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Simplifying Progress

Microsart® Mycoplasma Kits

2022

Microsart® Mycoplasma Kits

Features	Microsart® Research Mycoplasma	Microsart® ATMP Mycoplasma	Microsart® AMP Mycoplasma
Recommended use	Testing of cell culture materials in research and development	Testing of autologous cell transplants (ATMPs)	Regulated in-process and lot-release testing of cell cultures and cell-culture derived biologicals, with increased sample volume input
Type of PCR	5′ nuclease assay for qPCR	5′ nuclease assay for qPCR	5′ nuclease assay for qPCR
Device requirements	Kit can be applied on any qPCR cycler suitable to detect FAM™ and ROX™ dyes	Kit can be applied on any qPCR cycler suitable to detect FAM™ and ROX™ dyes	Kit can be applied on any qPCR cycler suitable to detect FAM™ and ROX™ dyes and accepting 100 µL reaction volume
Kit components	▪ Lyophilized primers | nucleotides | probes | polymerase | internal amplification control, aliquoted in 25 reactions ▪ Rehydration buffer ▪ Lyophilized positive control ▪ PCR grade water	▪ Lyophilized primers | nucleotides | probes | polymerase, aliquoted in 25 reactions ▪ Internal amplification control usable as DNA extraction control ▪ Rehydration buffer ▪ Lyophilized positive control ▪ PCR grade water	▪ Lyophilized primers | nucleotides | probes | polymerase, aliquoted in 25 reactions ▪ Internal amplification control usable as DNA extraction control ▪ Rehydration buffer ▪ Lyophilized positive control ▪ PCR grade water
Package sizes	Cat. No. SMB95-1005 25 tests	Cat. No. SMB95-1003 25 tests	Cat. No. SMB95-1001 25 tests
Sample volume	2 µL (optional 200 µL when combined with prior DNA extraction)	200 µL	200 µL to 18 mL
Sample volume per PCR	2 µL	10 µL	50 µL

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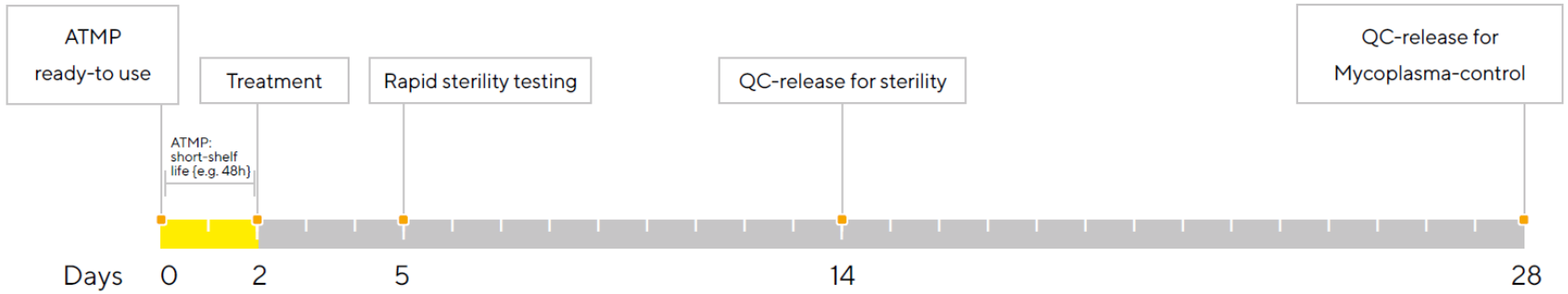
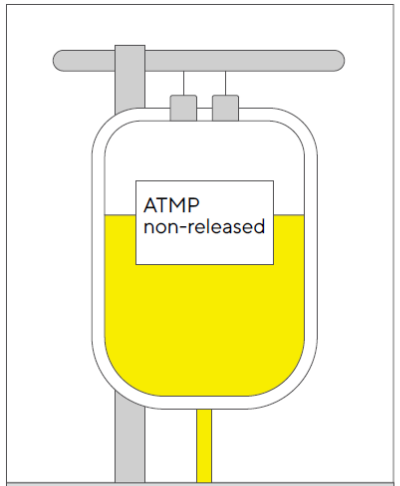
Microsart® AMP Extraction
Microsart® ATMP Mycoplasma

2022

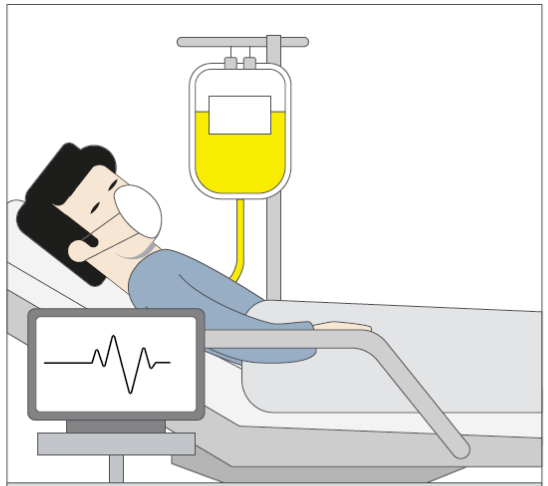
ATMPs put microbiological QC to novel challenges



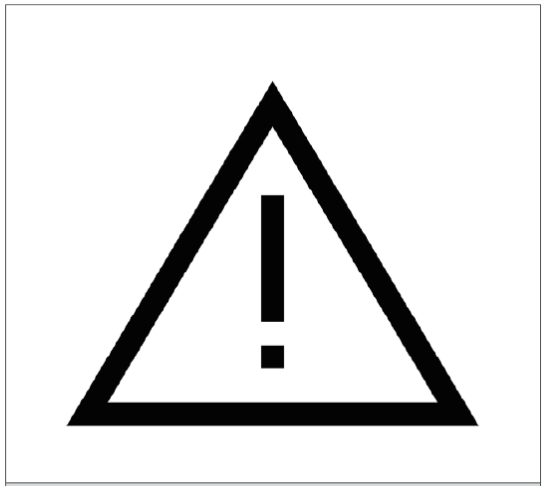
5, 14 or 28 days
of waiting
is too long
for ATMPs!



QC result pending

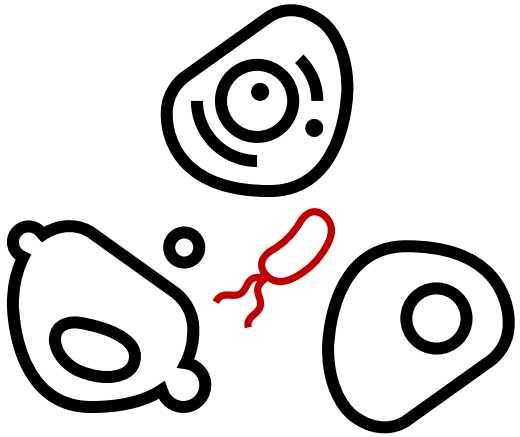


Treatment despite risk:
ATMP might be contaminated



Patient's life at risk

Nucleic acid techniques

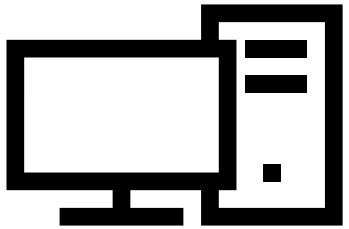


ATMP
(CHO, HEK,...)

DNA extraction
of a small sample

DNA

Amplification
of target sequence



Detection in
real-time PCR cycler:
Contamination? Yes/No

Results within 3 hours!

Mycoplasma contamination detection

- **Real-time PCR allows detection of Mycoplasma**
  - In 3 h
  - Down to 5-10 CFU/ml
- **Validated combination**
  - In accordance with EP 2.6.7 and USP 63
- Support
  - ***Product Validation Report*** containing all experimental details
  - ***Matrix Validation Proposal*** giving an overview of the required set up and materials
  - ***Matrix Validation Template*** containing detailed information for the customer specific matrix validation
  - **Technical support** during matrix validation process

Microsart® AMP Extraction

Microsart® ATMP Mycoplasma

Workflow Mycoplasma contamination detection

- **DNA isolation using the column-based Microsart® AMP Extraction kit**
  - Columns allow to isolate DNA from the whole ATMP sample (supernatant & cells)

Microsart® AMP Extraction

- **Real-time PCR using the Microsart® ATMP Mycoplasma kit**
  - Taq-Man® System → reduce false-positive signals
  - Duplex assay → reduce false-negative signals
  - Universal assay for different real-time PCR cycler → FAM™ and ROX™
  - High stability & no freezing → Lyophilized reagents

Microsart® ATMP Mycoplasma

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Technical background
DNA-based detection methods

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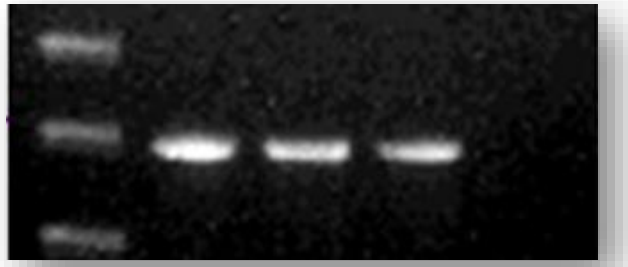
DNA-based detection methods

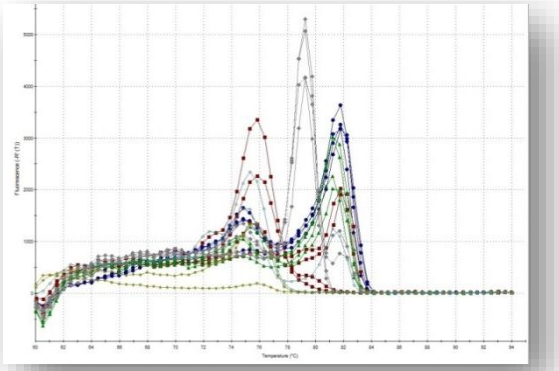
real-time PCR

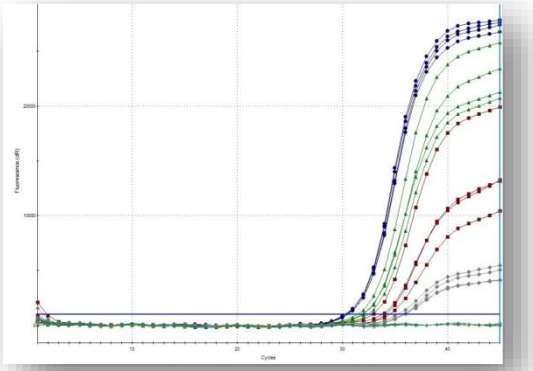
Conventional PCR

SYBR Green I

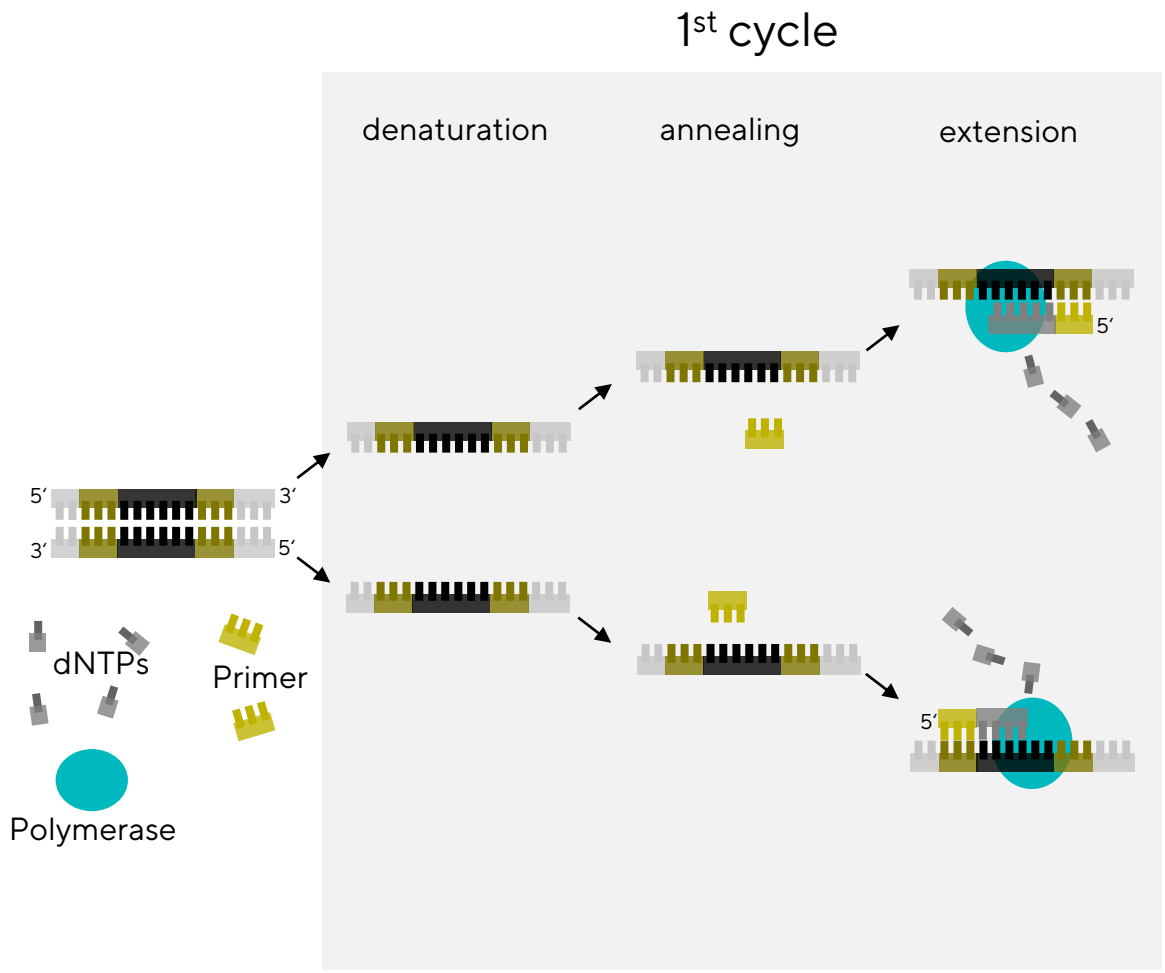
TaqMan® Probe



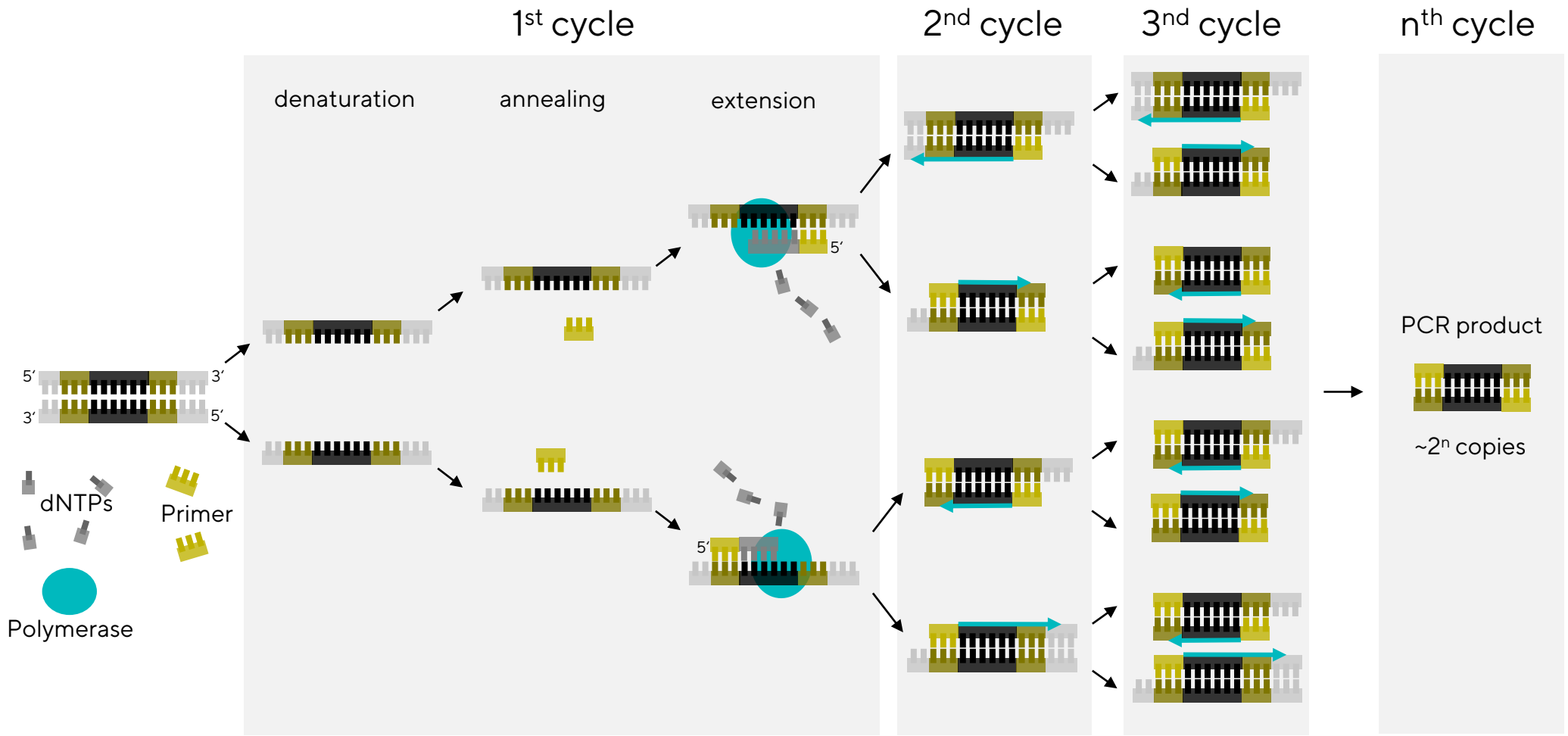




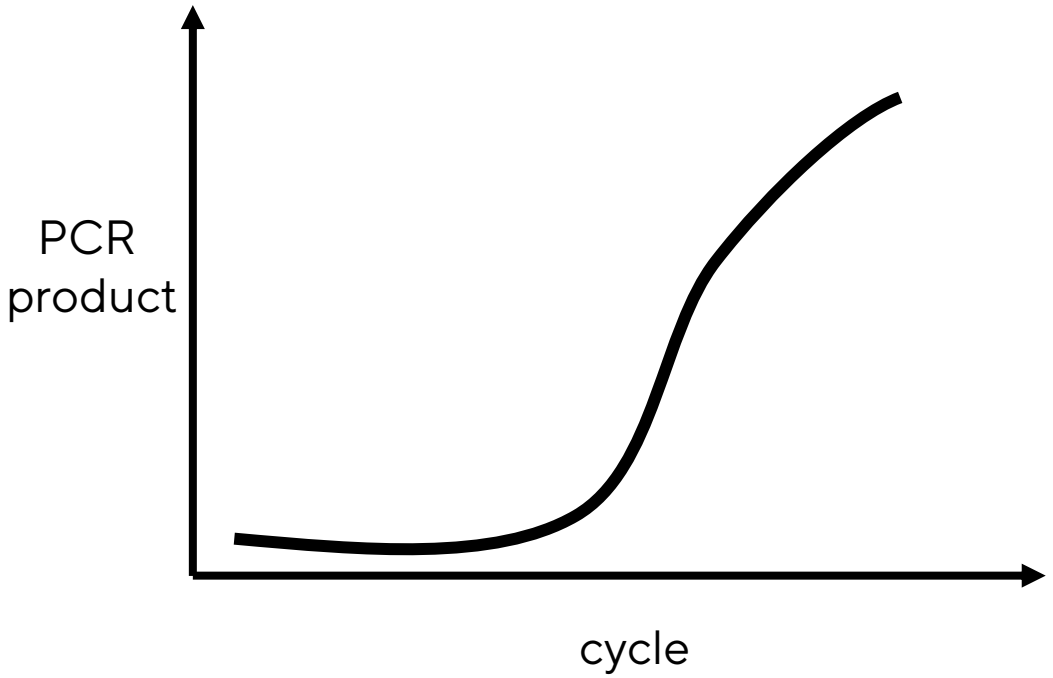
What is a conventional PCR?



What is a conventional PCR?

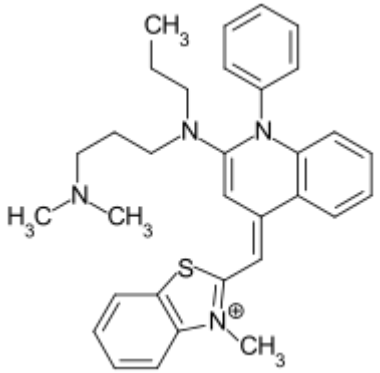


A real-time PCR visualizes the reaction

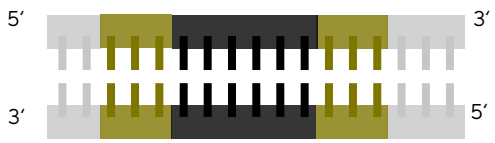


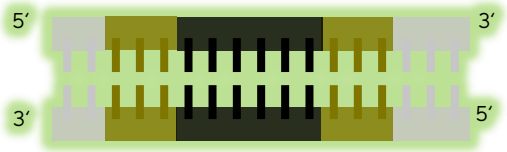
How does that work?

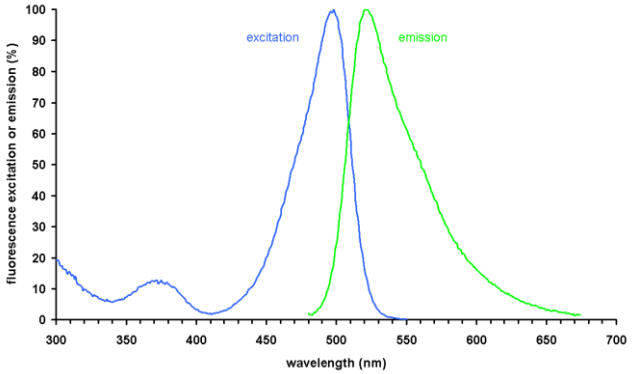
Real-time PCR



SYBR Green I







The dye SYBR Green I binds to double stranded DNA!

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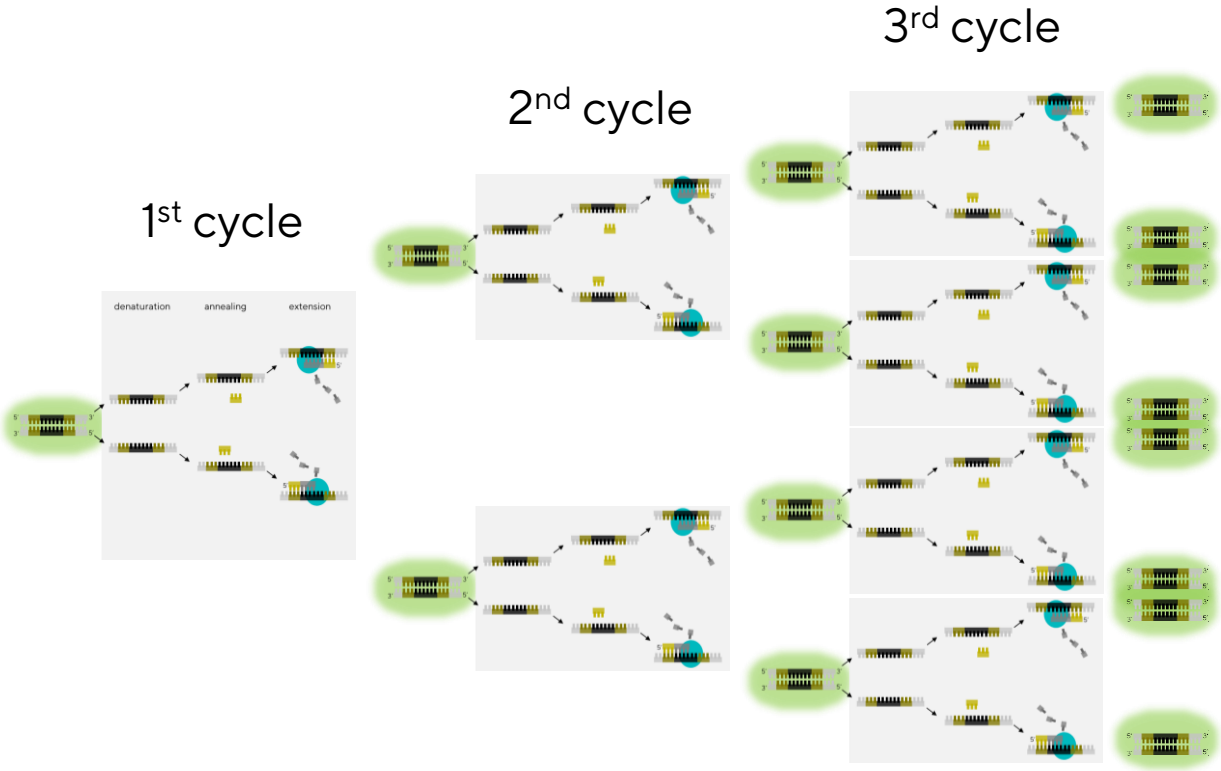
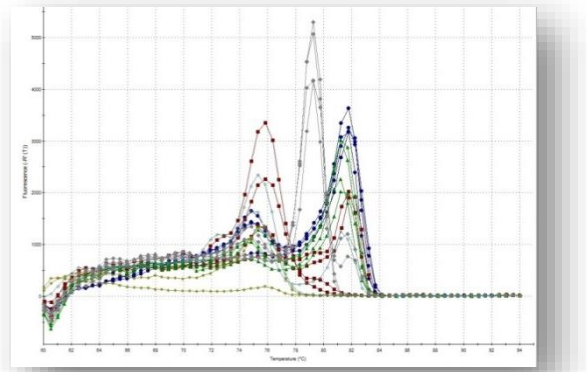
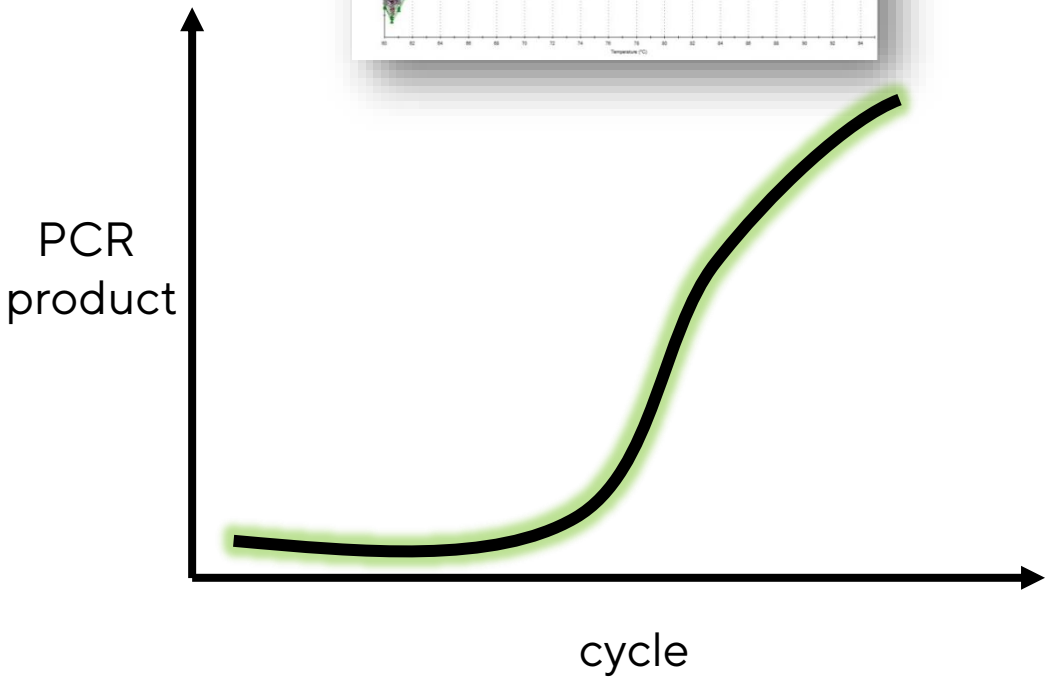
Real-time PCR



SYBR Green I





Unspecific binding of SYBR Green I can result in **false-positive signals!**

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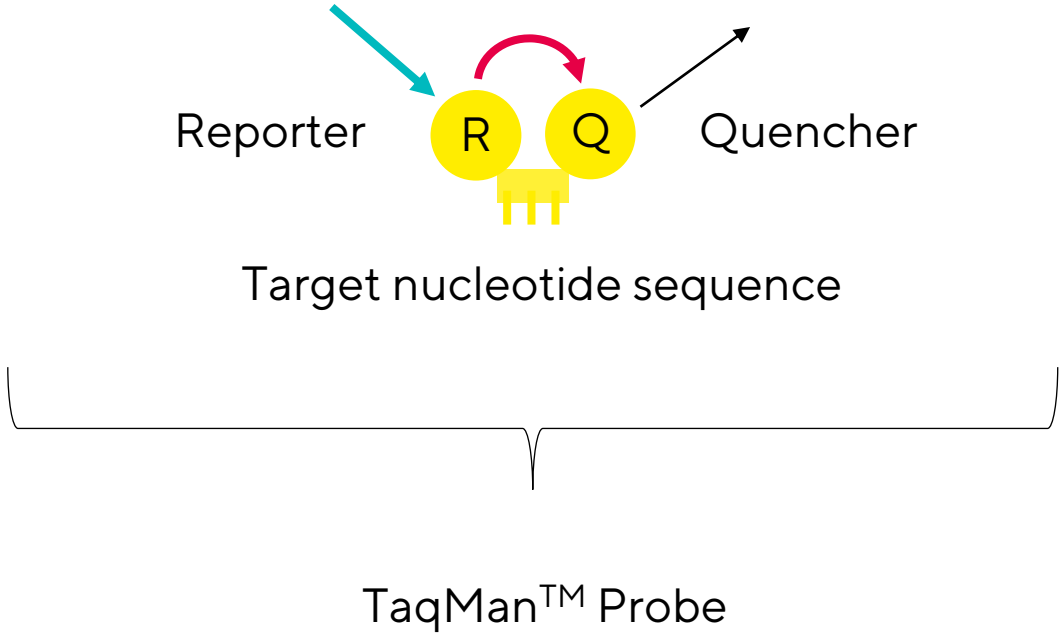
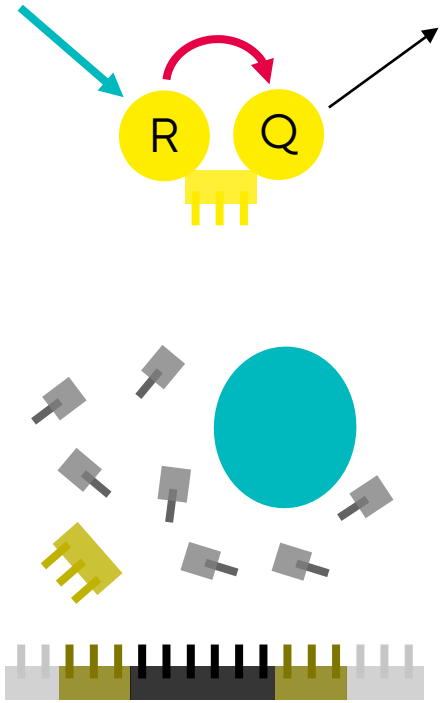
A TaqMan™ probe is more specific compared to SYBR Green I

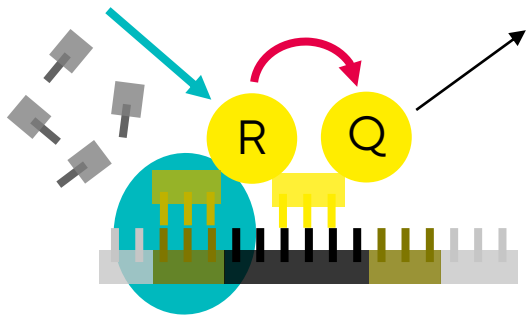


TaqMan™ real-time PCR

TaqMan™ probe is degraded during real-time PCR

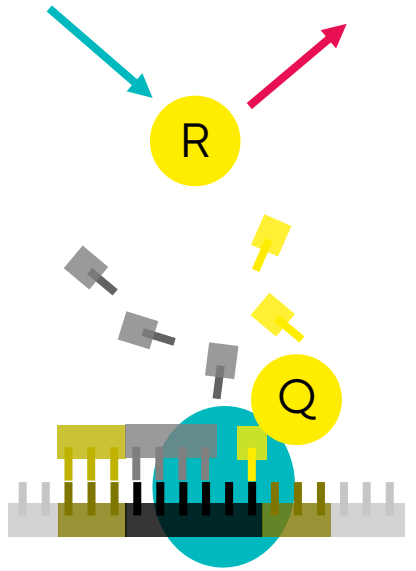


As long as the probe is complete no light signal can be detected



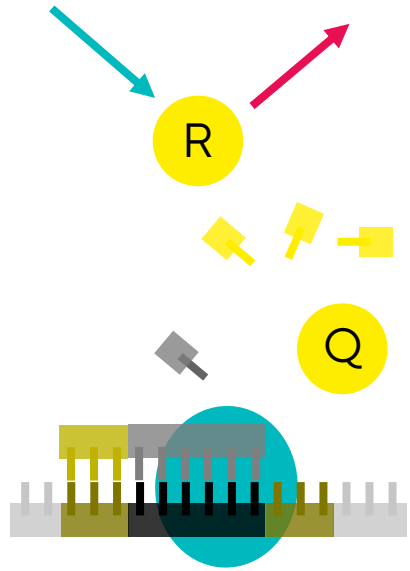
Taq Polymerase functions:

- DNA amplification
- 5’-3’ exonuclease activity

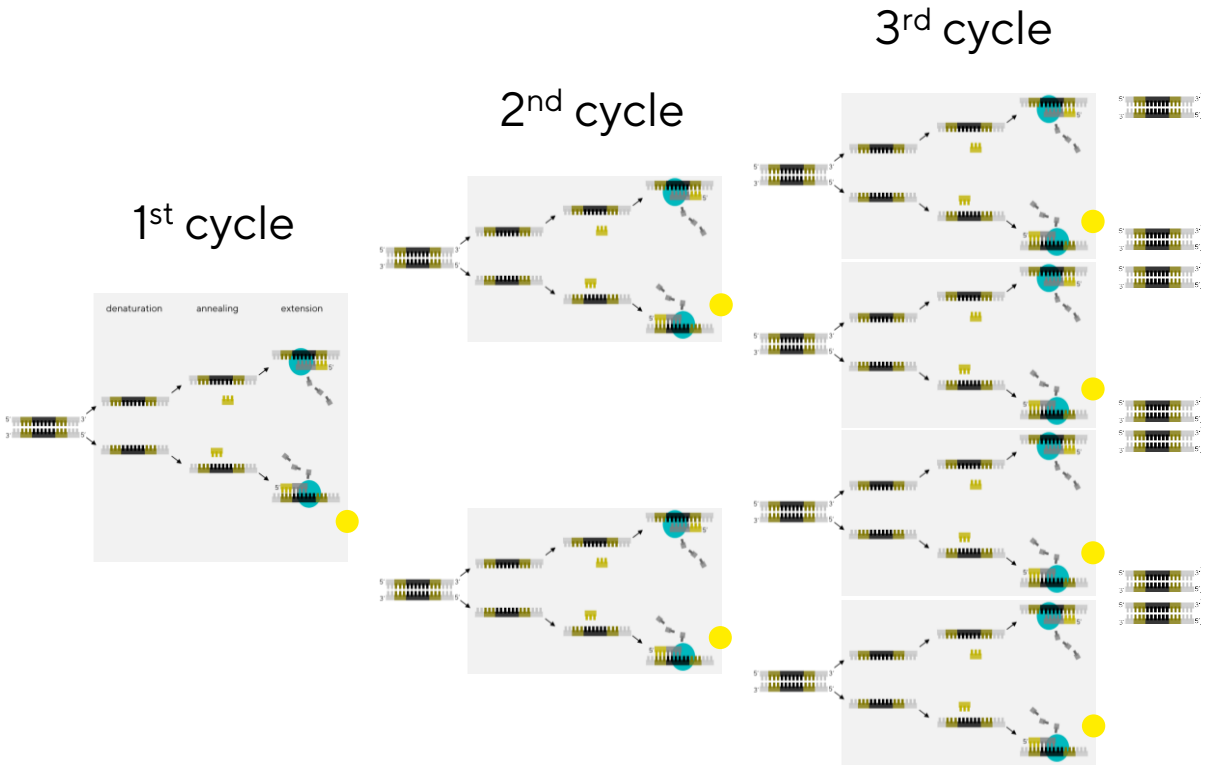


During elongation:

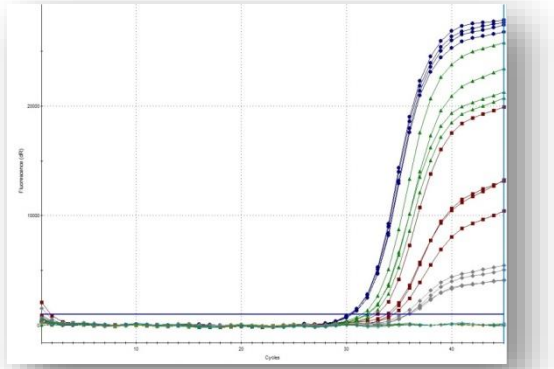
- Polymerase hydrolyses probe
- Dye and quencher are separated
- Reporter dye emits light signal

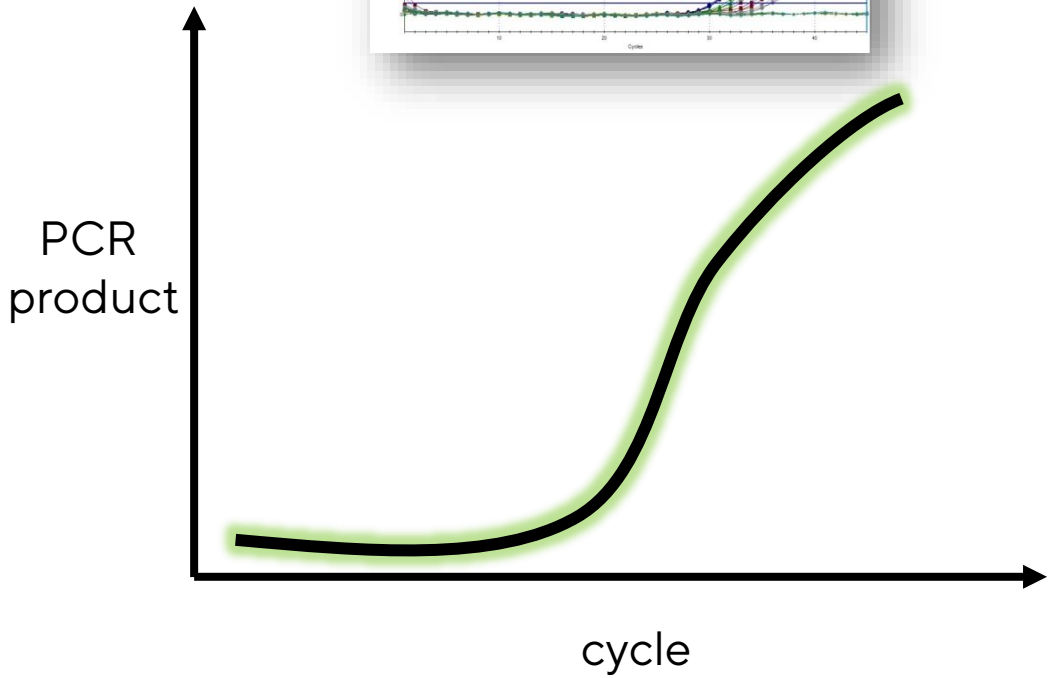


TaqMan™ real-time PCR



TaqMan® Probe





The specificity of TaqMan™ system **reduces false-positive results!**

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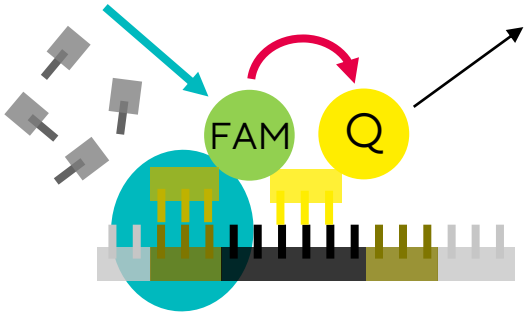
A duplex real-time PCR assay monitors PCR functionality

Problem:
What does **no signal** mean?
No microbial contamination?
PCR inhibition?

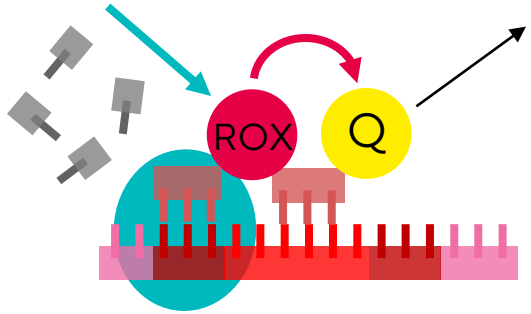
Solution:
Include a second real-time PCR and a control DNA that must lead to a signal!

→ If this internal control reaction does not lead to a signal, the PCR is inhibited.

Duplex assay
= two independent real-time PCRs in one run using different fluorophores



target-probe
target-DNA

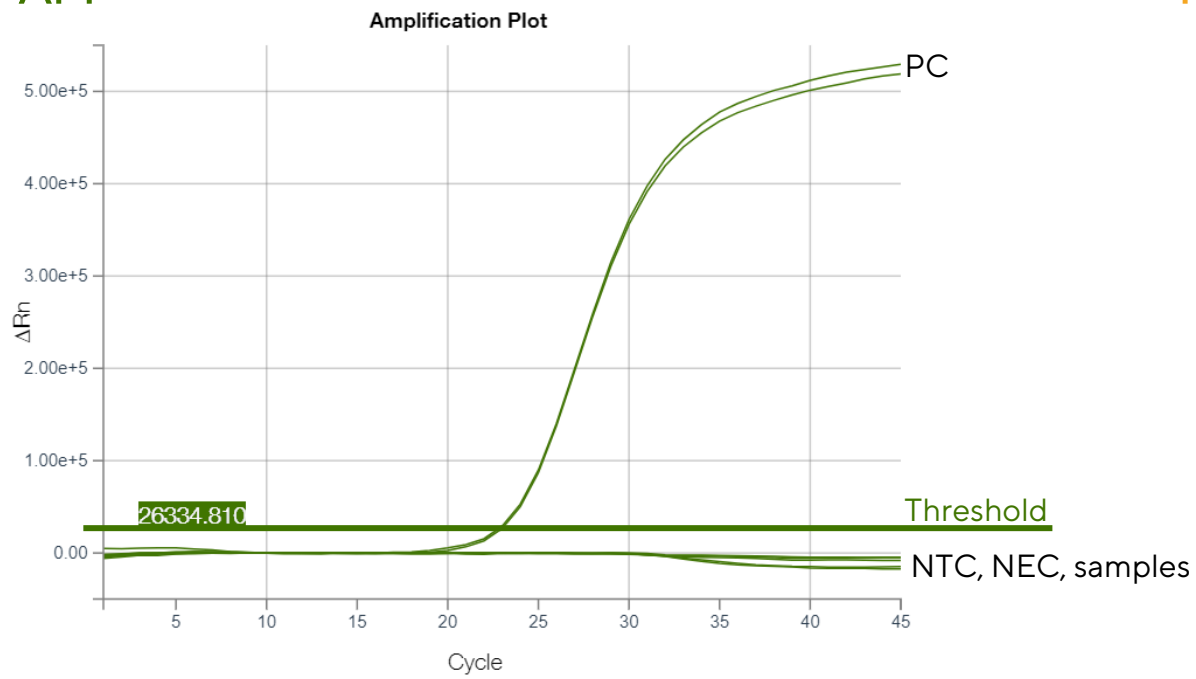


control-probe
control-DNA

The internal control reaction **reduces false-negative results!**

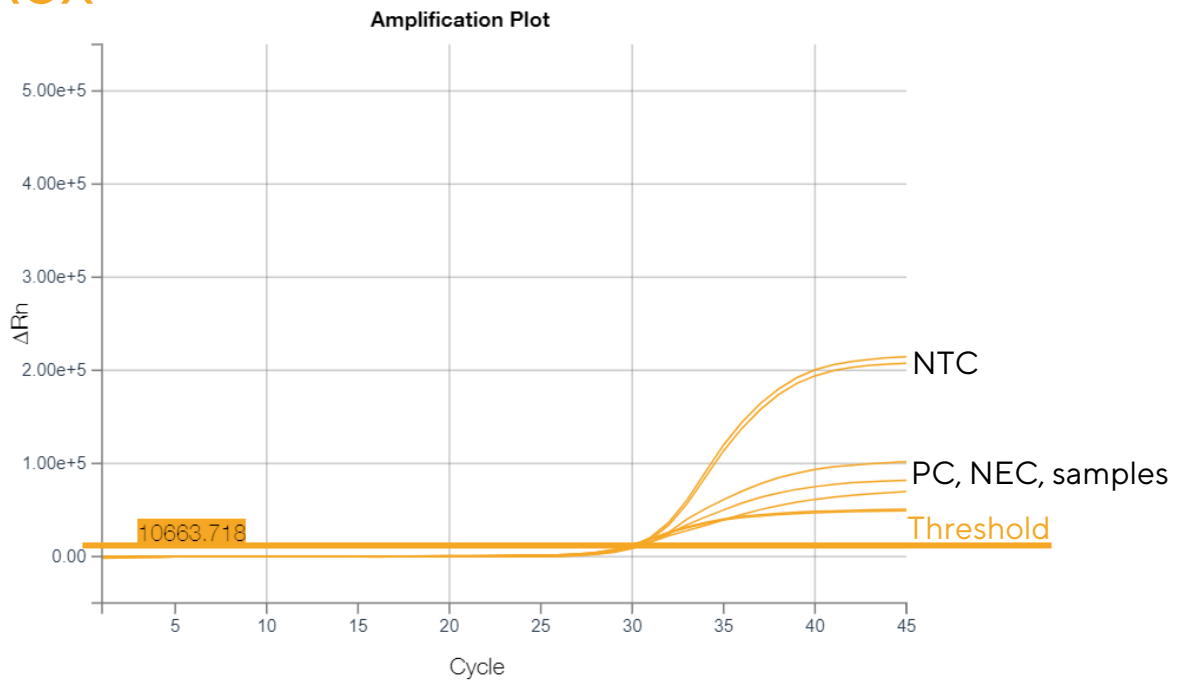
A duplex real-time PCR Analysis

FAM



There is no contamination in the samples, because only the positive control is detectable in the FAM channel

ROX



There is no PCR inhibition, because the internal control DNA was detected in all reactions.

What is in the kits?

real-time PCR master mix

- Primer for target DNA
- Primer for control DNA
- FAM probe for target DNA
- ROX probe for control DNA
- Taq polymerase
- Buffer

Rehydration buffer
Internal Control DNA
Positive Control DNA
Ultrapure Water

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Hands on Mycoplasma detection

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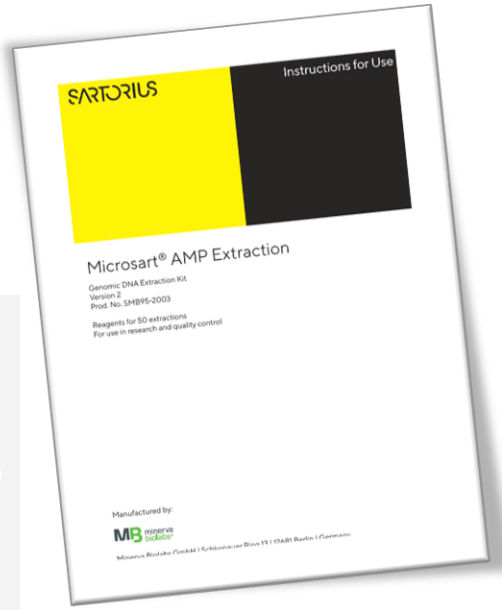
Microsart® AMP Extraction



1. Preparation of Cell Culture Material

1.5 ml
+ 200 µl sample
+ 200 µl Buffer A1
for 10 sec

& for 10 min at 70 °C

+ 400 µl Buffer A2
for 10 sec

2. DNA Isolation

Transfer sample
10,000 × g for 1 min
Discard flow-through

+ 500 µl Buffer B
10,000 × g for 1 min

Change tube

+ 500 µl Buffer C
10,000 × g for 1 min

Change tube

max. speed for 3 min

Change tube

+ 60 µl Buffer D
for 2 min at RT
8,000 × g for 2 min

DNA ready for PCR

incubate
vortex
centrifuge
add
shake

storage +18 - +25 °C (RT)

This procedure overview is not a substitute for the detailed manual.

Silica membrane based extraction

Microsart® ATMP Mycoplasma

1. Rehydration of Reagents

- Mycoplasma Mix
- Positive Control DNA
- Internal Control DNA

390 µl: Rehydration Buffer, Mycoplasma Mix
800 µl 300 µl: Internal Control, Positive Control

- for 5 min RT
- briefly
- for 5 sec

2. Preparation of PCR Reaction Mix

a) Internal Control added during DNA extraction

+15 µl Mycoplasma Mix (red cap)

* do not forget to add 1 µl Internal Control to NTC and PC

b) Internal Control not added during DNA extraction

Reaction Mix (for 1 reaction)
+15 µl Mycoplasma Mix (red cap)
+1 µl Internal Control (yellow cap)

+15 µl Reaction Mix

3. Addition of Samples and Controls

+ 10 µl sample
+ 10 µl Positive Control (green cap)
+ 10 µl elution buffer or PCR grade Water (white cap) (negative control)

Close lid tightly
briefly

4. Start of the qPCR Reaction

Start PCR program

95°C	95°C	45 cycles	60°C
3 min	30 sec	55°C 30 sec	45 sec

- Rehydration Buffer
- Mycoplasma Mix
- PCR grade Water
- Positive Control
- Internal Control

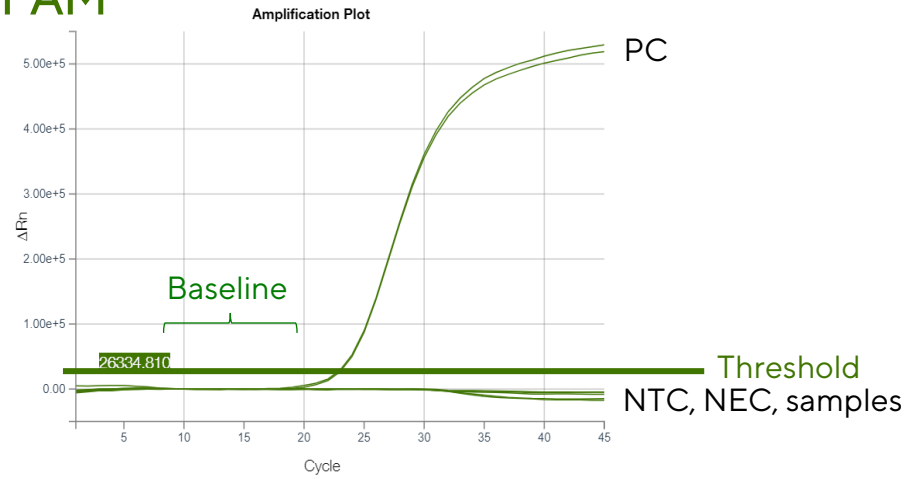
- incubate
- vortex
- centrifuge
- add

storage +2 - +8 °C
after rehydration ≤ -18 °C

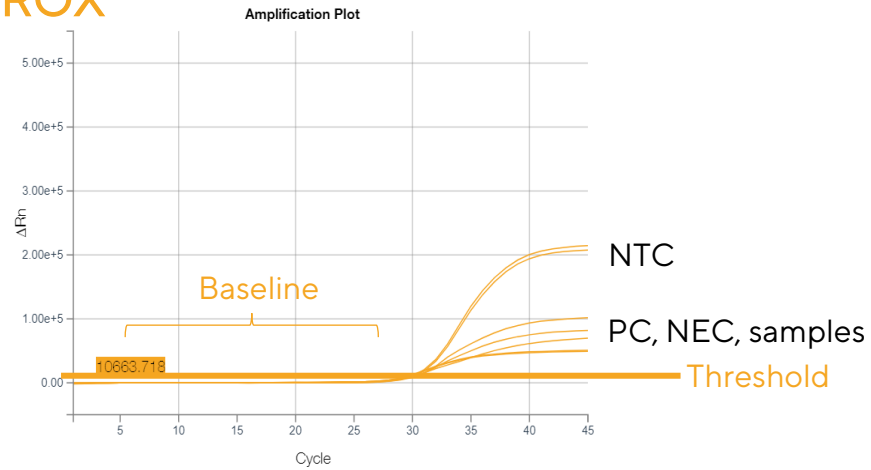
This procedure overview is not a substitute for the detailed manual. ST_SI_Microsart®-ATMP-Mycoplasma_03_EN

Microsart® ATMP Mycoplasma - Analysis

FAM



ROX



1. Set the baseline to level the curves
2. Set the threshold
3. Check if all controls are as expected

   → see next slide
4. Analyze your samples

Result interpretation

Templates added to the real-time PCR

Read-outs

		PC **P**ositive **C**ontrol	NTC **N**o **T**emplate **C**ontrol	Sample	NEC **N**egative **E**xtraction **C**ontrol
	FAM Template		Water	DNA ready for PCR	DNA extracted from sterile ATMP matrix
	ROX Template				
Target	FAM Signal	POSITIVE	NEGATIVE	To be tested!	NEGATIVE
Internal Control	ROX Signal	Does not matter	POSITIVE		POSITIVE

PCR grade Water
Positive Control
Internal Control

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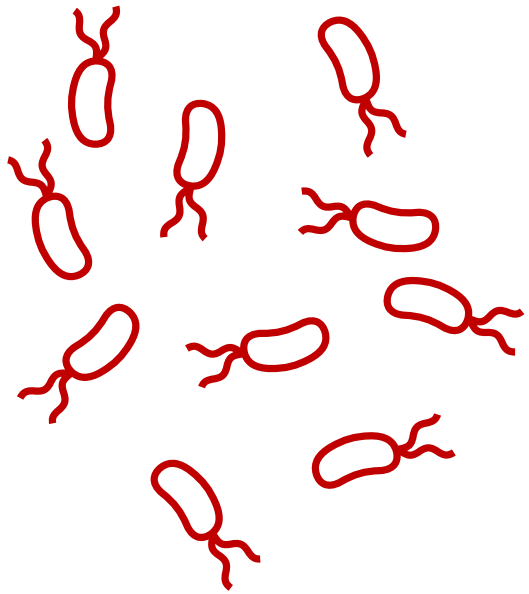
Tips and tricks

- Extensive cleaning with chlorine-based agents
- Avoid cleaning with ethanol → Ethanol precipitates DNA
- Work carefully e.g. do not touch the lids of open tubes
- It is recommended to wear gloves and mask

Mycoplasma DNA
is on our skin!



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Sartorius validation support

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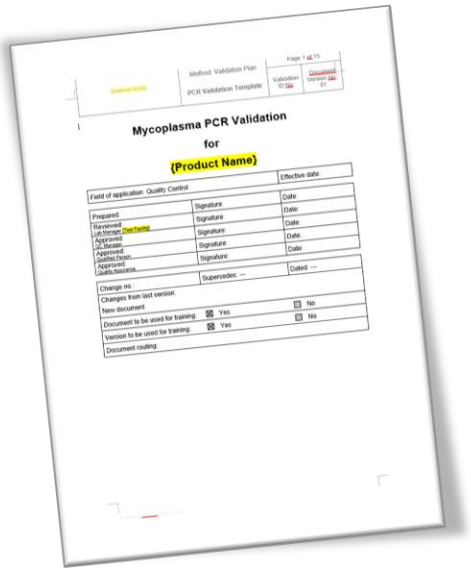
Validation reports, templates & testing proposals

- **Product Validation Report**
  - Microsart® ATMP Mycoplasma + Microsart® AMP Extraction



- **Validation Template**
  - Microsart® ATMP Mycoplasma + Microsart® AMP Extraction

- **Validation Proposal**
  - Standard matrix specific validation
  - Individual support

Further support for your validation

- Microsart® Validation Standard (10 CFU/Vial) &
  Microsart® Calibration Reagent (10^8 GC/Vial)
  - *Mycoplasma arginini*
  - *Mycoplasma orale*
  - *Mycoplasma gallisepticum*
  - *Mycoplasma pneumoniae*
  - *Mycoplasma synoviae*
  - *Mycoplasma fermentans*
  - *Mycoplasma hyorhinis*
  - *Acholeplasma laidlawii*
  - *Spiroplasma citri*
  - *Mycoplasma salivarium*

Non-viable CFU Standards!

DNA Standards!

Do you miss a species? Let us know!

Application Notes

Rapid, real-time PCR-based detection of microbial contaminations in high cell density Jurkat-, HPBMC-and CHO-cultures using Microsart® ATMP kits

In this study, we assessed the detection capability of Microsart® ATMP Extraction, combined with Microsart® ATMP Bacteria/Fungi/Mycoplasma assays, in high-density cell cultures.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Application No

Rapid, Real-Time PCR-Based Detection of Microbial Contaminations in High Cell Density Jurkat-, HPBMC- and CHO- Cultures using Microsart® ATMP Kits

Abstract

Detection limits in different cell types

Cell Type Background	Microorganism Spike	Background Cells /mL (In 10^6)	Detection
CHO	99 CFU *B. subtilis*	19.0	Successful
CHO	10 CFU *M. arginini*	15.0 and 15.6 (two individual experiments)	Successful
CHO	10 CFU *M. orale*	15.5 and 16.3 (two individual experiments)	Successful
Jurkat	99 CFU *K. rhizophila*	10 to 40	Successful up to 25×10^6 c/mL
Jurkat	50 CFU *C. albicans*	10 to 40	Successful up to 20×10^6 c/mL
Jurkat	10 CFU *M. orale*	10 to 40	Not successful: PCR inhibition > 15×10^6 c/mL No detection of Mycoplasma spike
Jurkat	10 CFU *M. synoviae*	10 to 35	Not successful: Partial PCR inhibition No detection of Mycoplasma spike
HPBMC	99 CFU *K. rhizophila*	10 to 40	Successful only up to 10×10^6 c/mL
HPBMC	10 CFU *M. arginini*	15.0	Successful
HPBMC	10 CFU *M. orale*	19.1	Successful
HPBMC	99 CFU *P. aeruginosa*	20 and 25	Successful

Table 1: Results of Real-Time PCR detection of respective microbial spikes in varying cell types with respective cell densities.

Status quo regulatory landscape

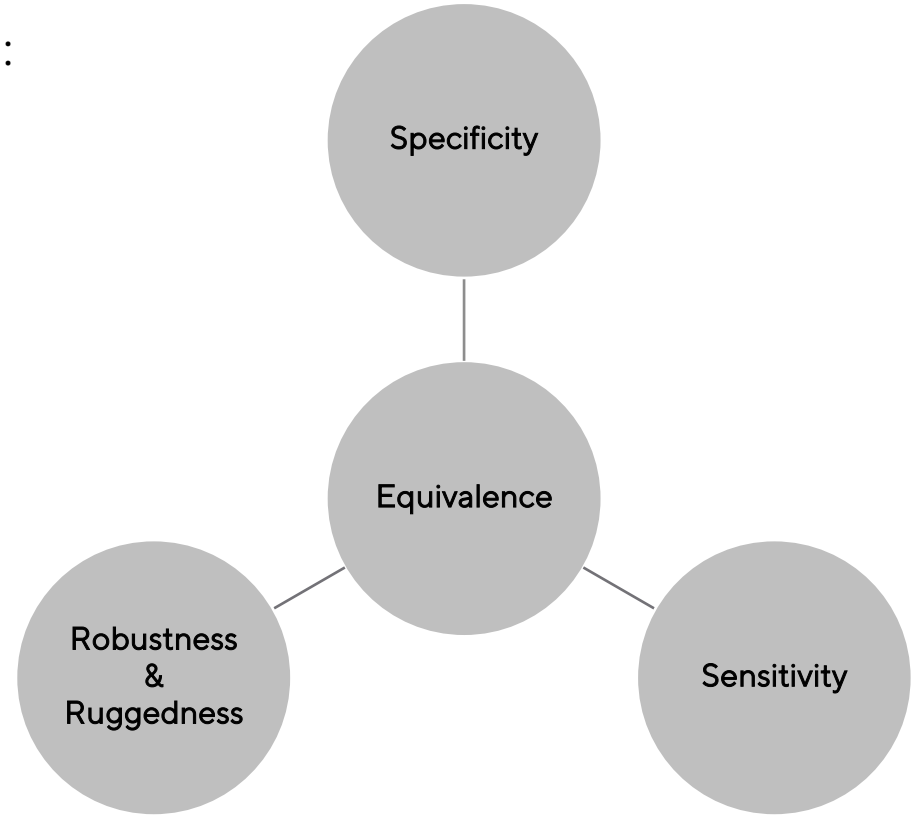
Method	Microbiological QC-Release testing		
	Mycoplasma	Bacteria	Fungi
Classical testing	USP<63> | EP 2.6.7 28 days	USP<71> | EP 2.6.1 Sterility testing 14 days	
real-time PCR-based	EP 2.6.7 (USP<1223>/EP 5.1.6)	USP<1071> EP 2.6.27 (USP<1223>/EP 5.1.6)	

Validation overview

Regulatory guidance for validation:

- EP 2.6.7 (Mycoplasma)
- EP 2.6.21 (NAT)
- ICH Q2B

- USP<63>
- USP<1223>
- EP 5.1.6
- PDA TR 33

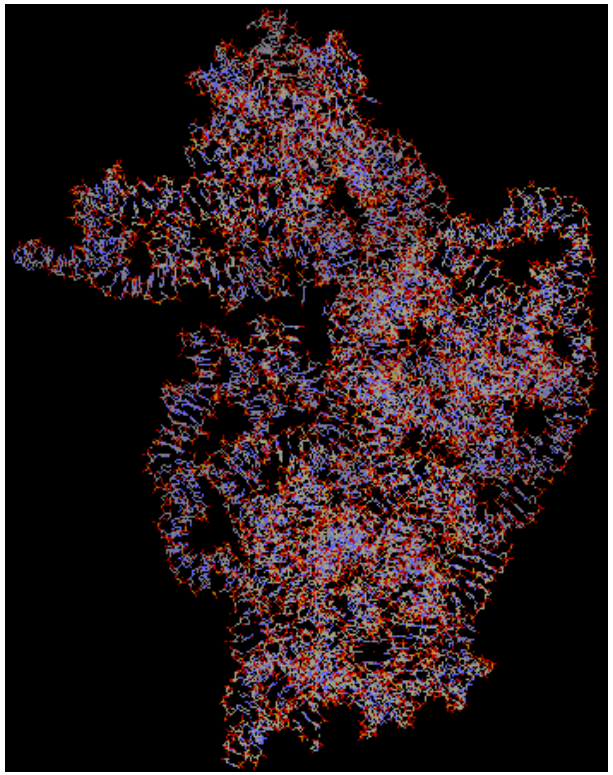


Including Guidance of the German Governmental Regulatory Agency (part of EMEA)

Paul-Ehrlich-Institut

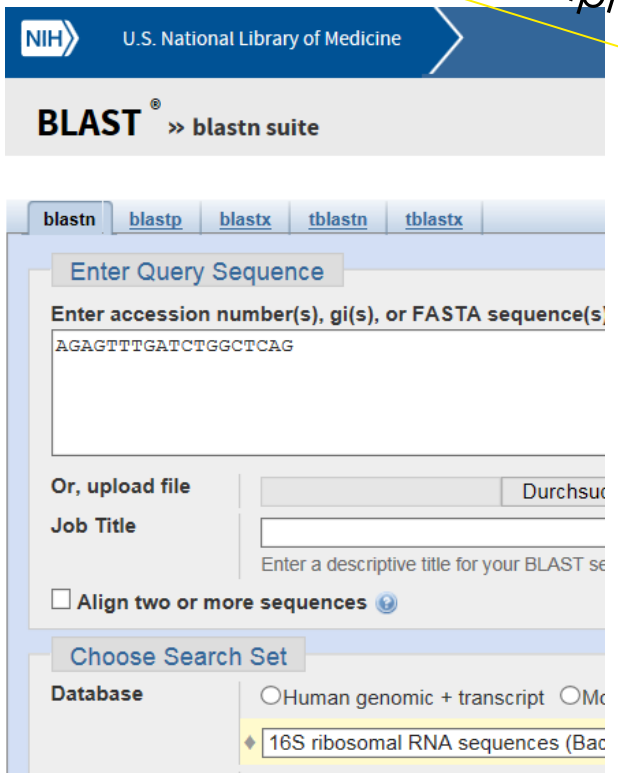
In silico prediction by sequence alignment and blast

16S rDNA



http://www.biochem.umd.edu/biochem/kahn/bchm465-01/ribosome/16SrRNA.html

example



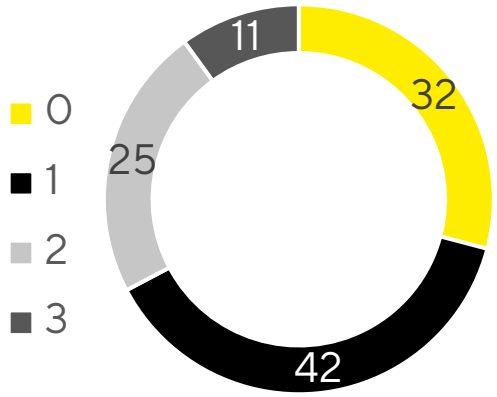
https://www.ncbi.nlm.nih.gov/

Detection range

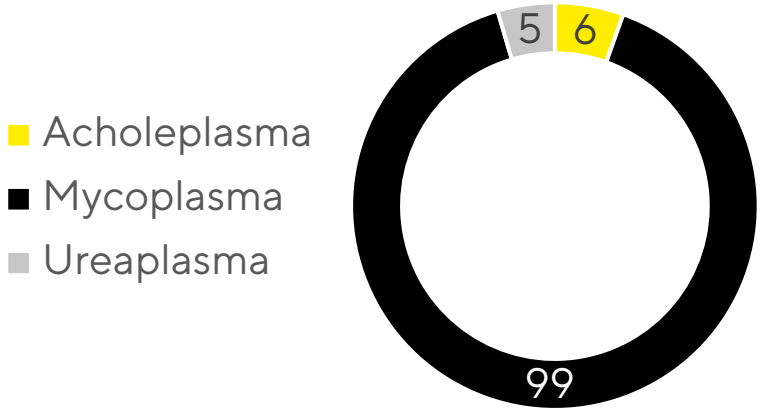
Specificity

Acceptance criterion:
≤ 3 nucleotides mismatch of primers and probe

Primer/Probe Mismatches



Genus



At least **110 species** are detectable based on sequence alignment.

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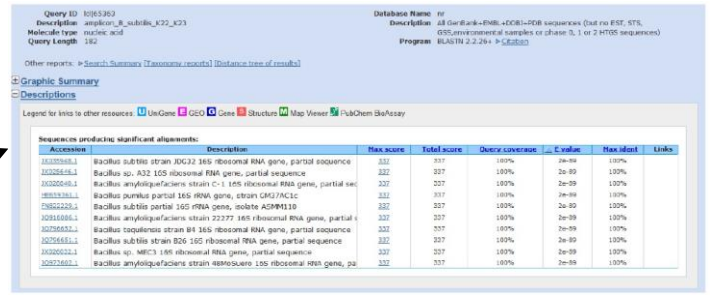
Matrix effects

Specificity

- Different matrices were tested for matrix effects and PCR inhibition
  - Typical cell culture media
  - Typical additives for cell culture media
  - Typical buffers

No.	Sample Matrix	Lot	Ct (FAM)	Ct (ROX)	Result
1	Hayflick Liquid Medium	107311	No Ct	35.01	negative
2	Frey Liquid Medium	107717	No Ct	32.39	negative
3	Dulbecco's MEM, bottle 1	1499W	No Ct	34.10	negative
4	Dulbecco's MEM, bottle 2	0407X	22.52	37.73	positive
5	RPMI Medium	0512X	No Ct	36.30	negative
6	PBS Dulbecco	0448W	No Ct	34.94	negative
7	anti-human-T-lymphocyte immune globulin, rabbit*	B 14 L-2	No Ct	34.24	negative
8	anti-human-T-lymphocyte immune globulin, rabbit*	B 13 L-1	No Ct	33.99	negative
9	FBS*	1231T	No Ct	35.13	negative
10	FBS*	11-3356	No Ct	34.65	negative
11	FBS Superior*	1201T	No Ct	34.61	negative
12	goat serum	B1060711	No Ct	33.96	negative
13	goat serum	A8070711	No Ct	33.78	negative
14	Tris buffer	118B1101	No Ct	33.67	negative
15	rabbit serum*	B03411-1243	No Ct	33.36	negative
16	donor horse serum	F11-2542	No Ct	34.11	negative
17	donor horse serum	0533W	No Ct	30.99	negative
18	calf serum*	11-3497	No Ct	32.46	negative
19	calf serum*	11-2875	No Ct	33.26	negative
20	horse serum*	10-2658	No Ct	34.32	negative
21	positive control	---	26.26	35.66	positive
22	NTC	---	No Ct	36.60	negative

* Due to confidentiality reasons no specifications can be provided for these matrices.



16S rDNA sequencing revealed an *M. hyorhinis* contamination in Dulbecco's MEM bottle 2

No.	Sample Matrix	Ct (FAM)	Ct (ROX)	Result
1	Dulbecco's MEM, bottle 1, repeat 1	No Ct	32.76	negative
2	Dulbecco's MEM, bottle 1, repeat 2	No Ct	34.13	negative
3	Dulbecco's MEM, bottle 1, repeat 3	No Ct	34.28	negative
4	Dulbecco's MEM, bottle 1, repeat 4	No Ct	34.24	negative
5	Dulbecco's MEM, bottle 1, repeat 5	No Ct	33.90	negative
6	Dulbecco's MEM, bottle 2, repeat 1	No Ct	34.00	negative
7	Dulbecco's MEM, bottle 2, repeat 2	No Ct	34.70	negative
8	Dulbecco's MEM, bottle 2, repeat 3	No Ct	32.88	negative
9	Dulbecco's MEM, bottle 2, repeat 4	No Ct	33.43	negative
10	Dulbecco's MEM, bottle 2, repeat 5	No Ct	33.93	negative
11	positive control	28.47	34.37	positive
12	NTC	No Ct	35.34	negative
13	DNA extraction control	No Ct	36.40	negative

Second test on Dulbecco's MEM was contamination free and showed as well as the other matrices no matrix effects

For the tested matrices, **no matrix effects** were detected.

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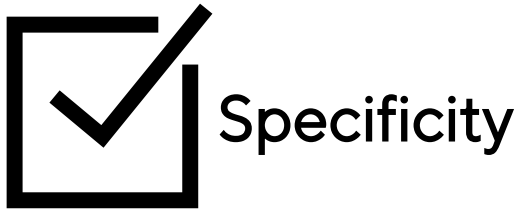
Cross reactivity



- To exclude detection of other microorganisms or eukaryotic cells, the cross reactivity was accessed
- ≥ 0.1 ng DNA/PCR for microorganisms and ≥ 30 ng for mammalian cells

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

For the tested organisms, **no cross reactivity** was detected.

Limit of detection

Sensitivity

EP 2.6.7

USP/EP required = 9

Mycoplasma arginini
Mycoplasma orale
Mycoplasma gallisepticum
Mycoplasma pneumoniae
Mycoplasma synoviae
Mycoplasma fermentans
Mycoplasma hyorhinis
Acholeplasma laidlawii
Spiroplasma citri

23/24 positive

Limit **o**f **d**etection (LOD_{95}): 80, 40, 20, 10, 5, 2.5 CFU/ml

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Limit of detection

Sensitivity

Mycoplasma synoviae

CFU/ml	80	40	20	10	5	2.5	NTC
	31.29	31.44	31.12	33.99	36.62	39.69	No Ct
	32.03	32.57	34.51	44.25	No Ct	33.78	
	31.94	32.02	33.28	39.00	33.39	35.09	
	30.14	33.07	30.80	33.77	35.26	39.36	No Ct
	30.69	31.24	30.85	33.72	No Ct	36.35	
	32.52	30.73	32.84	34.46	34.66	39.75	
	31.44	33.24	32.94	33.95	No Ct	36.92	No Ct
	31.27	32.38	36.52	33.12	38.97	36.72	
	29.34	33.88	34.26	No Ct	33.90	36.86	
	32.00	33.32	32.11	39.01	33.64	32.85	No Ct
	31.43	31.98	34.76	30.82	34.94	37.47	
	33.18	31.48	35.30	37.10	30.73	31.42	
	32.20	33.53	33.45	36.00	34.03	39.85	No Ct
	32.07	33.17	34.68	37.45	34.26	37.35	
	32.26	31.91	34.49	36.09	41.95	No Ct	
	29.82	32.51	31.45	33.07	39.94	33.94	No Ct
	32.54	33.48	31.96	33.44	31.55	32.08	
	30.38	33.88	32.56	32.98	34.10	33.19	
	32.92	34.47	34.45	36.28	32.61	36.36	No Ct
	33.86	36.67	37.23	36.72	35.16	38.16	
	33.90	33.95	35.18	35.89	34.98	39.63	
	31.63	32.52	32.16	31.06	33.58	29.66	No Ct
	30.75	31.36	32.00	29.83	33.52	33.00	
	33.32	28.08	32.42	28.90	34.03	32.52	
Mw	31.79	32.62	33.39	34.82	34.85	35.74	
STABWN	1.184	1.574	1.716	3.305	2.580	2.963	
positive	24	24	24	23	20	23	
total	24	24	24	24	24	24	

Detection Limit: 10 cfu/ml

Species (CFU-based)	Acceptance criterion	LOD_{95} (CFU/mL)
Mycoplasma arginini	23/24	10
Mycoplasma orale	23/24	10
Mycoplasma gallisepticum	23/24	10
Mycoplasma pneumoniae	23/24	≤ 5
Mycoplasma synoviae	23/24	10
Mycoplasma fermentans	23/24	10
Mycoplasma hyorhinis	23/24	≤ 5
Acholeplasma laidlawii	23/24	10
Spiroplasma citri	23/24	≤ 5

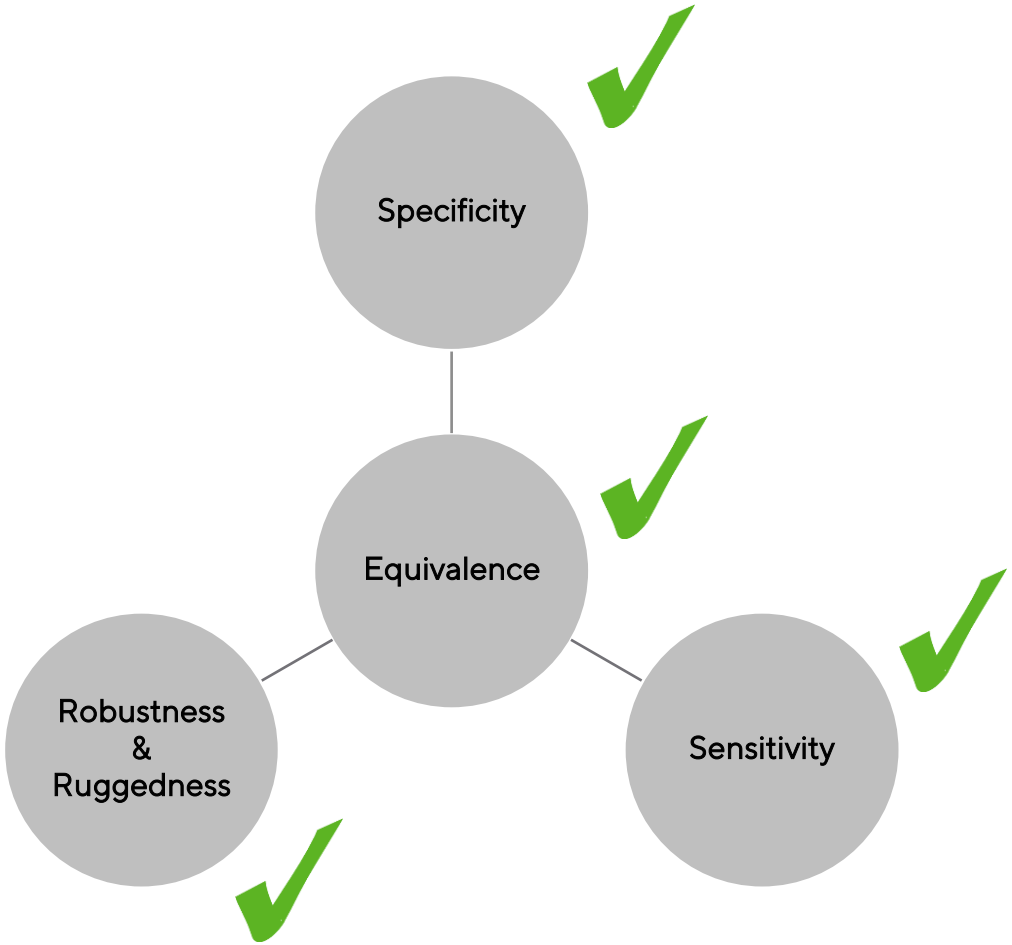
Device comparability

Robustness & Ruggedness

Cycler	Microsart® ATMP Mycoplasma
 Thermo Fischer ABI Prism 7500	PASSED
 Agilent Mx3005p	PASSED
 Qiagen Rotor-Gene 6000	PASSED

... by today many more devices are used by our customers.

Validation overview



- Sensitivity
  - LOD_{95} – limit of detection
  - Culture media comparison
- Specificity
  - Sequence alingment
  - Sample matrix effects
  - Cross reactivity
  - Mollicutes detection range
  - Identification of unspecific amplification
- Robustness
  - Cell culture material
  - Real-time PCR cycler compatibility

Simplifying Progress

Customer Validation Data
Service Lab **Labor Dr. Quade**

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Microsart® ATMP Mycoplasma Real-time PCR – Matrix specific Validation with automated DNA extraction

- Service Lab Labor Dr. Quade provides mycoplasma testing service for a cell therapy manufacturer (release testing of a T-cell-based product)
  - Samples
    - transduced **human T lymphocytes** at a concentration of **12.5 million cells per mL** in cultivation medium
  - Mycoplasma contamination detection system
    - automated DNA extraction using MagnaPure Compact Instrument
    - Microsart® ATMP Mycoplasma
  - Spikes
    - Sartorius Microsart® Validation Standards (10 CFU)
    - included species: *M. orale, M. fermentans, M. pneumoniae, A. laidlawii*
  - Acceptance criterion
    - 23 positive signals out of 24 replicates (each species)

Microsart® ATMP Mycoplasma Real-time PCR – Matrix specific Validation with automated DNA extraction

Table 1: *Mycoplasma pneumoniae*

Sample spiked with 10 CFU/mL	**Result CP 19.11.2019**	**Result CP 20.11.2019**	**Result CP 21.11.2019**	Inter-Assay CV%
Sample 1	29.93	29,23	32,99	6,51
Sample 2	29.85	29,60	32,85	5,88
Sample 3	29,33	29,27	32,27	5,66
Sample 4	30.00	29,63	32,47	5,03
Sample 5	29.87	29,58	32,24	4,77
Sample 6	30.08	29,50	32,45	5,09
Sample 7	29.73	29,79	32,09	4,41
Sample 8	29,87	30,03	31,92	3,73
Intra-Assay CV%	0.77	0,88	1.12	

CP: Crossing Point, Coefficient of Variance in %, Negative samples showed CP value >40 or a plane straight line

Microsart® ATMP Mycoplasma Real-time PCR – Matrix specific Validation with automated DNA extraction

Table 2: *Mycoplasma fermentans*

Sample spiked with 10 CFU/mL	Result CP 20.11.2019	Result CP 21.11.2019	Result CP 22.11.2019	Inter-Assay CV%
Sample 1	33,60	33,15	31,29	3,75
Sample 2	33,65	32,67	31,57	3,19
Sample 3	33,35	32,83	31,50	2,93
Sample 4	32,91	33,22	31,61	2,62
Sample 5	33,44	32,94	31,48	3,12
Sample 6	32,99	32,79	31,55	2,40
Sample 7	32,80	33,12	31,80	2,11
Sample 8	33,16	30,00	31,56	5,00
Intra-Assay CV%	0.97	3.27	0.45	

CP: Crossing Point, Coefficient of Variance in %, Negative samples showed CP value >40 or a plane straight line

Microsart® ATMP Mycoplasma Real-time PCR – Matrix specific Validation with automated DNA extraction

Table 3: *Mycoplasma orale*

Sample spiked with 10 CFU/mL	Result CP 20.11.2019	Result CP 21.11.2019	Result CP 22.11.2019	Inter-Assay CV%
Sample 1	31,53	32,47	31,64	1,61
Sample 2	31,56	32,68	31,93	1,78
Sample 3	31,68	33,02	31,21	2,94
Sample 4	31,21	32,36	31,84	1,81
Sample 5	31,19	32,48	31,27	2,28
Sample 6	31,20	32,86	31,54	2,75
Sample 7	31,23	32,14	31,43	1,51
Sample 8	31,00	32,47	32,04	3,50
Intra-Assay CV%	0.75	1.06	0.97	

CP: Crossing Point, Coefficient of Variance in %, Negative samples showed CP value >40 or a plane straight line

Microsart® ATMP Mycoplasma Real-time PCR – Matrix specific Validation with automated DNA extraction

Table 4: *Acholeplasma laidlawii*

Sample spiked with 10 CFU/mL	Result CP 20.11.2019	Result CP 21.11.2019	Result CP 22.11.2019	Inter-Assay CV%
Sample 1	31,46	28,61	31,06	5,08
Sample 2	31,28	28,17	30,73	5,52
Sample 3	31,07	29,00	31,27	4,13
Sample 4	31,52	29,00	30,93	4,32
Sample 5	31,12	31,29	31,01	0,45
Sample 6	30,97	31,33	30,94	0,70
Sample 7	31,38	31,55	31,15	0,64
Sample 8	30,74	31,44	30,98	1,15
Intra-Assay CV%	0.85	4.9	0.52	

CP: Crossing Point, Coefficient of Variance in %, Negative samples showed CP value >40 or a plane straight line

Microsart® ATMP Mycoplasma Real-time PCR – Matrix specific Validation with automated DNA extraction

- Matrix specific validation was **successful**
- The transduced **human T lymphocytes** at a concentration of **12.5 million cells per mL** in cultivation medium are suitable for release testing using automated extraction and the Microsart® ATMP Mycoplasma kit

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Simplifying Progress

Microsart® AMP Extraction
Microsart® AMP Mycoplasma

2022

Mycoplasma contamination detection

- **Real-time PCR allows detection of Mycoplasma**
  - In 3 h
  - Down to 5-10 CFU/ml
- **Validated combination**
  - In accordance with EP 2.6.7 and USP 63
- Support
  - ***Product Validation Report*** containing all experimental details
  - ***Matrix Validation Template*** containing detailed information for the customer specific matrix validation
  - **Technical support** during matrix validation process

Microsart® AMP Extraction

Microsart® AMP Mycoplasma

Workflow Mycoplasma contamination detection

20 mL

6 mL

100.000 MWCO

- **Concentration of the matrix to be tested**
  - Vivaspin® 6 or Vivaspin® 20
  - **Volume is reduced to 200 µl sample**

- **DNA isolation using the column-based Microsart® AMP Extraction kit**
  - Columns allow to isolate DNA from the whole 200 µl ATMP sample (supernatant & cells)

- **Real-time PCR using the Microsart® AMP Mycoplasma kit**
  - **PCR contains 50 µl sample DNA → complete analysis of 20 ml sample**
  - Taq-Man® System → reduce false-positive signals
  - Duplex assay → reduce false-negative signals
  - Universal assay for different real-time PCR cycler →High volume thermo cycling, FAM™ and ROX™
  - High stability & no freezing → Lyophilized reagents

RESEARCH

SARTORIUS

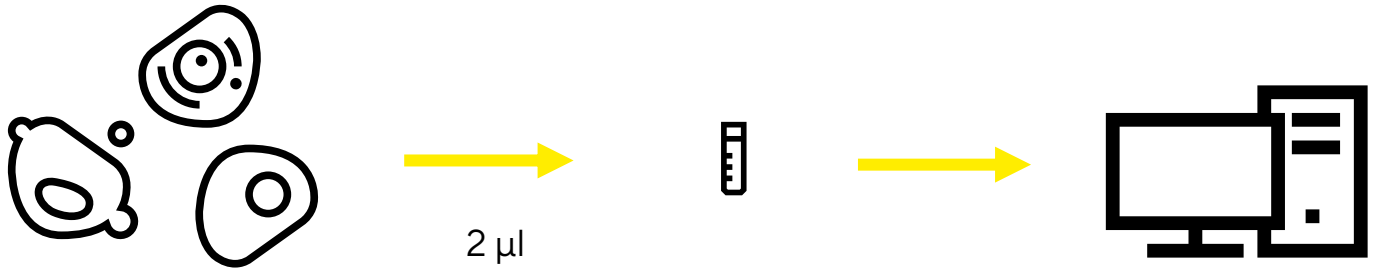
Simplifying Progress

Microsart® RESEARCH Mycoplasma

2022

In-process contamination control of Mycoplasma

- Key advantages
  - Very robust towards inhibitors
  - No prior DNA extraction required
  - Internal control DNA included in real-time PCR master mix
  - One step preparation



→Quick 'n' Dirty for process monitoring

- Taq-Man® System → reduce false-positive signals
- Duplex assay → reduce false-negative signals
- Universal assay for different real-time PCR cycler → FAM™ and ROX™
- High stability & no freezing → Lyophilized reagents

Microsart® RESEARCH Mycoplasma

Thank you.

PCR@Sartorius.com