internal Control DNA are added directly to the sample volume per 10 µl DNA extract. The sample is vortexed briefly prior extraction. No additional Internal Control DNA is used in the reaction mix for these samples.

The isolation of DNA will be carried out according to the update version of the instruction manual. In detail:

Transfer 200 µl of sample material into a fresh 1.5 ml reaction tube.

Add 200 µl Conditioner, vortex for at least 10 sec.

Add 200 µl of absolute ethanol to the mixture. Vortex immediately and very thoroughly in order to prevent any precipitation of nucleic acids.

Take one spin column per sample from the kit and insert it into a collection tube. Mark the sample identification on the lid of the spin column. Fill the sample lysate into the spin column without moistening the rim of the spin column.

Centrifuge the system for 1 min at 10.600 x g (approx. 10,000 rpm with a bench top centrifuge). Discard the flow through from the collection tube and reassemble the spin column and the collection tube.

Add 500 µl of Buffer A1. Centrifuge the system for 1 min at 10,600 x g (approx. 10,000 rpm with a bench top centrifuge), discard the flow through and re-assemble the spin column.

Fill the spin column with 500 µl Buffer A2. Centrifuge the system for 1 min at 10.600 rpm (10,000 x g), take the spin column out of the collection tube, dump the containing Buffer A2, discard the flow through and re-assemble the spin column.

Centrifuge for 1 min at full speed (approx. 13.200 rpm) in order to remove the remaining Buffer A2.

Discard the collection tube containing the Buffer A2 and place the spin column into a sample storage tube.

Pipette 60 µl of pre-heated Buffer E (70 °C) into the spin column directly onto the center of the silica membrane. The complete membrane should get in touch with the Buffer E. Secure the sample storage tube and incubate for 2 min at room temperature.

Following the incubation, centrifuge the system for 2 min at 10,600 rpm (10,000 x g).

Remove the spin column and use the eluate directly for the PCR procedure.

6.2 Analytical procedures

The detection of mycoplasma DNA will be carried out according to the update version of the instruction manual. In detail:

Rehydration of the Reagents:

1. Centrifuge tubes with lyophilized components (5 sec at maximum speed)
2. Add 390 µl of Rehydration Buffer to the Mycoplasma Mix
3. Add appropriate amount of deionized DNA-free water
   Positive Control DNA 300 µl
   Internal Control DNA 800 µl
3. Incubate for 5 minutes at room temperature
4. Vortex and centrifuge again

PCR Master Mix Setup:

Total volume per reaction is 25 µl including 10 µl of sample. When setting up reactions, calculations include positive (PC) and negative controls (NTC). Pipet master mix on ice into a 1.5 ml reaction tube and mix gently.

Pipetting scheme:

	for 1 reaction	for 25 reactions
Mycoplasma Mix	15 µl	375.0 µl
Internal Control DNA	1.0 µl	25.0 µl

The reagents are mixed by carefully snipping the tube and 15 µl are added to each PCR tube. The remaining liquid is discarded.

If the Internal Control DNA was added to the sample prior to DNA extraction 15 µl of the Mycoplasma Mix (red cap) are added directly to each PCR tube.

After pipetting the negative control (10 µl of water or elution buffer of DNA extraction kit), the tube must be sealed before proceeding with the samples. Add 10 µl of sample to each PCR reaction tube. Seal the tubes completely before proceeding with the positive control (10 µl) in order to avoid cross contamination.

Programming the qPCR cycler Rotorgene 6000 (5-plex):

Program Step 1: Pre-incubation

Setting	Hold
Hold Temperature	95°C
Hold Time	3 min 0 sec

Program Step 2: Amplification

Setting	Cycling
Cycles	45
Denaturation	95 °C for 30 sec
Annealing	55 °C for 30 sec
Detection/ Elongation	60 °C for 45 sec
Gain setting	automatic (auto gain)
Slope Correct	activated
Ignore First	deactivated

Programming the qPCR cycler ABI Prism® 7500:

Program Step 1: Pre-incubation

Setting	Hold
Hold Temperature	95°C
Hold Time	3 min 0 sec

Program Step 2: Amplification

Setting	Cycling
Cycles	45
Denaturation	95 °C for 30 sec
Annealing	55 °C for 30 sec
Detection/ Elongation	60 °C for 45 sec

Programming the qPCR cycler Mx3005P®:

Segment 1 (Pre-Melt)	95°C. 3 min 0 sec
Segment 2	95 °C for 30 sec
	55 °C for 30 sec
	60 °C for 45 sec. data collection
Cycles	45
Analysis mode:	non adaptive baseline (baseline correction)

Programming the qPCR cycler LightCycler 1.3:

Program 1:	Pre-incubation
Cycles	1
Analysis Mode	None
Temperature Targets	Segment 1
Target Temperature [°C]	95
Incubation time [min]	180
Temperature Transition Rate [°C/s]	20.0
Secondary Target Temperature [°C]	0
Step Size [°C]	0.0
Step Delay [Cycles]	0
Acquisition Mode	None

Program 2:	Amplification		
Cycles	45		
Analysis Mode	Quantification		
Temperature Targets	Segment 1	Segment 2	Segment 3
Target Temperature [°C]	95	55	60
Incubation time [s]	10	30	45
Temperature Transition Rate [°C/s]	20.0	20.0	20.0
Secondary Target Temperature [°C]	0	0	0
Step Size [°C]	0	0	0
Step Delay [Cycles]	0	0	0
Acquisition Mode	None	None	Single

Program 3:	Cooling
Cycles	1
Analysis Mode	None
Temperature Targets	Segment 1
Target Temperature [°C]	40
Incubation time [s]	60
Temperature Transition Rate [°C/s]	20.0
Secondary Target Temperature [°C]	0
Step Size [°C]	0
Step Delay [Cycles]	0
Acquisition Mode	None

Result Interpretation:

The presence of mycoplasma in the sample is indicated by an increasing fluorescence signal in the mycoplasma FAM™ channel during PCR.

A successfully performed PCR without inhibition is indicated by an increasing fluorescence signal in the internal control channel, provided the Internal Control DNA was added to the master mix. Mycoplasma DNA and Internal Control DNA are competitors in PCR. Because of the very low concentration of internal control in the PCR mix, the signal strength in this channel is reduced with increasing mycoplasma DNA loads in the sample.

Table 9. Result interpretation

Detection of Mollicutes FAM™ channel	Internal Control ROX™ channel	Interpretation
positive (Ct < 40)	irrelevant	*Mollicutes* positive
negative (no Ct)	negative (no Ct)	PCR inhibition
negative (no Ct)	positive (Ct < 40)	*Mollicutes* negative
borderline (Ct > 40)	positive (Ct < 40)	Result not valid. repeat process including DNA extraction
borderline (Ct > 40)	negative (no Ct)	PCR inhibition

6.3 System Suitability Test Criteria

Internal control must show Ct-values in the range of +/- 2 cycles of the no-template control (master mix control) if used as PCR control. If the internal control is used as process control it must show Ct-values in the range of +/- 3 cycles of the no-template control (master mix control). To exclude contaminations the extraction control and the master mix control must be negative. The PCR positive control must show Ct-values of > 23 cycles.

6.4 Reporting Requirements

The reports generated by the qPCR machine will be printed in color. All run information will be printed, including protocol, sample identification, internal amplification control curves (ROX™ channel) and target curves (FAM™ channel) and filed according to the chapter structure of this validation plan. Sample identification should contain information on the species, the contained concentration in CFU/ml or alternatively the type of control (PC for positive control, NTC for no-template control).

6.5 Calculations

N/A

6.6 Overview of experimental settings

Specificity				
Day 1	2 matrices + IC		PCR NTC: 10 µl Elution Buffer	PCR PC: 10 µl of Positive Control DNA
	4x each		2x	2x
	Extract DNA and perform PCR with 10 µl DNA extract in a final volume of 25 µl w/o IC		Perform PCR in a final volume of 25 µl +IC	
Day 2	Test of Mollicutes DNA: 10 µl DNA	Test of DNA from microorganisms and cells: 10 µl DNA	PCR NTC: 10 µl Tris Buffer	PCR PC: 10 µl of Positive Control DNA
	6x each	3x each	2x	2x
	Perform PCR in a final volume of 25 µl +IC			

Detection limit				
Day 3	Spike DMEM + 5 % FCS with mycoplasma			
	20 CFU/ml *Acholeplasma laidlawii* →Split into 8 samples	10 CFU/ml *Acholeplasma laidlawii* →Split into 8 samples	5 CFU/ml *Acholeplasma laidlawii* →Split into 8 samples	1x extraction NTC with DMEM + 5 % FCS
	20 CFU/ml *Mycoplasma fermentans* →Split into 8 samples	10 CFU/ml *Mycoplasma fermentans* →Split into 8 samples	5 CFU/ml *Mycoplasma fermentans* →Split into 8 samples	1x extraction NTC with DMEM + 5 % FCS
	Extract DNA and perform PCR with 10 µl DNA extract in a final volume of 25 µl + IC Add 2x PCR NTC and 2x PCR PC.			
Day 4	Spike DMEM + 5 % FCS with mycoplasma			
	20 CFU/ml *Acholeplasma laidlawii* →Split into 8 samples	10 CFU/ml *Acholeplasma laidlawii* →Split into 8 samples	5 CFU/ml *Acholeplasma laidlawii* →Split into 8 samples	1x extraction NTC with DMEM + 5 % FCS
	20 CFU/ml *Mycoplasma fermentans* →Split into 8 samples	10 CFU/ml *Mycoplasma fermentans* →Split into 8 samples	5 CFU/ml *Mycoplasma fermentans* →Split into 8 samples	1x extraction NTC with DMEM + 5 % FCS
	Extract DNA and perform PCR with 10 µl DNA extract in a final volume of 25 µl + IC Add 2x PCR NTC and 2x PCR PC.			
Day 5	Spike DMEM + 5 % FCS with mycoplasma			
	20 CFU/ml *Acholeplasma laidlawii* →Split into 8 samples	10 CFU/ml *Acholeplasma laidlawii* →Split into 8 samples	5 CFU/ml *Acholeplasma laidlawii* →Split into 8 samples	1x extraction NTC with DMEM + 5 % FCS
	20 CFU/ml *Mycoplasma fermentans* →Split into 8 samples	10 CFU/ml *Mycoplasma fermentans* →Split into 8 samples	5 CFU/ml *Mycoplasma fermentans* →Split into 8 samples	1x extraction NTC with DMEM + 5 % FCS
	Extract DNA and perform PCR with 10 µl DNA extract in a final volume of 25 µl + IC Add 2x PCR NTC and 2x PCR PC.			

Detection limit				
Day 6	Spike DMEM + 5 % FCS with mycoplasma			
	20 CFU/ml *Mycoplasma hyorhinis* →Split into 8 samples	10 CFU/ml *Mycoplasma hyorhinis* →Split into 8 samples	5 CFU/ml *Mycoplasma hyorhinis* →Split into 8 samples	1x extraction NTC with DMEM + 5 % FCS
	20 CFU/ml *Mycoplasma orale* →Split into 8 samples	10 CFU/ml *Mycoplasma orale* →Split into 8 samples	5 CFU/ml *Mycoplasma orale* →Split into 8 samples	1x extraction NTC with DMEM + 5 % FCS
	Extract DNA and perform PCR with 10 µl DNA extract in a final volume of 25 µl. Add 2x PCR NTC and 2x PCR PC.			
Day 7	Spike DMEM + 5 % FCS with mycoplasma			
	20 CFU/ml *Mycoplasma hyorhinis* →Split into 8 samples	10 CFU/ml *Mycoplasma hyorhinis* →Split into 8 samples	5 CFU/ml *Mycoplasma hyorhinis* →Split into 8 samples	1x extraction NTC with DMEM + 5 % FCS
	20 CFU/ml *Mycoplasma orale* + IC →Split into 8 samples	10 CFU/ml *Mycoplasma orale* + IC →Split into 8 samples	5 CFU/ml *Mycoplasma orale* + IC →Split into 8 samples	1x extraction NTC with DMEM + 5 % FCS
	Extract DNA and perform PCR with 10 µl DNA extract in a final volume of 25 µl. Add 2x PCR NTC and 2x PCR PC.			
Day 8	Spike DMEM + 5 % FCS with mycoplasma			
	20 CFU/ml *Mycoplasma hyorhinis* →Split into 8 samples	10 CFU/ml *Mycoplasma hyorhinis* →Split into 8 samples	5 CFU/ml *Mycoplasma hyorhinis* →Split into 8 samples	1x extraction NTC with DMEM + 5 % FCS
	20 CFU/ml *Mycoplasma orale* →Split into 8 samples	10 CFU/ml *Mycoplasma orale* →Split into 8 samples	5 CFU/ml *Mycoplasma orale* →Split into 8 samples	1x extraction NTC with DMEM + 5 % FCS
	Extract DNA and perform PCR with 10 µl DNA extract in a final volume of 25 µl IC Add 2x PCR NTC and 2x PCR PC.			

Detection limit				
Day 9	Spike DMEM + 5 % FCS with mycoplasma			
	20 CFU/ml *Mycoplasma pneumoniae* →Split into 8 samples	10 CFU/ml *Mycoplasma pneumoniae* →Split into 8 samples	5 CFU/ml *Mycoplasma pneumoniae* →Split into 8 samples	1x extraction NTC
	20 CFU/ml *Mycoplasma gallisepticum* →Split into 8 samples	10 CFU/ml *Mycoplasma gallisepticum* →Split into 8 samples	5 CFU/ml *Mycoplasma gallisepticum* →Split into 8 samples	1x extraction NTC
	Extract DNA and perform PCR with 10 µl DNA extract in a final volume of 25 µl + IC Add 2x PCR NTC and 2x PCR PC.			
Day 10	Spike DMEM + 5 % FCS with mycoplasma			
	20 CFU/ml *Mycoplasma pneumoniae* →Split into 8 samples	10 CFU/ml *Mycoplasma pneumoniae* →Split into 8 samples	5 CFU/ml *Mycoplasma pneumoniae* →Split into 8 samples	1x extraction NTC
	20 CFU/ml *Mycoplasma gallisepticum* →Split into 8 samples	10 CFU/ml *Mycoplasma gallisepticum* →Split into 8 samples	5 CFU/ml *Mycoplasma gallisepticum* →Split into 8 samples	1x extraction NTC
	Extract DNA and perform PCR with 10 µl DNA extract in a final volume of 25 µl + IC Add 2x PCR NTC and 2x PCR PC.			
Day 11	Spike DMEM + 5 % FCS with mycoplasma			
	20 CFU/ml *Mycoplasma pneumoniae* →Split into 8 samples	10 CFU/ml *Mycoplasma pneumoniae* →Split into 8 samples	5 CFU/ml *Mycoplasma pneumoniae* →Split into 8 samples	1x extraction NTC
	20 CFU/ml *Mycoplasma gallisepticum* →Split into 8 samples	10 CFU/ml *Mycoplasma gallisepticum* →Split into 8 samples	5 CFU/ml *Mycoplasma gallisepticum* →Split into 8 samples	1x extraction NTC
	Extract DNA and perform PCR with 10 µl DNA extract in a final volume of 25 µl + IC Add 2x PCR NTC and 2x PCR PC.			

Detection limit				
Day 12	Spike DMEM + 5 % FCS with mycoplasma			
	20 CFU/ml *Mycoplasma synoviae* →Split into 8 samples	10 CFU/ml *Mycoplasma synoviae* →Split into 8 samples	5 CFU/ml *Mycoplasma synoviae* →Split into 8 samples	1x extraction NTC
	20 CFU/ml *Mycoplasma arginini* →Split into 8 samples	10 CFU/ml *Mycoplasma arginini* →Split into 8 samples	5 CFU/ml *Mycoplasma arginini* →Split into 8 samples	1x extraction NTC
	Extract DNA and perform PCR with 10 µl DNA extract in a final volume of 25 µl +IC Add 2x PCR NTC and 2x PCR PC.			
Day 13	Spike DMEM + 5 % FCS with mycoplasma			
	20 CFU/ml *Mycoplasma synoviae* + IC →Split into 8 samples	10 CFU/ml *Mycoplasma synoviae* + IC →Split into 8 samples	5 CFU/ml *Mycoplasma synoviae* →Split into 8 samples	1x extraction NTC
	20 CFU/ml *Mycoplasma arginini* →Split into 8 samples	10 CFU/ml *Mycoplasma arginini* →Split into 8 samples	5 CFU/ml *Mycoplasma arginini* →Split into 8 samples	1x extraction NTC
	Extract DNA and perform PCR with 10 µl DNA extract in a final volume of 25 µl IC Add 2x PCR NTC and 2x PCR PC.			
Day 14	Spike DMEM + 5 % FCS with mycoplasma			
	20 CFU/ml *Mycoplasma synoviae* →Split into 8 samples	10 CFU/ml *Mycoplasma synoviae* →Split into 8 samples	5 CFU/ml *Mycoplasma synoviae* →Split into 8 samples	1x extraction NTC
	20 CFU/ml *Mycoplasma arginini* →Split into 8 samples	10 CFU/ml *Mycoplasma arginini* →Split into 8 samples	5 CFU/ml *Mycoplasma arginini* →Split into 8 samples	1x extraction NTC
	Extract DNA and perform PCR with 10 µl DNA extract in a final volume of 25 µl + IC Add 2x PCR NTC and 2x PCR PC.			

Detection limit				
Day 15	Spike DMEM + 5 % FCS with mycoplasma			
	20 CFU/ml *Spiroplasma citri* →Split into 8 samples	10 CFU/ml *Spiroplasma citri* →Split into 8 samples	5 CFU/ml *Spiroplasma citri* →Split into 8 samples	1x extraction NTC
	Extract DNA and perform PCR with 10 µl DNA extract in a final volume of 25 µl + IC Add 2x PCR NTC and 2x PCR PC.			
Day 16	Spike DMEM + 5 % FCS with mycoplasma			
	20 CFU/ml *Spiroplasma citri* →Split into 8 samples	10 CFU/ml *Spiroplasma citri* →Split into 8 samples	5 CFU/ml *Spiroplasma citri* →Split into 8 samples	1x extraction NTC
	Extract DNA and perform PCR with 10 µl DNA extract in a final volume of 25 µl + IC Add 2x PCR NTC and 2x PCR PC.			
Day 17	Spike DMEM + 5 % FCS with mycoplasma			
	20 CFU/ml *Spiroplasma citri* →Split into 8 samples	10 CFU/ml *Spiroplasma citri* →Split into 8 samples	5 CFU/ml *Spiroplasma citri* →Split into 8 samples	1x extraction NTC
	Extract DNA and perform PCR with 10 µl DNA extract in a final volume of 25 µl + IC Add 2x PCR NTC and 2x PCR PC.			

Robustness				
Day 18	Vero cell culture supernatant + 10 CFU/ml *M. fermentans*			
	10x			
	Extract DNA and perform PCR with 10 µl DNA extract in a final volume of 25 µl + IC			
	Vero cell culture supernatant + IC	Tris buffer + IC	RPMI + IC	
	1x	1x	1x	
	Extract DNA and perform PCR with 10 µl DNA extract in a final volume of 25 µl w/o IC Add 2x PCR NTC and 2x PCR PC.			
Day 19	Mycoplasma negative ATMP samples from random + 10 CFU/ml *M. fermentans*		extraction NTC	
	2x 20		2x 1	
	Extract DNA and perform PCR with 10 µl DNA extract in a final volume of 25 µl + IC Add 2x PCR NTC and 2x PCR PC.			
Day 20	DMEM + 5 % FCS + 10 CFU/ml *M. fermentans* tested on			
	RotorGene 6000	ABI 7500	LightCycler 1.2	Mx3005p
	10x	10x	10x	10x
	Extract DNA and perform PCR with 10 µl DNA extract in a final volume of 25 µl + IC Add 2x PCR NTC and 2x PCR PC.			

NOTE: Days did not imperatively succeed.

7. Study Results

The study conditions had to provide information on all relevant validation parameters requested by ICH Q2B, EP 2.6.7 and EP 2.6.21.

7.1. Specificity

7.1.1 Sequence Alignment

Procedure	Acceptance Criterion	Results
Comparison of all primer sequences with the genomic database. Mycoplasma sequence alignments will be performed. Even though this technique is not recommended by EP 2.6.7 for specificity determination it provides additional information for species not available for testing.	*Mycoplasma* species showing ≤ 3 nucleotides mismatch in the alignment of the primer sequence with the 16S rRNA genome are considered specifically detectable.	At least 1 species are putatively detectable based on sequence alignment.

Species; Type Strain	**Primer Mismatches**		
	Forward Primer	**Probe**	**Reverse Primer**
Acholeplasma equifetale (T); C112.	0	1	2
Acholeplasma granularum (T); BTS-39.	0	1	0
Acholeplasma hippikon (T); C1.	0	1	1
Acholeplasma laidlawii (T); PG8 ATCC 23206.	0	2 (1)	0
Acholeplasma oculi (T); 19L ATCC 27350.	0	1	1
Acholeplasma pleciae (T); ATCC 49582; PS-1.	0	1	0
Mycoplasma adleri (T); G145.	1	0	0
Mycoplasma agalactiae (T).	0	0	0
Mycoplasma agassizii (T).	0	0	2
Mycoplasma alkalescens (T); PG51.	0	0	1
Mycoplasma alligatoris (T); A21JP2(T).	0	0	2
Mycoplasma alvi (T); Isley.	0	0	2
Mycoplasma amphoriforme (T); A39.	0	0	2
Mycoplasma anatis (T); 1340(T).	0	0	2
Mycoplasma anseris (T); 1219(T).	0	0	1
Mycoplasma arginini (T); G230(T).	0	0	1
Mycoplasma arthritidis (T).	0	0	1
Mycoplasma auris (T); UIA.	0	0	1
Mycoplasma bovigenitalium (T).	0	0	0
Mycoplasma bovirhinis (T); PG43.	0	0	0
Mycoplasma bovis (T); Donetta (type strain); pMb16S.	0	0	0
Mycoplasma bovoculi (T); M165/69.	0	0	2
Mycoplasma buccale (T); CH20247(T).	0	0	1
Mycoplasma buteonis (T); BbT2g(T).	0	0	1
Mycoplasma californicum (T).	0	0	0
Mycoplasma canadense (T); 275c.	0	0	1

Species; Type Strain	Primer Mismatches		
	Forward Primer	Probe	Reverse Primer
Mycoplasma canis (T); PG14.	0	0	1
Mycoplasma capricolum.	0	0	1
Mycoplasma caviae (T); G122(T).	0	0	0
Mycoplasma citelli (T); RG-2C(T).	0	0	0
Mycoplasma cloacale (T); 383(T).	0	0	1
Mycoplasma columbinasale (T); 694(T).	0	0	0
Mycoplasma columbinum (T); MMP-1(T).	0	0	0
Mycoplasma columborale (T); MMP-4(T).	0	0	1
Mycoplasma cricetuli (T); CH(T).	0	1	2
Mycoplasma crocodyli (T); MP145(T).	0	0	2
Mycoplasma cynos (T); H831(T).	0	0	1
Mycoplasma edwardii; PG24.	0	0	2
Mycoplasma elephantis (T); E42(T).	0	0	1
Mycoplasma equigenitalium (T); T37(T).	0	0	1
Mycoplasma equirhinis (T); M432/72(T).	0	0	1
Mycoplasma falconis (T); H/T1(T).	0	0	0
Mycoplasma faucium (T); DC333(T).	0	0	0
Mycoplasma felifaucium (T); ATCC 43428.	1	0	0
Mycoplasma felis (T); ATCC 23391.	1	0	1
Mycoplasma fermentans (T).	0	0	0
Mycoplasma gallinaceum (T); DD.	0	0	0
Mycoplasma gallinarum (T); PG16.	0	0	0
Mycoplasma gallisepticum str. F; 1.	0	0	0
Mycoplasma gallopavonis (T); WR1(T).	0	0	1
Mycoplasma gateae (T); ATCC 23392.	0	0	1
Mycoplasma genitalium (T); G37.	0	0	2
Mycoplasma glycophilum (T); 486(T).	0	0	1
Mycoplasma gypis (T); B1/T1(T).	0	1	1
Mycoplasma hominis (T); PG21; ATCC 23114.	0	0	2
Mycoplasma hyopharyngis (T).	0	0	1
Mycoplasma hyorhinis (T); BTS7(T).	0	0	0
Mycoplasma hyosynoviae (T); S-16.	0	0	1
Mycoplasma iguanae (T); 2327.	0	1	1
Mycoplasma imitans (T); 4229.	0	0	0
Mycoplasma indiense (T); 3T(T).	0	0	1
Mycoplasma iners (T); PG30(T).	0	0	0
Mycoplasma iowae (T).	0	0	1
Mycoplasma lagogenitalium (T); 12MS(T).	0	1	2
Mycoplasma leonicaptivi (T); ATCC 49890.	1	0	1
Mycoplasma leopharyngis (T); ATCC 49889.	1	0	0
Mycoplasma lipofaciens (T); R171(T).	0	0	1
Mycoplasma lipophilum (T).	0	0	0
Mycoplasma maculosum (T); PG15(T).	0	0	0
Mycoplasma meleagridis (T); 17529.	0	0	0
Mycoplasma microti (T); IL371.	0	0	1
Mycoplasma moatsii (T); MK405(T).	0	0	0
Mycoplasma mobile (T).	0	0	1
Mycoplasma molare (T); H542.	0	1	2
Mycoplasma mucosicanis (T); type strain: 1642.	0	0	1
Mycoplasma muris (T).	0	0	2
Mycoplasma mustelae (T); MX9(T).	0	0	0

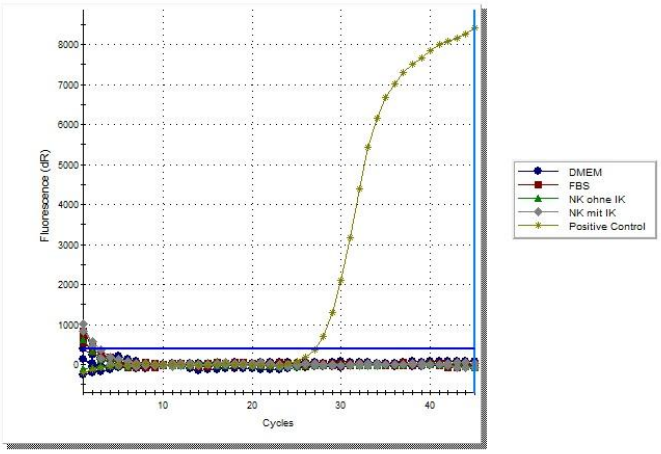
Species; Type Strain	Primer Mismatches		
	Forward Primer	Probe	Reverse Primer
Mycoplasma opalescens (T); MH5408(T).	0	0	0
Mycoplasma orale (T); NC10112; CH 19299; ATCC 23714.	0	0	1
Mycoplasma oxoniensis (T); 128(T).	0	0	0
Mycoplasma penetrans HF-2.	0	0	1
Mycoplasma phocae; CSL 4693.	0	0	2
Mycoplasma phocicerebrale (T); 1049; ATCC 49640.	0	0	2
Mycoplasma phocidae (T); 105; ATCC 33657.	0	0	2
Mycoplasma phocirhinis (T); 852; ATCC 49639.	0	0	0
Mycoplasma pirum (T).	0	0	2
Mycoplasma pneumoniae (T); ATCC 15531.	0	0	2
Mycoplasma primatum (T); HRC292(T).	0	0	0
Mycoplasma pullorum (T); CKK.	0	0	2
Mycoplasma pulmonis (T); PG34(T).	0	0	1
Mycoplasma salivarium (T); PG20(T).	0	0	1
Mycoplasma simbae (T); ATCC 49888.	0	0	1
Mycoplasma spermatophilum (T); AH159(T).	0	0	0
Mycoplasma sphenisci; UCMJ.	0	0	1
Mycoplasma spumans (T); PG13(T).	0	0	1
Mycoplasma sturni (T); UC/MF; p170/171.	0	0	0
Mycoplasma sualvi (T); Mayfield B(T).	0	0	0
Mycoplasma subdolum (T); TB(T).	0	0	1
Mycoplasma synoviae (T); WVU 1853; pMSk3-4 pMSF16S.	0	0	0
Mycoplasma testudineum (T); H3110.	1	0	1
Mycoplasma testudinis (T); ATCC 43263.	0	0	3
Mycoplasma timone.	0	0	1
Mycoplasma verecundum (T); GIH(T).	0	0	0
Mycoplasma vulturii; Gb-V33.	0	0	3
Mycoplasma zalophidermidis; CSL 4779.	0	0	1
Ureaplasma canigenitalium (T); D6P-C.	0	0	3
Ureaplasma diversum (T); A417.	0	0	3
Ureaplasma felinum (T); FT2-B.	0	0	3
Ureaplasma parvum (T); ATCC27815.	0	0	3
Ureaplasma urealyticum (T); ATCC27618.	0	0	3

7.1.2 Sample Matrix Effects

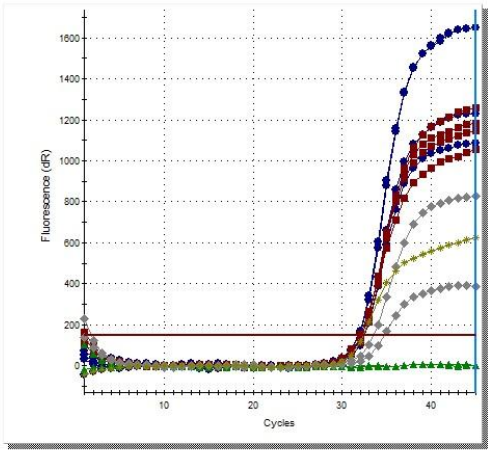
Procedure	Acceptance Criterion	Results
Testing of at least 4 different samples using the media components according to Table 5 to exclude the possibility of false-positive results. The internal amplification control will be added to the sample matrix as extraction control according to chapter 8.2.	All tested samples shall show a negative result.	passed

No.	Sample Matrix	Manufacturer	Catalog No.	Lot No.	Ct FAM	Ct ROX
1	Dulbecco's MEM	Biochrom AG	FG 0415	0580A	No Ct	31.92
2	Dulbecco's MEM	Biochrom AG	FG 0415	0580A	No Ct	31.85
3	Dulbecco's MEM	Biochrom AG	FG 0415	0580A	No Ct	32.57
4	Dulbecco's MEM	Biochrom AG	FG 0415	0580A	No Ct	32.21
5	FCS	Biochrom AG	S0615	0248W	No Ct	31.95
6	FCS	Biochrom AG	S0615	0248W	No Ct	32.25
7	FCS	Biochrom AG	S0615	0248W	No Ct	32.42
8	FCS	Biochrom AG	S0615	0248W	No Ct	32.23
9	Positive control	---	---	---	27.16	No CT
10	NTC with IC	---	---	---	No Ct	33.59
11	NTC with IC	---	---	---	No Ct	34.76
12	NTC without IC	---	---	---	No Ct	No Ct
13	NTC without IC	---	---	---	No Ct	No Ct

FAM Channel



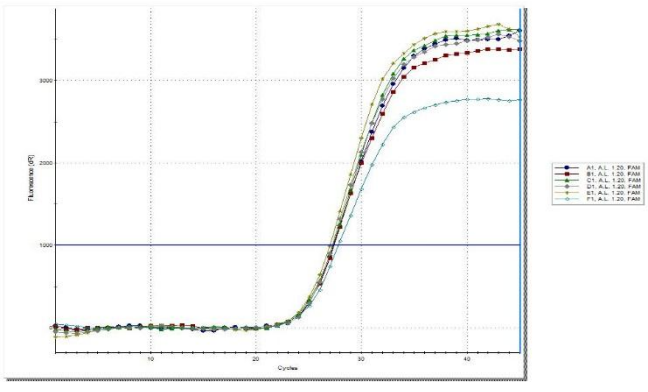
ROX Channel



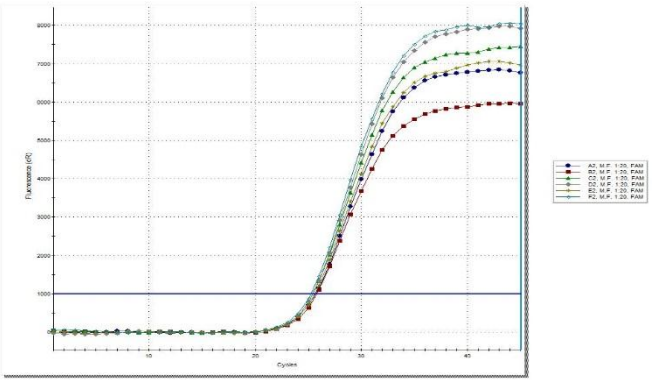
7.1.3 Mollicutes Detection Range

Procedure	Acceptance Criterion	Results
All DNA extracts listed in Table 3 derived from *Mollicutes* will be tested at a load of ≥ 0.1 ng/test. At least 6 repeats shall be tested for each sample.	All tested samples shall show a positive result.	passed

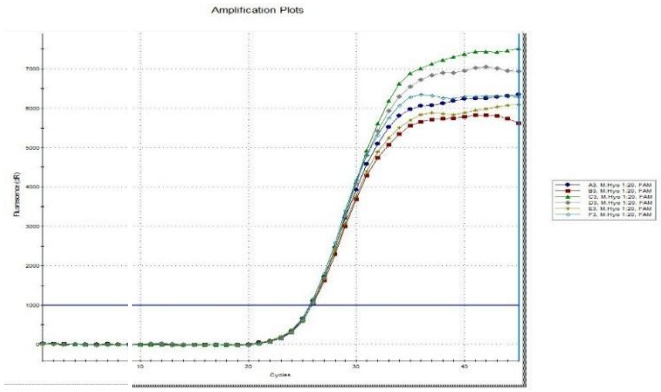
No	Species	Ct	Result
1	*Acholeplasma laidlawii*	27.43	positive
2	*Acholeplasma laidlawii*	27.46	positive
3	*Acholeplasma laidlawii*	27.33	positive
4	*Acholeplasma laidlawii*	27.28	positive
5	*Acholeplasma laidlawii*	27.01	positive
6	*Acholeplasma laidlawii*	27.85	positive



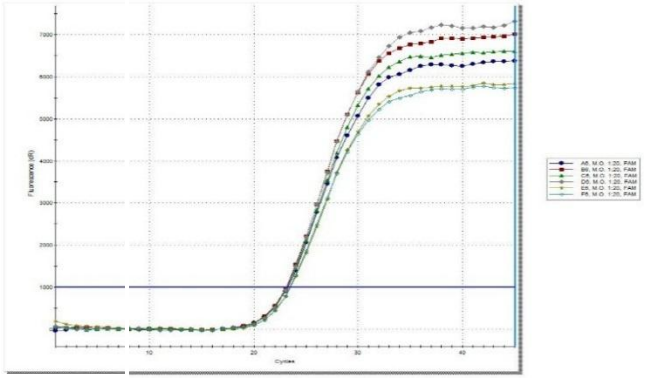
No	Species	Ct	Result
1	*Mycoplasma fermentans*	25.71	positive
2	*Mycoplasma fermentans*	25.81	positive
3	*Mycoplasma fermentans*	25.46	positive
4	*Mycoplasma fermentans*	25.43	positive
5	*Mycoplasma fermentans*	25.64	positive
6	*Mycoplasma fermentans*	25.26	positive



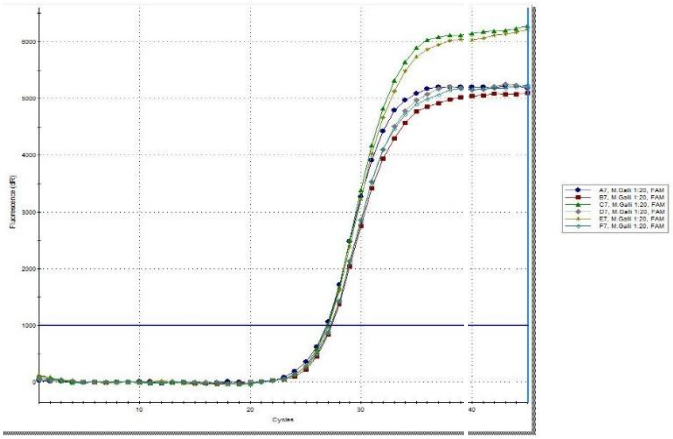
No	Species	Ct	Result
1	*Mycoplasma hyorhinis*	25.80	positive
2	*Mycoplasma hyorhinis*	25.93	positive
3	*Mycoplasma hyorhinis*	25.92	positive
4	*Mycoplasma hyorhinis*	25.89	positive
5	*Mycoplasma hyorhinis*	25.79	positive
6	*Mycoplasma hyorhinis*	25.72	positive



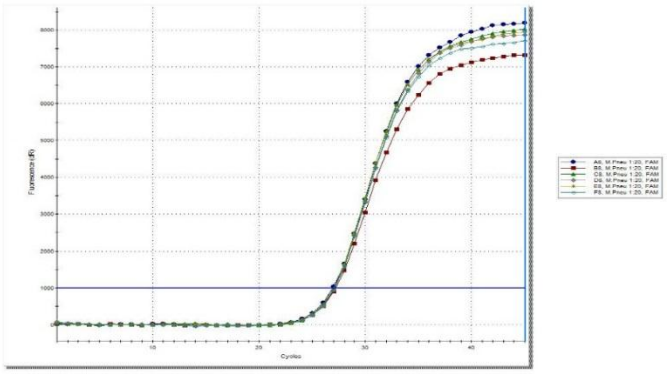
No	Species	Ct	Result
1	*Mycoplasma orale*	23.25	positive
2	*Mycoplasma orale*	23.12	positive
3	*Mycoplasma orale*	23.18	positive
4	*Mycoplasma orale*	23.16	positive
5	*Mycoplasma orale*	23.53	positive
6	*Mycoplasma orale*	23.47	positive



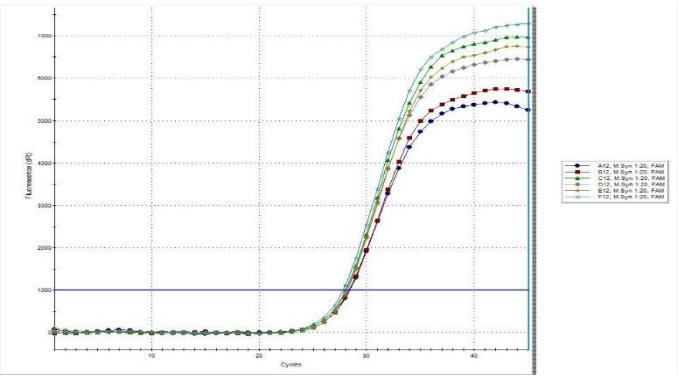
No	Species	Ct	Result
1	*Mycoplasma gallisepticum*	26.88	positive
2	*Mycoplasma gallisepticum*	27.35	positive
3	*Mycoplasma gallisepticum*	26.97	positive
4	*Mycoplasma gallisepticum*	27.27	positive
5	*Mycoplasma gallisepticum*	27.04	positive
6	*Mycoplasma gallisepticum*	27.23	positive



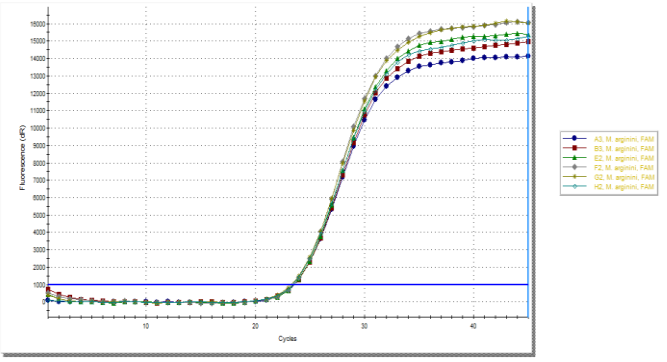
No	Species	Ct	Result
1	*Mycoplasma pneumoniae*	26.94	positive
2	*Mycoplasma pneumoniae*	27.21	positive
3	*Mycoplasma pneumoniae*	27.03	positive
4	*Mycoplasma pneumoniae*	27.11	positive
5	*Mycoplasma pneumoniae*	27.06	positive
6	*Mycoplasma pneumoniae*	27.09	positive



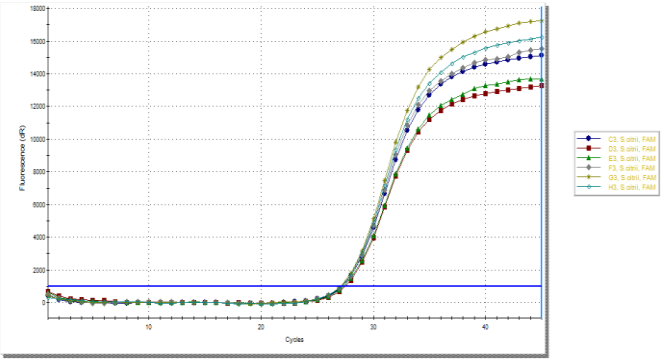
No	Species	Ct	Result
1	*Mycoplasma synoviae*	28.47	positive
2	*Mycoplasma synoviae*	28.40	positive
3	*Mycoplasma synoviae*	28.04	positive
4	*Mycoplasma synoviae*	28.12	positive
5	*Mycoplasma synoviae*	28.25	positive
6	*Mycoplasma synoviae*	27.83	positive



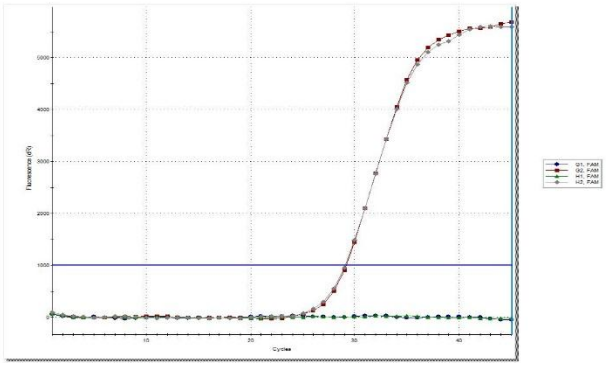
No	Species	Ct	Result
1	*Mycoplasma arginini*	23.59	positive
2	*Mycoplasma arginini*	23.52	positive
3	*Mycoplasma arginini*	23.34	positive
4	*Mycoplasma arginini*	23.44	positive
5	*Mycoplasma arginini*	23.65	positive
6	*Mycoplasma arginini*	23.60	positive



No	Species	Ct	Result
1	*Spiroplasma citri*	27.28	positive
2	*Spiroplasma citri*	27.54	positive
3	*Spiroplasma citri*	27.40	positive
4	*Spiroplasma citri*	27.22	positive
5	*Spiroplasma citri*	27.15	positive
6	*Spiroplasma citri*	27.19	positive



No	Sample	Ct	Result
1	Negative control	No Ct	negative
2	Positive control	29.10	positive
3	Positive control	29.20	positive



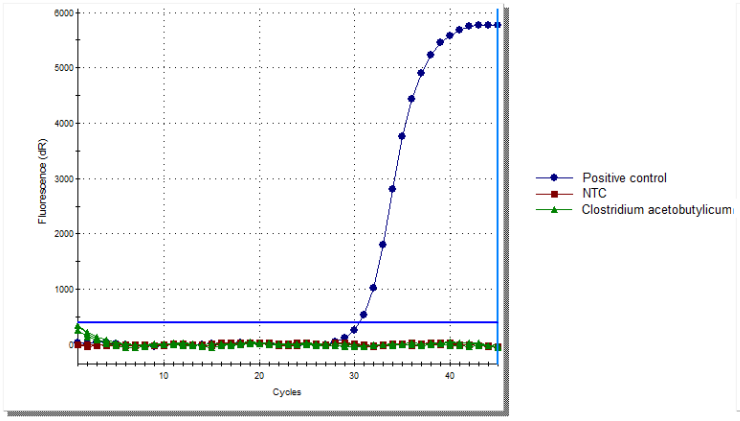
7.1.4 Cross Reactivity

Procedure	Acceptance Criterion	Results
All DNA extracts listed in Table 4 derived from microorganisms and cells will be tested at a load of ≥ 0.1 ng/test for microorganisms and ≥ 30 ng for mammalian cells. 3 repeats shall be tested for each sample.	All tested samples shall show a negative result.	passed

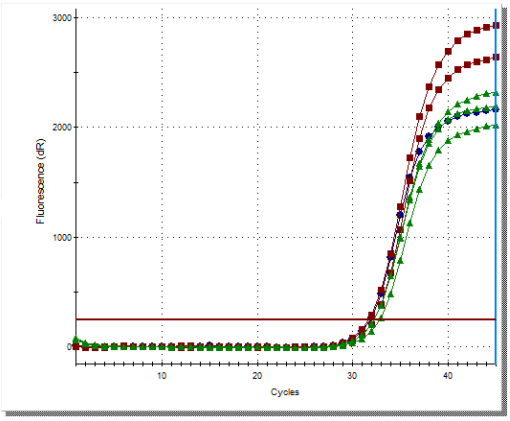
Species	Results
Clostridium acetobutylicum	negative
Lactobacillus acidophilus	negative
Streptococcus pneumoniae	negative
Vero-B4	negative
Per.C6	negative
RK13	negative
CHO-K1	negative
Murine Genomic DNA	negative
Calf Thymus DNA	negative

No	Species	Ct FAM	Ct ROX	Result
1	*Clostridium acetobutylicum*	No Ct	32.30	negative
2	*Clostridium acetobutylicum*	No Ct	32.90	negative
3	*Clostridium acetobutylicum*	No Ct	32.33	negative

FAM Channel

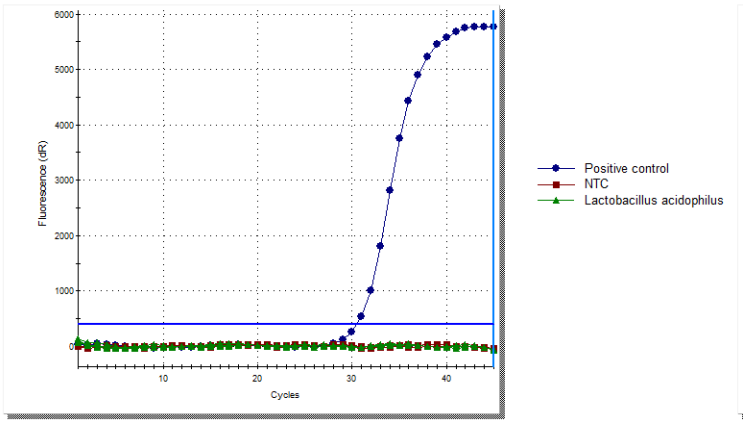


ROX Channel

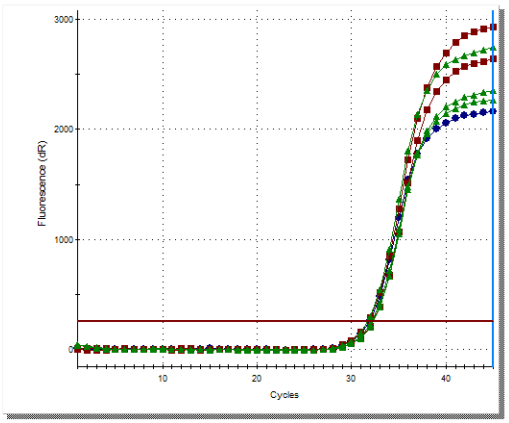


No	Species	Ct FAM	Ct ROX	Result
1	*Lactobacilus acidophilus*	No Ct	32.35	negative
2	*Lactobacilus acidophilus*	No Ct	32.15	negative
3	*Lactobacilus acidophilus*	No Ct	31.73	negative

FAM Channel

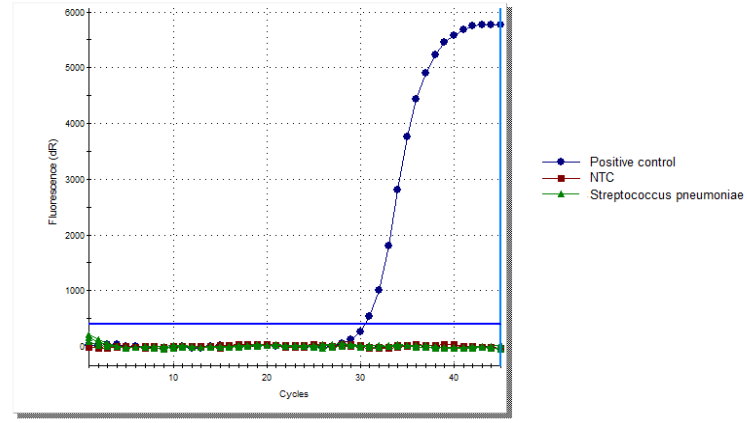


ROX Channel

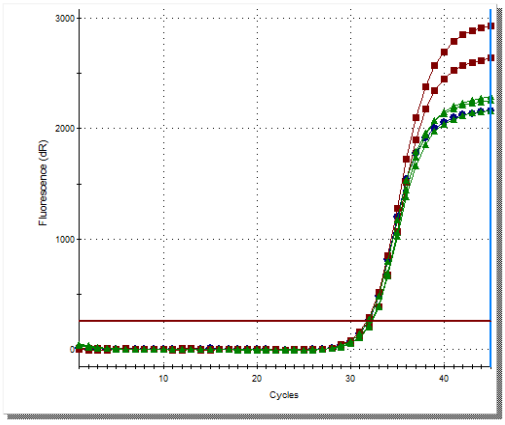


No	Species	Ct FAM	Ct ROX	Result
1	*Streptococcus pneumoniae*	No Ct	32.31	negative
2	*Streptococcus pneumoniae*	No Ct	31.89	negative
3	*Streptococcus pneumoniae*	No Ct	32.24	negative

FAM Channel

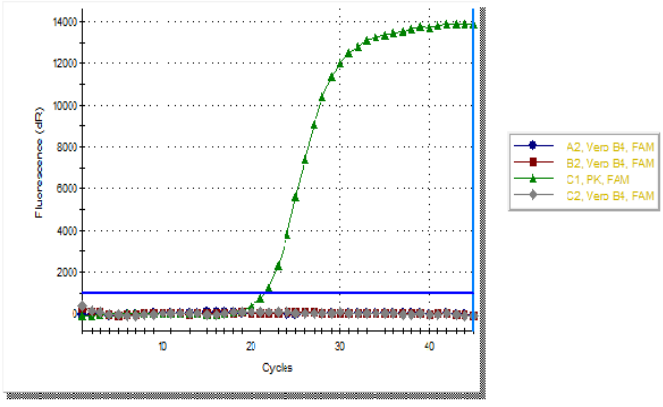


ROX Channel

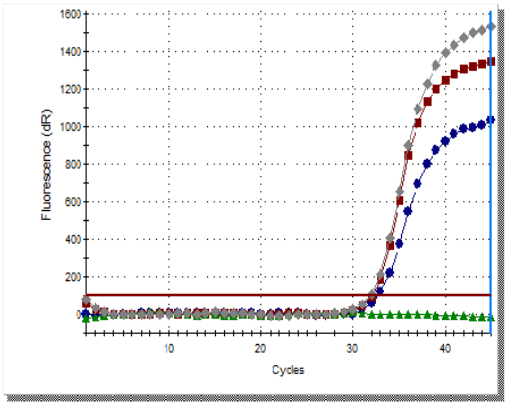


No	Species	Ct FAM	Ct ROX	Result
1	Vero B4	No Ct	32.37	negative
2	Vero B4	No Ct	32.20	negative
3	Vero B4	No Ct	32.64	negative

FAM Channel

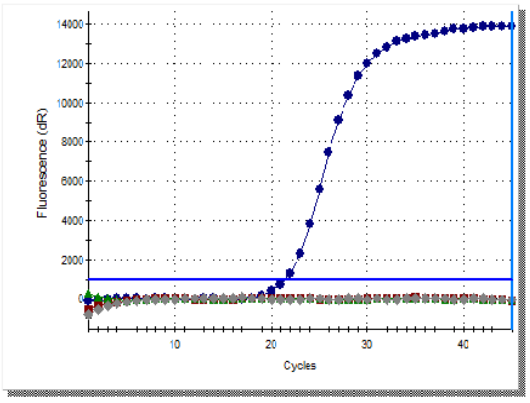


ROX Channel

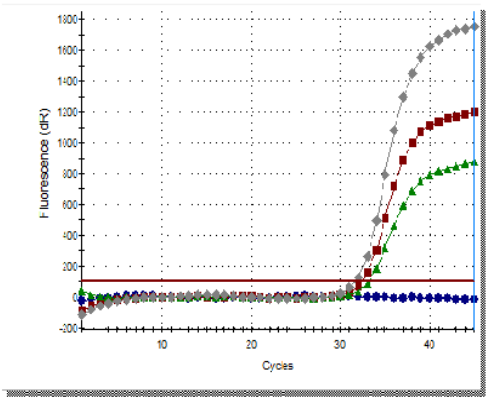


No	Species	Ct FAM	Ct ROX	Result
1	Per C6	No Ct	32.64	negative
2	Per C6	No Ct	33.45	negative
3	Per C6	No Ct	31.89	negative

FAM Channel

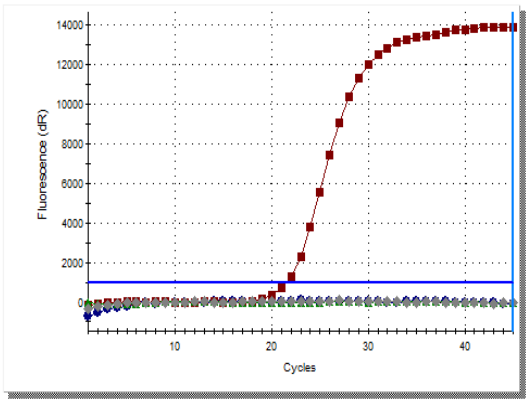


ROX Channel

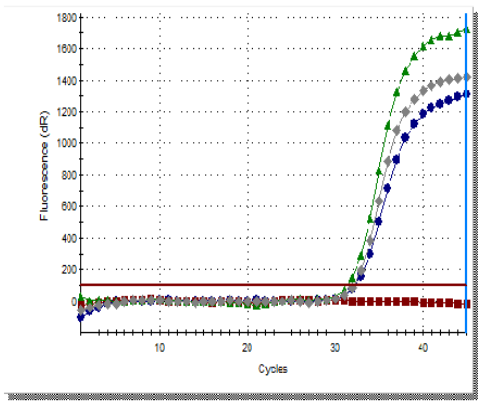


No	Species	Ct FAM	Ct ROX	Result
1	RK13	No Ct	31.74	negative
2	RK13	No Ct	32.45	negative
3	RK13	No Ct	32.62	negative

FAM Channel

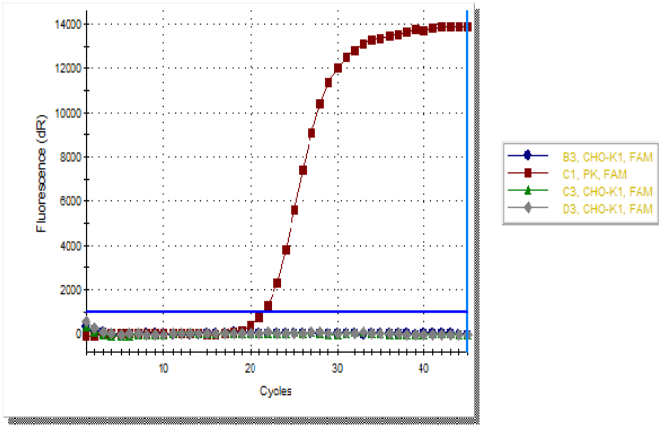


ROX Channel

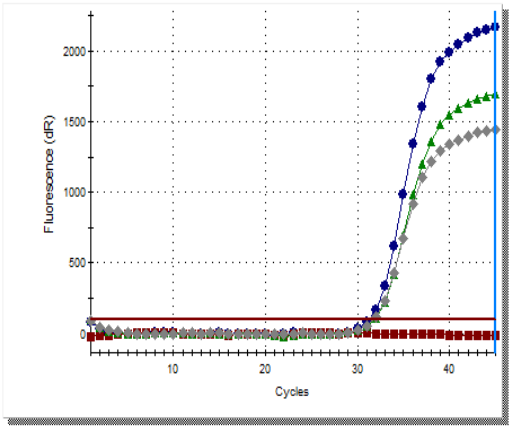


No	Species	Ct FAM	Ct ROX	Result
1	CHO-K1	No Ct	31.56	negative
2	CHO-K1	No Ct	32.11	negative
3	CHO-K1	No Ct	32.05	negative

FAM Channel

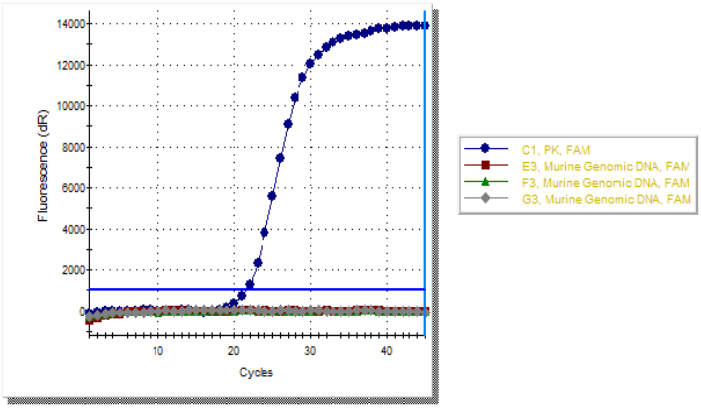


ROX Channel

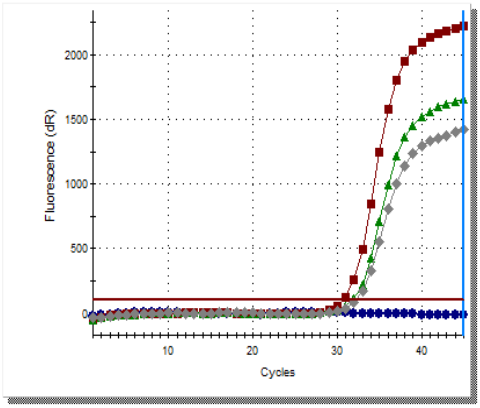


No	Species	Ct FAM	Ct ROX	Result
1	Murine Genomic DNA	No Ct	30.96	negative
2	Murine Genomic DNA	No Ct	32.13	negative
3	Murine Genomic DNA	No Ct	32.49	negative

FAM Channel

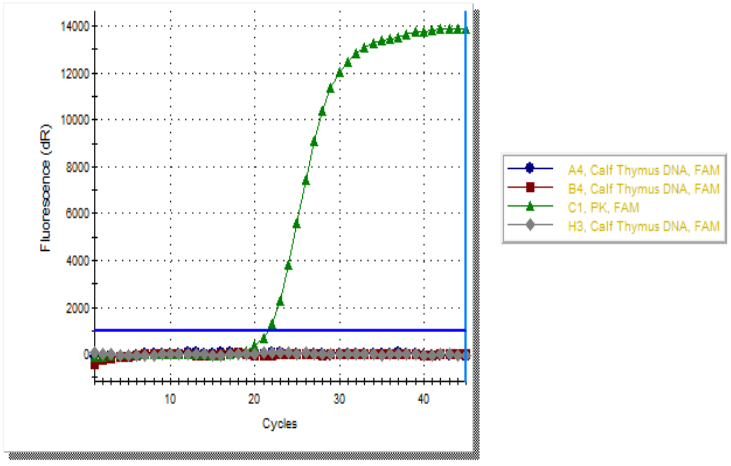


ROX Channel

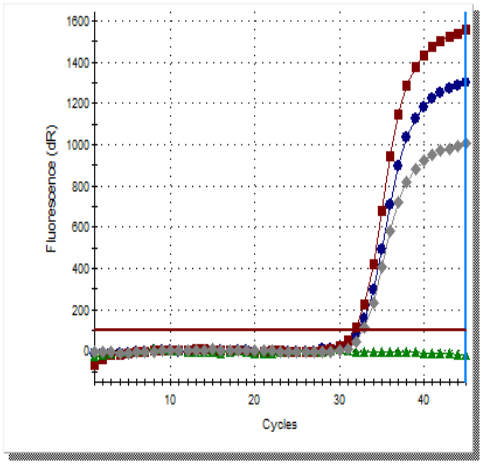


No	Species	Ct FAM	Ct ROX	Result
1	Calf Thymus DNA	No Ct	33.07	negative
2	Calf Thymus DNA	No Ct	32.61	negative
3	Calf Thymus DNA	No Ct	32.09	negative

FAM Channel



ROX Channel



7.1.5 Identification of Unspecific Amplification

Procedure	Acceptance Criterion	Results
Further identification of the result of amplification is conducted by sequence analysis using the primer portion of the kit for sequencing.	In case of amplification. amplicon should be identified as homologous to the *Mycoplasma* genome.	N/A

7.2 Detection Limit

As the method employed is used only to obtain a qualitative result, proof of linearity is not required. However, if the concept of linearity is extended to cover the working range, the detection limit becomes extremely important. In practice, the detection limit is determined in the form of the positive threshold (i.e. the cut-off point in the form of the minimum number of amplified target sequences by volume positively detected in 95% of the sample series).

7.2.1 Culture Media Comparison

Procedure	Acceptance Criterion	Results
For spiking, all *Mollicutes* species listed in EP 2.6.7 (see Table 3) are used. The spike will be prepared according to chapter 5.4 from fresh cultures and will be quantified immediately on culture plates. The ability of the culture method used to sustain the growth of mycoplasma in acceptable performance is confirmed by parallel testing with the EDQM Reference Standard listed in Table 6. The EDQM Reference Standards and the spikes of the same species will be diluted in culture broth with 3 replicates per dilution.	The prepared spikes shall show a calculated titre within the estimated titre range of the EDQM Reference Standard.	passed

Article No.	Article name	Estimated Titer	Result
Y0000692	*Mycoplasma fermentans* Ph Eur BRP, batch 1	$9.55x10^7$ CFU/ml with a range from $1.58x10^7$ to $5.75x10^8$ CFU/ml	$4.78x10^7$ CFU/ml
Y0000691	*Mycoplasma orale* Ph Eur BRP, batch 1	$4.90x10^5$ CFU/ml with a range from $9.33x10^4$ to $2.57x10^6$ CFU/ml	$5.88x10^5$ CFU/ml
Y0000689	*Mycoplasma synoviae* BRP, batch 1	$1.86x10^7$ CFU/ml with a range from $5.89x10^6$ to $5.89x10^7$ CFU/ml	$2.79x10^7$ CFU/ml
Y0000690	*Mycoplasma hyorhinis* BRP, batch 1	$1.17x10^8$ CFU/ml with a range from $6.76x10^7$ to $2.34x10^8$ CFU/ml	$1.99x10^8$ CFU/ml

7.2.2 Detection Limit

Procedure	Acceptance Criterion	Results
The prepared *Mollicutes* spikes according to chapter 5.4 will be diluted in 1:10 dilution steps (one deviating dilution step for accurate adjustment of concentration) in DMEM supplemented with 5 % (v/v) FCS to prepare suspensions with concentration of 20, 10 and 5 CFU/ml. 3 individually dilution series will be prepared and each of the 3 dilutions tested according to chapter 8 with 8 repeats each so that at least 24 results per dilution are obtained (n = 3 x 8 = 24).	All samples containing at least 10 CFU/ml must be tested positive for all species.	passed A Detection Limit of at least 10 cfu/ml was found for all species listed in the EP 2.6.7.

Acholeplasma laidlawii

CFU/ ml	**20**			**10**			**5**		
	run 1	**run 2**	**run 3**	**run 1**	**run 2**	**run 3**	**run 1**	**run 2**	**run 3**
	32.03	30.23	32.24	31.66	33.41	31.93	No Ct	30.00	No Ct
	33.43	31.79	33.53	29.17	31.09	28.64	32.98	33.93	31.79
	30.16	32.25	30.04	34.75	29.17	33.65	33.83	No Ct	34.51
	30.35	30.2	30.34	33.32	29.44	33.02	34.68	No Ct	34.09
	31.25	29.57	31.16	34.42	32.58	34.47	33.7	29.94	34.61
	29.85	30.42	29.59	32.9	31.00	32.89	30.33	29.26	30.36
	29.51	27.70	28.95	31.96	30.23	32.73	32.70	31.70	32.48
	32.13	31.15	32.62	34.31	33.75	35.21	31.96	No Ct	32.59
Mean Ct	31.09	30.41	31.06	32.81	31.33	32.82	32.88	30.97	32.92
		24/24			24/24			19/24	

Mycoplasma fermentans

CFU/ ml	20			10			5		
	run 1	run 2	run 3	run 1	run 2	run 3	run 1	run 2	run 3
	29.62	32.12	30.43	29.74	33.34	31.04	29.97	33.76	32.29
	29.15	32.18	29.83	30.78	36.02	29.71	31.09	No Ct	32.25
	29.22	32.68	30.03	30.45	35.10	30.69	30.96	No Ct	32.26
	29.73	32.52	30.37	29.72	34.39	30.72	29.74	No Ct	32.04
	29.15	31.77	30.21	29.67	32.53	31.49	31.07	33.75	32.76
	29.38	31.95	30.41	29.1	35.19	30.96	31.61	33.63	32.31
	29.57	32.88	29.77	29.59	33.3	30.80	30.99	35.12	32.93
	28.59	32.17	30.6	30.16	34.09	31.3	31.80	35.09	30.86
Mean Ct	29.30	32.28	30.21	29.90	34.25	30.84	30.90	34.27	32.21
		24/24			24/24			21/24	

Mycoplasma hyorhinis

CFU/ ml	20			10			5		
	run 1	run 2	run 3	run 1	run 2	run 3	run 1	run 2	run 3
	33.39	32.35	33.81	33.11	33.87	33.48	35.80	34.05	34.68
	33.08	32.59	32.96	32.85	33.45	33.22	35.49	34.23	33.99
	32.78	31.99	33.97	32.97	34.24	34.78	35.59	34.13	35.04
	33.39	32.33	33.89	33.08	33.69	33.80	36.92	34.71	34.70
	32.61	32.90	33.90	33.58	33.31	33.51	34.88	34.39	34.74
	32.70	33.23	33.40	34.02	33.08	34.07	35.61	33.73	34.67
	32.36	32.79	33.58	33.00	33.05	33.97	34.31	34.13	34.51
	32.46	32.59	33.85	33.74	33.23	33.67	34.47	34.6	36.11
Mean Ct	32.85	32.60	33.67	33.29	33.49	33.81	35.38	34.25	34.81
		24/24			24/24			24/24	

Mycoplasma orale

CFU/ ml	20			10			5		
	run 1	run 2	run 3	run 1	run 2	run 3	run 1	run 2	run 3
	31.86	32.81	34.01	32.45	33.86	34.50	33.67	35.19	35.37
	31.76	33.30	33.33	32.39	34.30	34.42	33.57	36.06	36.37
	31.79	32.77	33.06	32.79	33.34	35.78	33.59	39.12	35.94
	31.85	32.99	33.13	32.62	33.57	34.83	33.48	36.17	36.11
	31.76	32.61	34.11	32.96	34.97	34.84	33.60	41.62	No Ct
	31.98	32.96	33.90	32.78	33.37	34.86	33.76	35.83	35.00
	31.76	33.04	33.47	33.18	33.97	35.17	33.44	35.69	36.99
	31.79	32.75	33.84	32.59	34.60	34.33	34.04	38.04	36.57
Mean Ct	31.82	32.90	33.61	32.72	34.00	34.84	33.64	37.22	36.05
		24/24			24/24			22/24	

Mycoplasma pneumoniae

CFU/ ml	20			10			5		
	run 1	run 2	run 3	run 1	run 2	run 3	run 1	run 2	run 3
	32.42	31.49	31.64	32.61	32.53	32.47	33.83	33.26	33.23
	31.88	29.85	31.20	32.15	32.04	32.36	36.88	32.21	33.08
	31.40	29.57	30.81	31.39	32.24	32.00	33.44	33.06	30.88
	31.13	31.88	29.98	33.02	32.69	31.84	33.62	34.15	33.10
	31.21	29.57	32.10	32.35	31.21	32.48	No Ct	32.82	34.10
	31.46	29.46	30.69	32.48	31.03	33.21	33.37	30.01	33.24
	31.54	30.57	30.84	32.17	30.92	32.25	32.18	31.47	34.16
	31.58	30.76	32.04	33.56	32.68	32.87	33.45	32.19	32.69
Mean Ct	31.58	30.39	31.16	32.47	31.92	32.44	33.82	32.40	33.06
		24/24			24/24			23/24	

Mycoplasma gallisepticum

CFU/ ml	20			10			5		
	run 1	run 2	run 3	run 1	run 2	run 3	run 1	run 2	run 3
	N/V	32.56	31.65	34.08	32.95	32.52	33.77	34.49	33.99
	32.61	30.49	31.19	36.74	34.19	32.34	35.77	35.75	33.90
	32.95	32.59	32.15	32.59	35.26	34.29	34.79	No Ct	34.00
	32.00	32.49	32.98	34.04	33.80	33.26	34.76	No Ct	42.06
	32.86	31.57	31.98	32.76	33.33	31.58	No Ct	33.59	33.64
	32.61	34.11	32.17	34.39	33.30	32.24	35.85	34.19	35.15
	33.52	33.05	31.94	34.82	31.96	31.37	No Ct	42.44	32.43
	34.01	31.04	32.17	34.00	33.01	32.28	36.09	39.59	33.15
Mean Ct	32.94	32.24	32.03	34.18	33.48	32.49	35.17	36.68	34.79
	23/24			24/24			19/24		

Mycoplasma synoviae

CFU/ml	20			10			5		
	run 1	run 2	run 3	run 1	run 2	run 3	run 1	run 2	run 3
	32.95	33.01	34.37	33.80	33.28	34.47	No Ct	31.95	34.06
	34.36	31.44	32.49	33.60	33.58	33.12	No Ct	33.91	35.62
	33.27	33.18	32.62	35.01	35.17	33.63	No Ct	32.86	33.09
	32.76	32.43	32.47	32.14	32.31	34.24	No Ct	33.11	32.33
	32.57	33.22	32.56	32.98	33.30	33.35	No Ct	33.73	33.15
	32.01	32.24	33.39	33.32	34.3	33.12	No Ct	33.04	34.71
	31.93	33.50	31.33	No Ct	33.83	35.22	No Ct	33.26	33.34
	33.17	34.23	32.79	34.03	33.59	32.48	No Ct	33.51	36.05
Mean Ct	32.88	32.91	32.75	33.55	33.67	33.70	No Ct	33.17	34.04
	24/24			23/24			16/24		

Mycoplasma arginini

CFU/ ml	20			10			5		
	run 1	run 2	run 3	run 1	run 2	run 3	run 1	run 2	run 3
	34.57	32.09	32.13	35.48	34.42	32.94	37.31	34.70	34.39
	33.09	32.19	32.15	32.93	33.14	33.12	37.44	33.79	33.85
	32.22	32.39	32.70	35.08	33.22	33.67	No Ct	33.05	33.77
	33.58	32.37	32.11	No Ct	33.64	33.68	No Ct	34.07	35.85
	34.36	32.55	32.41	34.49	33.83	34.59	40.74	34.29	33.88
	32.78	32.76	32.15	37.88	32.64	32.09	No Ct	35.11	35.36
	34.40	31.77	32.57	34.95	32.64	32.38	34.72	34.97	35.18
	No Ct	31.79	32.27	36.73	32.88	32.59	40.51	34.43	34.05
Mean Ct	33.57	32.24	32.31	35.36	33.30	33.13	38.14	34.30	34.54
		23/24			23/24			18/24	

Spiroplasma citri

CFU/ ml	20			10			5		
	run 1	run 2	run 3	run 1	run 2	run 3	run 1	run 2	run 3
	35.14	35.96	34.6	36.83	35.92	34.67	37.21	36.7	44.44
	35.16	35.88	35.02	35.76	35.69	34.36	36.77	37.28	35.88
	34.78	36.07	34.86	36.03	35.38	35.04	35.69	36.99	37.79
	35.06	35.96	35.33	36.98	35.66	34.84	35.98	36.73	38.34
	34.09	34.88	34.72	35.44	35.14	34.52	36.72	36.78	37.64
	34.75	35.51	34.92	35.47	35.12	33.96	37.30	36.07	35.72
	33.61	36.09	34.73	35.36	36.68	34.56	36.65	36.55	36.48
	34.15	35.38	34.55	35.67	36.33	34.49	37.03	38.61	36.4
Mean Ct	34.59	35.72	34.84	35.94	35.74	34.56	36.67	36.96	37.84
		24/24			24/24			23/24	

7.3 Precision, Linearity, Range, Accuracy and Quantification Limit

As the requirement of the method is to provide qualitative results only, this parameter is irrelevant.

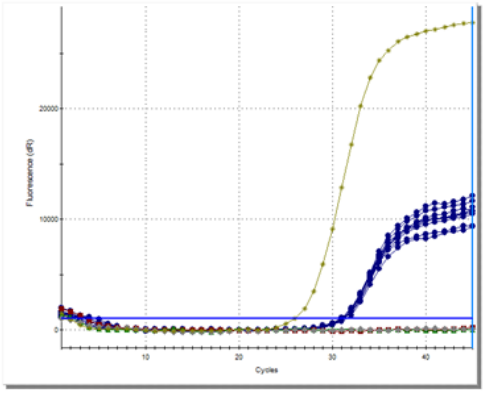
7.4 Robustness

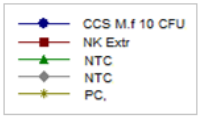
7.4.1 Cell Culture Material

Procedure	Acceptance Criterion	Results
The robustness of the method will be demonstrated by the determination of 10 CFU/ml of *Mycoplasma fermentans* in a representative cell culture matrix according to Table 2. At least 10 repeats shall be tested for each sample matrix.	9 out of 10 samples show a positive result.	passed

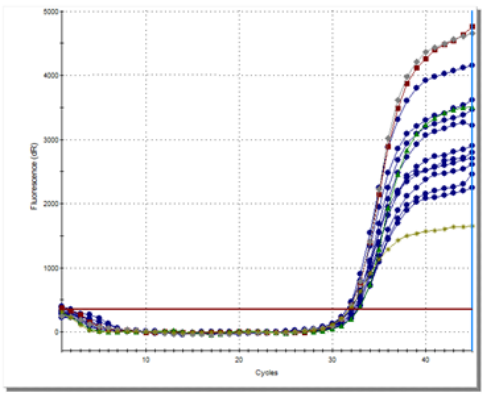
No	Sample	Ct - FAM	Ct - ROX	Result
1	Supernatant of a 100 % confluent Vero cell culture including cell debris after heat treatment + 10 CFU/ml *M. fermentans*	31.09	33.26	positive
2		31.05	32.53	positive
3		31.54	32.87	positive
4		31.29	32.01	positive
5		31.33	33.06	positive
6		31.03	33.22	positive
7		30.96	32.09	positive
8		31.15	33.29	positive
9		31.10	32.74	positive
10		30.89	32.50	positive
11	NTC	No Ct	33.30	passed
12	NTC	No Ct	32.23	passed
13	Negative control (extraction)	No Ct	32.25	passed
14	PC	25.93	32.43	passed

Channel FAM





Channel ROX



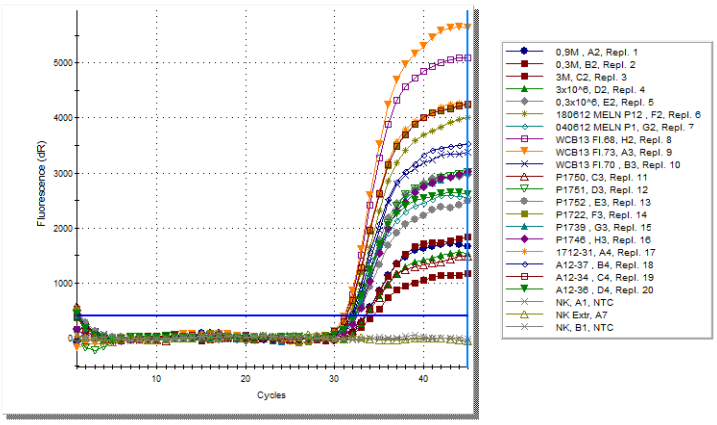
7.4.2 ATMPs

Procedure	Acceptance Criterion	Results
To demonstrate robustness, at least 20 mycoplasma negative ATMP samples (selected at random from samples submitted by customers for mycoplasma detection) spiked with 10 CFU/ml of *Mycoplasma fermentans*. At least 2 repeats shall be tested for each sample.	19 out of 20 samples show a positive result.	passed

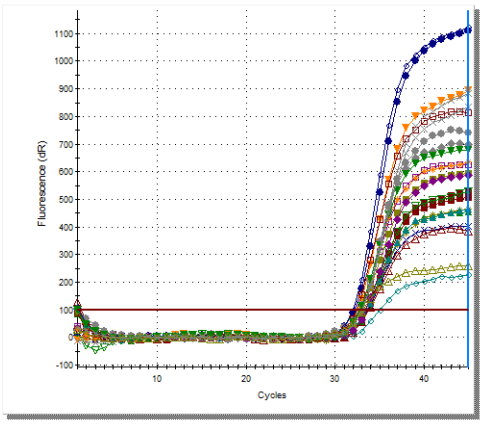
N°	**Sample Code / Characteristics**		**Ct FAM**	**Ct ROX**	**Ct FAM**	**Ct ROX**	**Result**
			Repeat 1		Repeat 2		
1	0.9M	GMP010	33.53	32.20	32.69	33.92	positive
2	0.3M	GMP010	33.60	33.68	32.32	32.51	positive
3	3M	GMP008	34.32	33.55	33.81	32.71	positive
4	3x10^6	GMP009	33.65	33.83	33.71	34.16	positive
5	0.3x10^6	GMP009	32.14	32.92	32.28	32.29	positive
6	180612 MELN P12	D1906912	31.61	33.68	31.98	32.85	positive
7	040612 MELN P1	D050612-7	32.39	35.00	31.88	32.57	positive
8	WCB13 FI.68	D311012-4	31.13	33.03	31.10	33.36	positive
9	WCB13 FI.73	D311012-4	31.07	32.58	30.97	32.66	positive
10	WCB13 FI.70	D311012-4	32.01	33.77	31.78	32.53	positive
11	P1750	D210612-1	33.44	33.86	32.05	32.23	positive
12	P1751	D260612-2	32.29	33.51	32.54	32.36	positive
13	P1752	D260612-2	32.37	32.74	33.38	32.12	positive
14	P1722	D150612-1	31.40	33.12	33.18	32.30	positive
15	P1739	D150612-1	32.42	33.65	32.98	32.97	positive
16	P1746	D120612-2	32.60	33.55	32.65	32.88	positive
17	1712-31	D191012-1	31.27	32.87	31.81	33.19	positive
18	A12-37	D191012-1	31.84	32.01	31.27	32.64	positive
19	A12-34	D191012-1	31.34	32.47	31.55	32.41	positive
20	A12-36	D191012-1	32.15	32.85	31.42	32.05	positive
21	Extraction control		No Ct	33.23			negative
22	NTC		No Ct	33.07			negative
21	Positive control		25.70	32.40			positive

Repeat 1:

Channel FAM

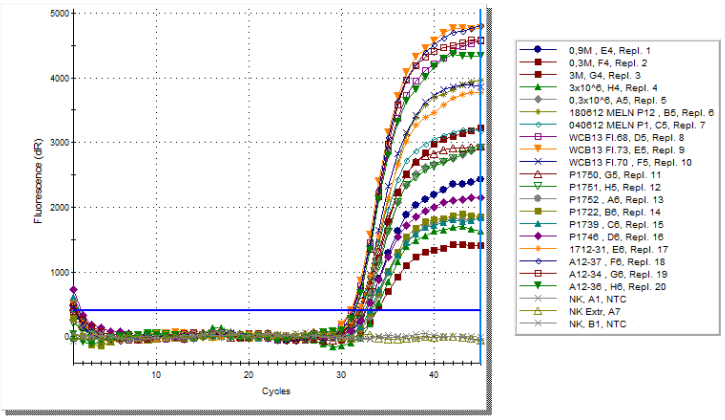


Channel ROX

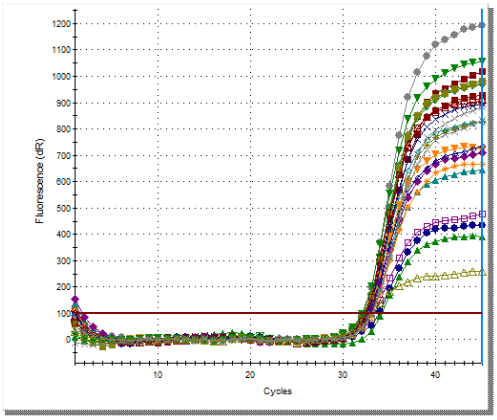


Repeat 2:

Channel FAM



Channel ROX

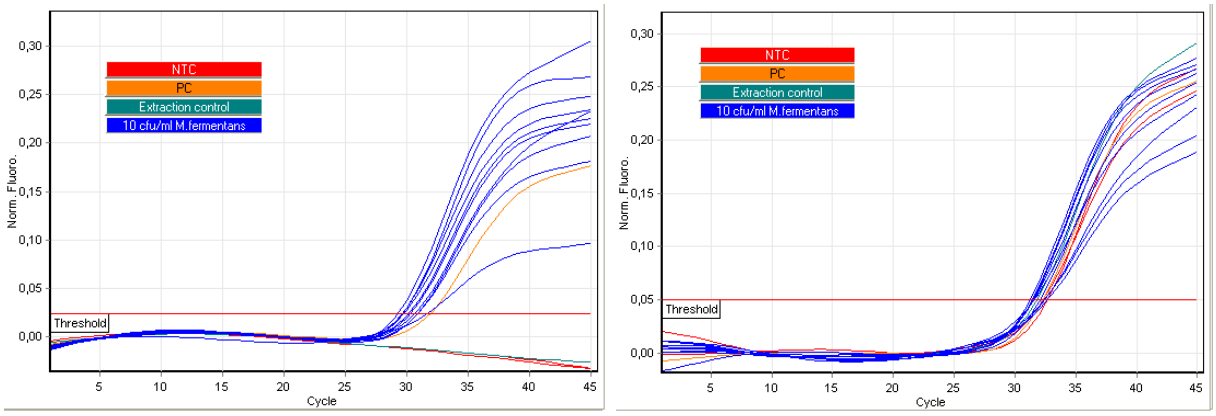


7.4.3 qPCR Cycler Compatibility

Procedure	Acceptance Criterion	Results
As the test can basically be performed with any qPCR cycler capable of interpreting FAM™ and ROX™ signals performance of the test on these machines needs to be validated. As not all qPCR cycler commercially available are accessible for validation the following three devices representing block and air heating systems are tested: RotorGene 6000, LC 1.2, ABI Prism 7500 and Mx3005p. The robustness of the method will be demonstrated by the determination of 10 CFU/ml of *Mycoplasma fermentans* in DMEM + 5 % FCS. At least 10 replicates shall be tested on each machine.	9 out of 10 samples show a positive result.	passed

RotorGene 6000

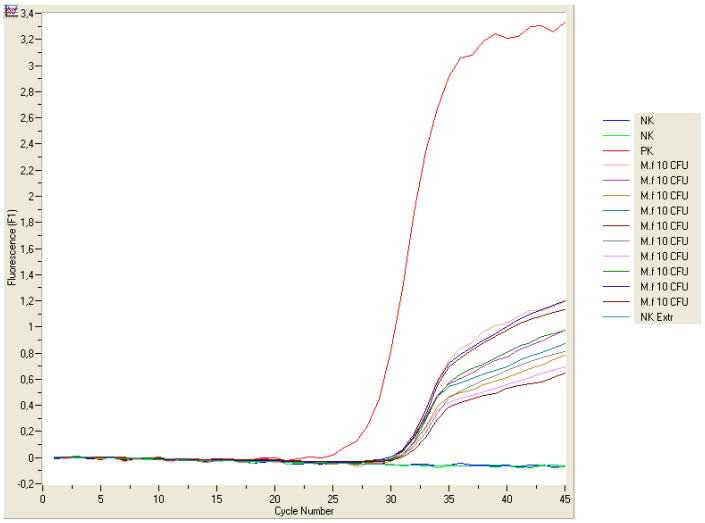
No	Species	Concentration	Ct (FAM)	Ct (ROX)	Result
1	*Mycoplasma fermentans*	10 CFU / ml	29.66	32.71	positive
2	*Mycoplasma fermentans*	10 CFU / ml	30.92	32.45	positive
3	*Mycoplasma fermentans*	10 CFU / ml	30.67	32.48	positive
4	*Mycoplasma fermentans*	10 CFU / ml	31.71	29.61	positive
5	*Mycoplasma fermentans*	10 CFU / ml	30.91	32.11	positive
6	*Mycoplasma fermentans*	10 CFU / ml	30.19	31.46	positive
7	*Mycoplasma fermentans*	10 CFU / ml	29.79	31.29	positive
8	*Mycoplasma fermentans*	10 CFU / ml	30.31	31.53	positive
9	*Mycoplasma fermentans*	10 CFU / ml	29.32	31.60	positive
10	*Mycoplasma fermentans*	10 CFU / ml	29.72	31.59	positive
	NTC		No Ct	32.36	passed
	NTC		No Ct	32.71	passed
11	Extraction control	---	No Ct	31.85	passed
12	PC	---	31.90	32.36	passed



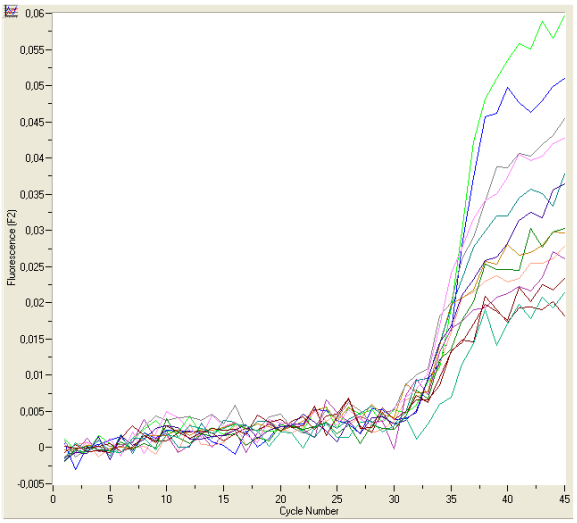
LightCycler 1.2

No	Species	Concentration	Ct FAM	Ct ROX	Result
1	*Mycoplasma fermentans*	10 CFU / ml	30.25	30.74	positive
2	*Mycoplasma fermentans*	10 CFU / ml	29.94	30.00	positive
3	*Mycoplasma fermentans*	10 CFU / ml	29.96	31.33	positive
4	*Mycoplasma fermentans*	10 CFU / ml	29.91	31.51	positive
5	*Mycoplasma fermentans*	10 CFU / ml	30.34	30.84	positive
6	*Mycoplasma fermentans*	10 CFU / ml	30.54	31.30	positive
7	*Mycoplasma fermentans*	10 CFU / ml	30.00	31.42	positive
8	*Mycoplasma fermentans*	10 CFU / ml	30.23	31.36	positive
9	*Mycoplasma fermentans*	10 CFU / ml	30.19	31.26	positive
10	*Mycoplasma fermentans*	10 CFU / ml	30.54	31.71	positive
11	NTC	---	No Ct	32.89	passed
12	Extraction control	---	No Ct	32.75	passed
13	PC	---	28.22	---	passed

Channel F1 - FAM



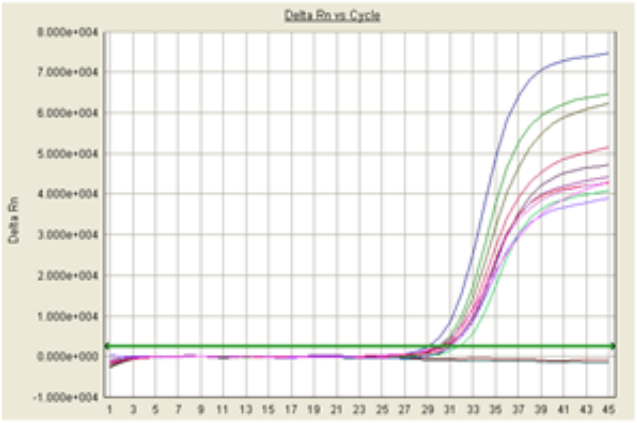
Channel F2 - ROX



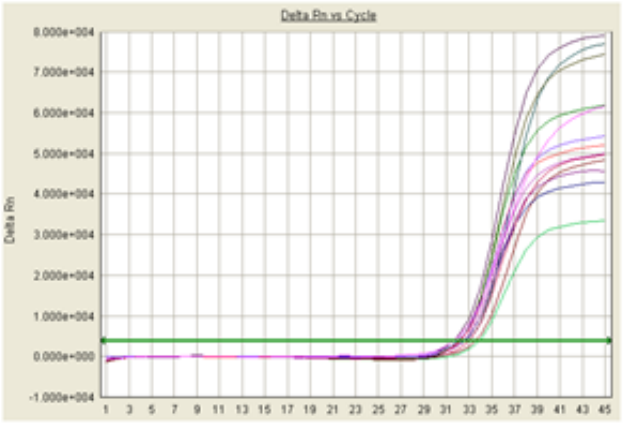
ABI Prism 7500

No	Species	Concentration	Ct (FAM)	Ct (ROX)	Result
1	*Mycoplasma fermentans*	10 CFU / ml	30.05	32.10	positive
2	*Mycoplasma fermentans*	10 CFU / ml	29.09	32.58	positive
3	*Mycoplasma fermentans*	10 CFU / ml	31.11	31.70	positive
4	*Mycoplasma fermentans*	10 CFU / ml	30.25	32.14	positive
5	*Mycoplasma fermentans*	10 CFU / ml	30.26	33.03	positive
6	*Mycoplasma fermentans*	10 CFU / ml	30.91	32.19	positive
7	*Mycoplasma fermentans*	10 CFU / ml	30.63	32.55	positive
8	*Mycoplasma fermentans*	10 CFU / ml	30.56	32.34	positive
9	*Mycoplasma fermentans*	10 CFU / ml	31.09	32.08	positive
10	*Mycoplasma fermentans*	10 CFU / ml	29.93	31.72	positive
11	NTC	---	No Ct	33.55	negative
12	Extraction control	---	No Ct	31.48	passed
13	PC	---	31.81	33.91	passed

Channel FAM



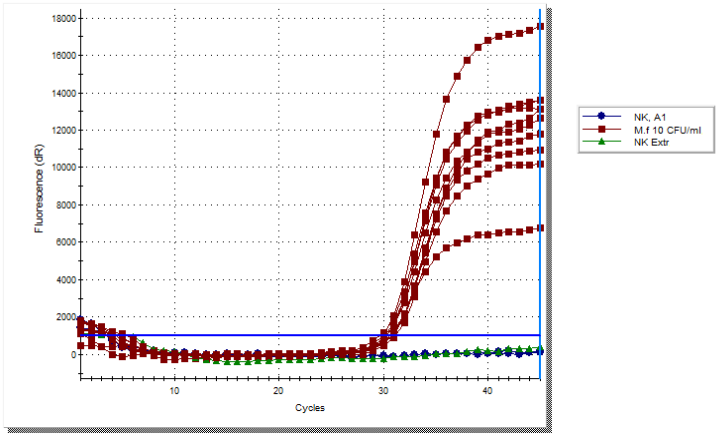
Channel ROX



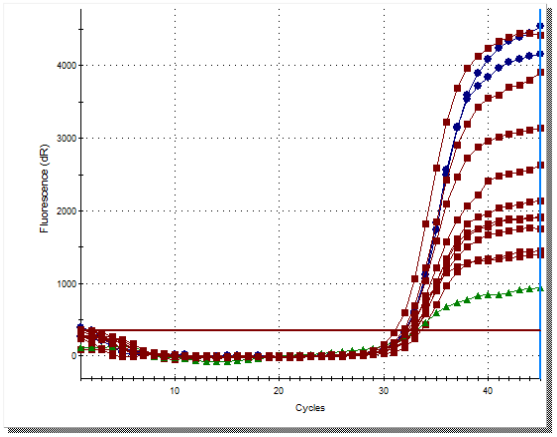
Mx3005p

No	Species	Concentration	Ct FAM	Ct ROX	Result
1	*Mycoplasma fermentans*	10 CFU / ml	30.25	32.54	positive
2	*Mycoplasma fermentans*	10 CFU / ml	30.38	33.65	positive
3	*Mycoplasma fermentans*	10 CFU / ml	30.87	32.92	positive
4	*Mycoplasma fermentans*	10 CFU / ml	29.69	32.80	positive
5	*Mycoplasma fermentans*	10 CFU / ml	30.20	33.04	positive
6	*Mycoplasma fermentans*	10 CFU / ml	30.71	32.34	positive
7	*Mycoplasma fermentans*	10 CFU / ml	30.87	33.11	positive
8	*Mycoplasma fermentans*	10 CFU / ml	30.66	31.89	positive
9	*Mycoplasma fermentans*	10 CFU / ml	31.16	31.13	positive
10	*Mycoplasma fermentans*	10 CFU / ml	29.91	32.22	positive
11	NTC	---	No Ct	32.17	passed
12	Extraction control	---	No Ct	33.19	passed
13	PC	---	26.06	34.09	passed

Channel FAM



Channel ROX



7.5 Handling of deviations

No deviations from the validation protocol occurred.

8. Conclusions

Microsart® ATMP Mycoplasma was designed to be applied at any customer site equipped with qPCR cyclers as part of a routine or research PCR lab by personal trained on good PCR practise.

Microsart® ATMP Mycoplasma was validated intensively in compliance with the validation protocol and EP 2.6.7 recommendations providing detailed information about the performance of the kit:

1. by reflecting influences of the sample matrices frequently used for manufacturing of ATMPs and in cell culture technology in general.
2. for all species of mycoplasma that are requested by the EP 2.6.7.
3. comparable to other qPCR devices not tested within the study but equally designed as block or air heated cyclers.

By following these criteria the validation protocol reflected the method itself and variations expected by the diversity of samples from different customers during QC testing in the manufacturing process of ATMPs.

The kit and the introduced protocol allow for the save and robust detection of 10 CFU/ml of all *Mycoplasma* species relevant for the production of ATMPs as listed in EP 2.6.7. The sensitivity of the kit complies in full as required for an alternative to both official methods, the indicator cell culture as well as the culture method. Anyhow, it needs to be considered that sample material is limited with 10 µl out of 60 µl DNA extract by using 200 µl sample material only. Using low sample volumes, statistical uncertainty is unavoidable. When using larger sample volumes, as for example by using the Microsart AMP Mycoplasma kit in conjunction with the Vivaspin concentration method which allows for the testing of 50 µl DNA extract per PCR using up to 18 ml of sample, the sensitivity can be further increased.

According to the sequence alignment the kit detects at least 110 different *Acholeplasma*, *Mycoplasma*, *Spiroplasma* and *Ureaplasma* species. This feature increases the chance to detect mycoplasmas which contaminate cell culture rarely, have not been described as contaminants so far or are not culturable by using the traditional *Mycoplasma* testing method.

All representatives of the phylogenetically closely related genera *Clostridium*, *Lactobacillus* and *Streptococcus* were not detected as required by the EP 2.6.7. DNA of various eukaryotic origin was not detected by Microsart® ATMP Mycoplasma even at concentrations higher than 30 ng/PCR.

As robustness is a key issue of a release test, the product has been validated with different relevant sample materials and qPCR cyclers. The species *Mycoplasma fermentans* was used for spiking. *Mycoplasma fermentans* was easily detectable at the required concentration of 10 CFU/ml even in complex sample matrices and all replicates.

Microsart® ATMP Mycoplasma applies with all requirements for *Mycoplasma* testing according to *European Pharmacopoeia* 2.6.7. It´s the state-of-the-art product for *Mycoplasma* detection in cell cultures and other cell culture-based materials, including cryo stocks, cell culture supernatants, cell suspensions and ATMPs as Microsart® ATMP Mycoplasma combines highest sensitivity with minute sample volumes. Whenever larger sample volumes, e.g. cell culture media, additives or bulk harvest have to be tested, Microsart AMP Mycoplasma is the method of choice.

9. Reference Documents

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