

SARTORIUS

Simplifying Progress

Microsart® PCR Portfolio Overview

2022

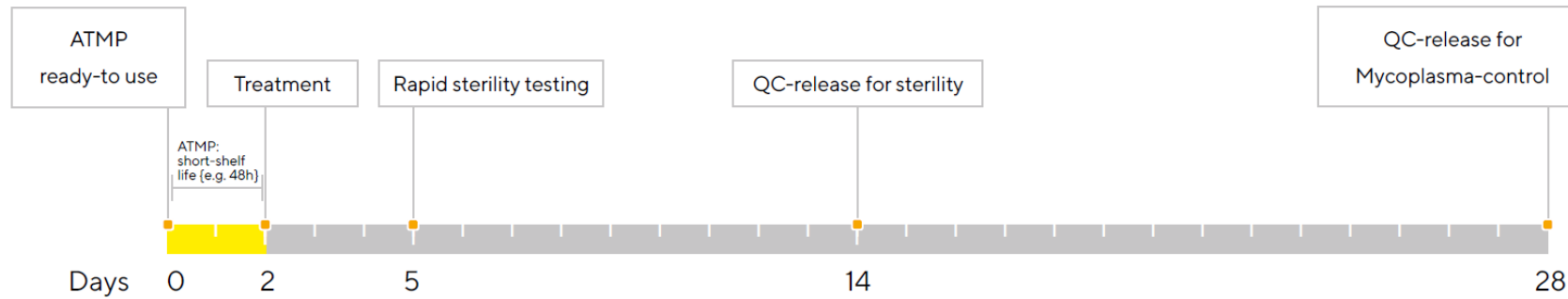


Product overview

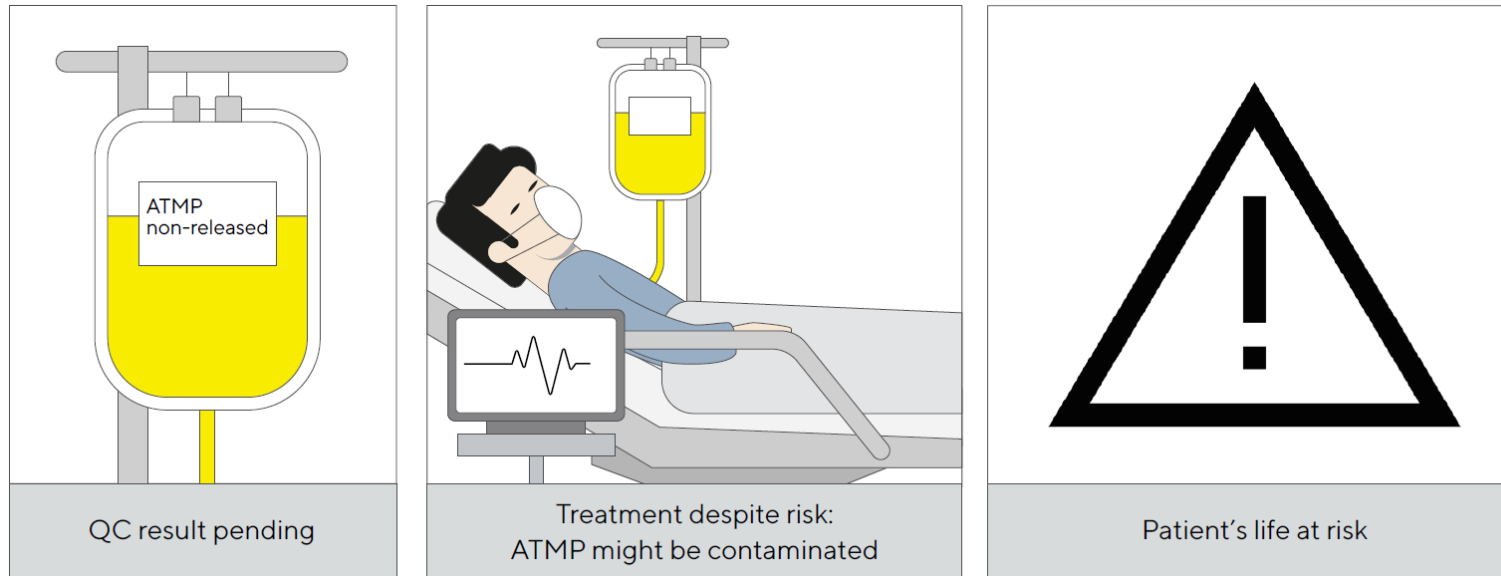
- **Validated solutions for QC release**
 - Microsart® ATMP Sterile Release
 - Microsart® ATMP Mycoplasma & Microsart® AMP Extraction
 - Microsart® AMP Mycoplasma & Microsart® AMP Extraction (special application)
- **Non-validated solutions for in-process control**
 - Microsart® Research Mycoplasma
 - Microsart® Research Bacteria
 - Microsart® Research Fungi
- **Standards**
 - Microsart® Validation Standards (inactivated CFU)
 - Microsart® Calibration Standards (DNA)



ATMPs put microbiological QC to novel challenges

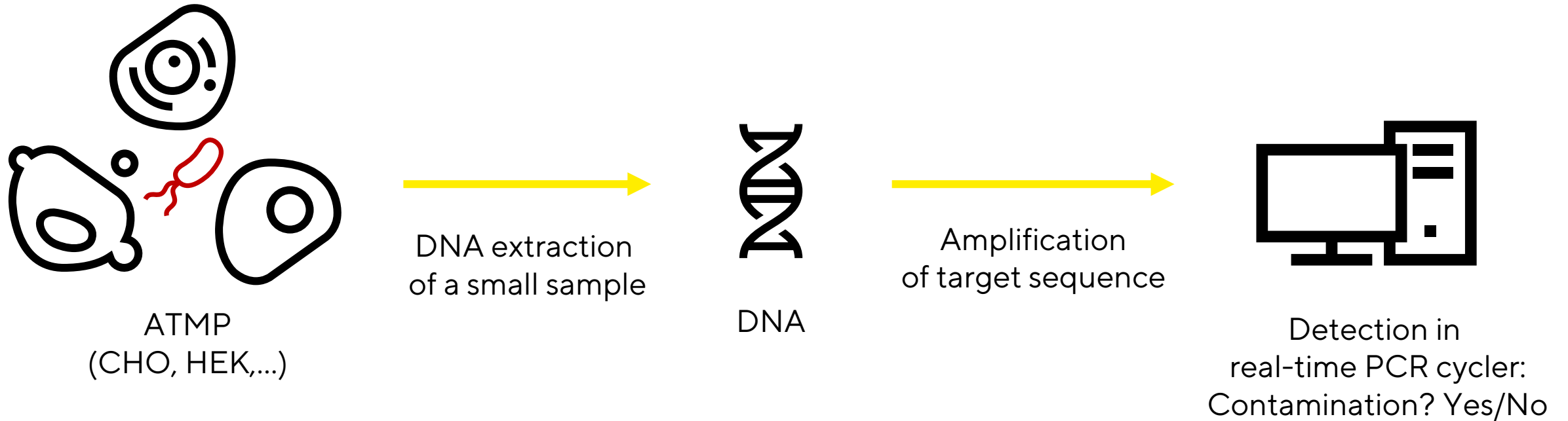


5, 14 or 28 days
of waiting
is too long
for ATMPs!



Why new methods?

Nucleic acid techniques



Results within 3 hours!

Validated solutions for QC sterile release

Mycoplasma Detection



Microsart® AMP Extraction
Microsart® ATMP Mycoplasma

Bacteria and Fungi Detection



Microsart® ATMP Extraction
Microsart® ATMP Bacteria
Microsart® ATMP Fungi
or
Microsart® ATMP Sterile Release



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)

- 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 cyclers → FAM™ and ROX™
 - High stability & no freezing → Lyophilized reagents



Microsart® AMP Extraction



Microsart® ATMP Mycoplasma

Bacteria & fungi contamination detection

- Real-time PCR allows detection of bacteria and fungi
 - In 3 h
 - Down to 2.5-99 CFU/ml
- Validated combination
 - In accordance with EP 5.1.6, USP 1223, EP 2.6.27, and USP 1071
- 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® ATMP Extraction



Microsart®
ATMP Bacteria



Microsart®
ATMP Fungi



Microsart® ATMP Sterile Release

Workflow bacteria & fungi contamination detection

- DNA isolation using the Microsart® ATMP Extraction kit
 - Extraction protocol includes centrifugation step to remove free bacterial DNA
 - Harsh extraction allows to isolate fungi & bacteria
- 2 real-time PCRs using the Microsart® ATMP Fungi & Microsart® ATMP Bacteria kit
 - Taq-Man® System → reduce false-positive signals
 - Duplex real-time PCR assay → reduce false-negative signals
 - Universal assay for different real-time PCR cycler → FAM™ and ROX™
 - Highly stability & no freezing → Lyophilized reagents



Microsart® ATMP Extraction



Microsart® ATMP Bacteria



Microsart® ATMP Fungi



Microsart® ATMP Sterile Release



Simplifying Progress

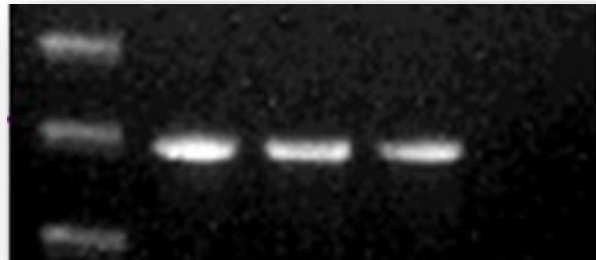
Technical background
DNA-based detection methods

SARTORIUS

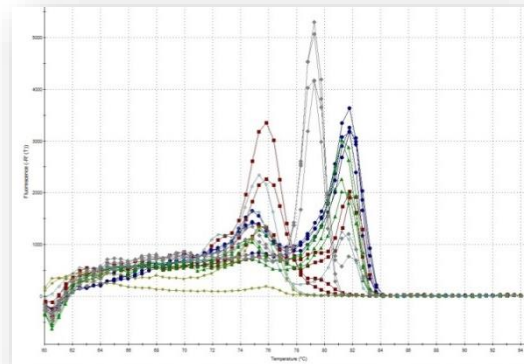
DNA-based detection methods

real-time PCR

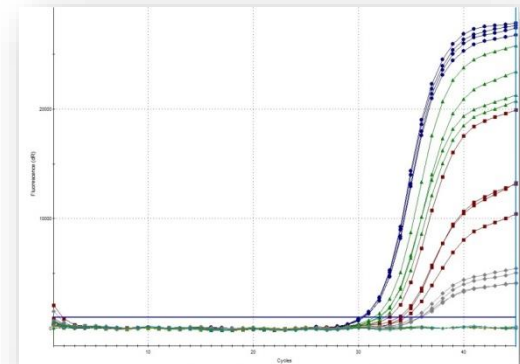
Conventional PCR



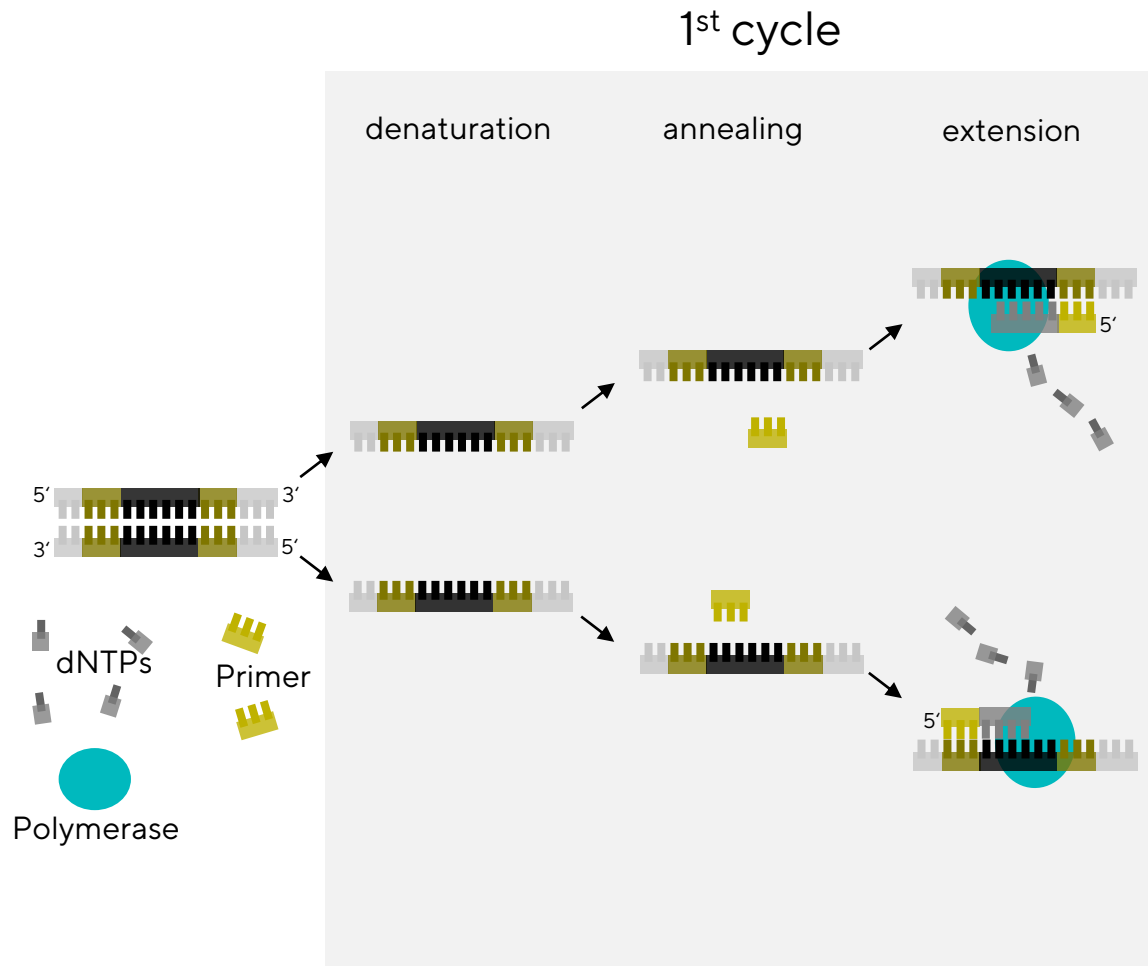
SYBR Green I



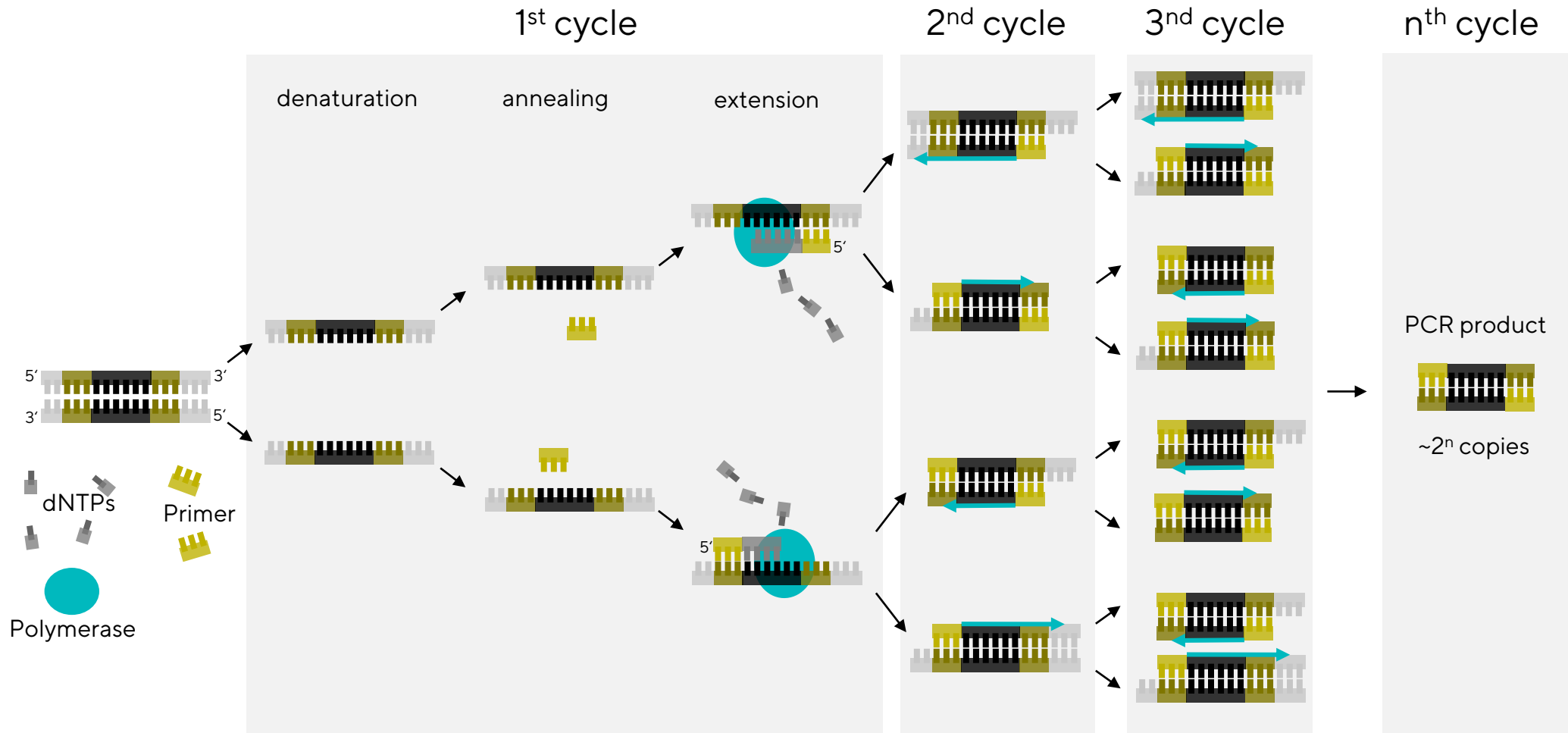
TaqMan® Probe



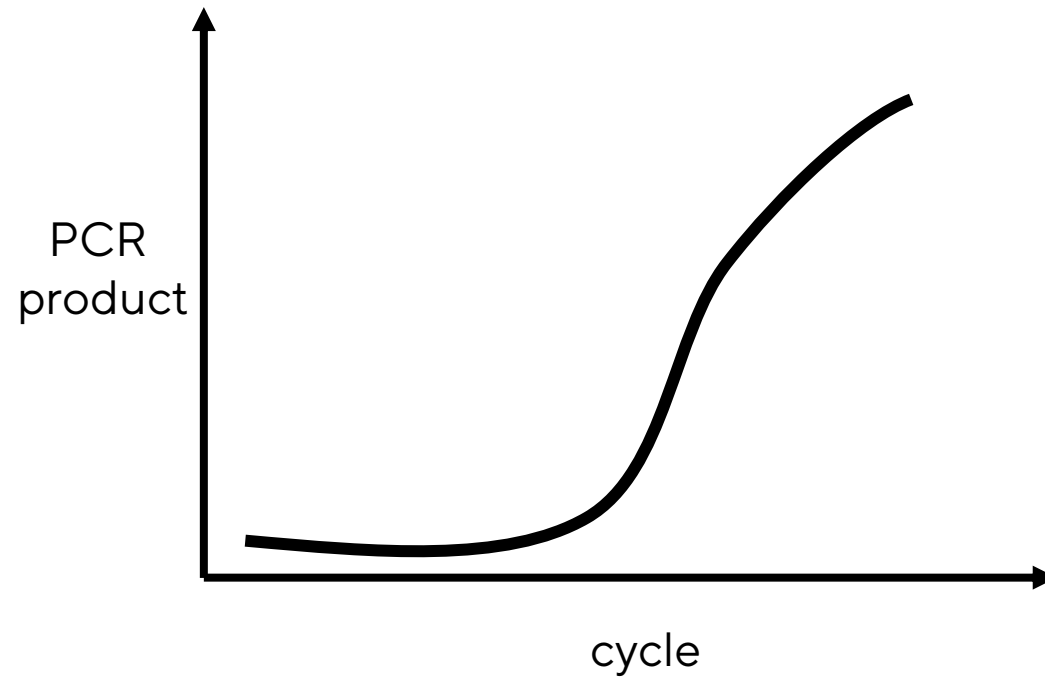
What is a conventional PCR?



What is a conventional PCR?

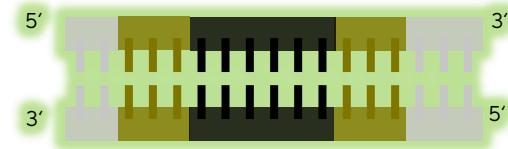
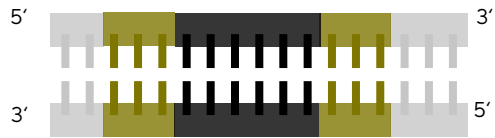
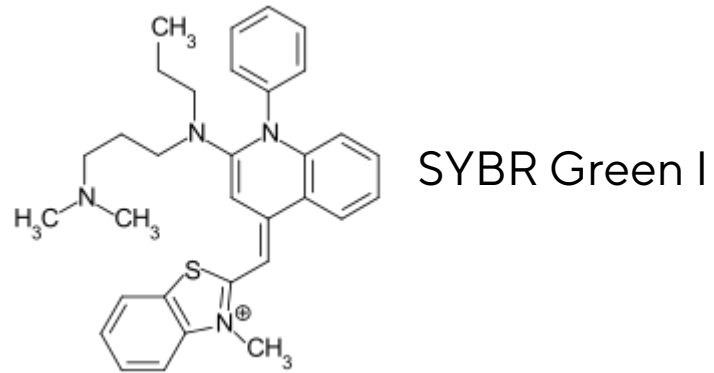


A real-time PCR visualizes the reaction

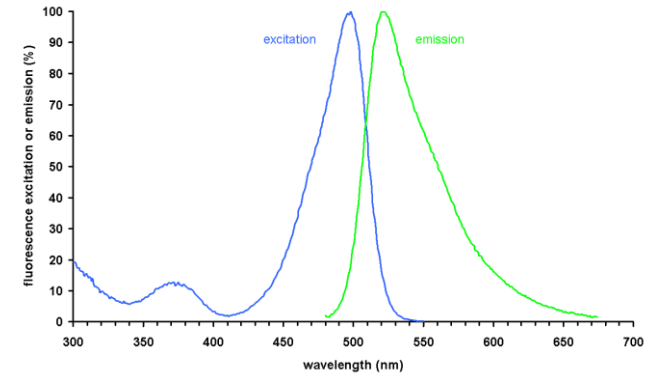


How does that work?

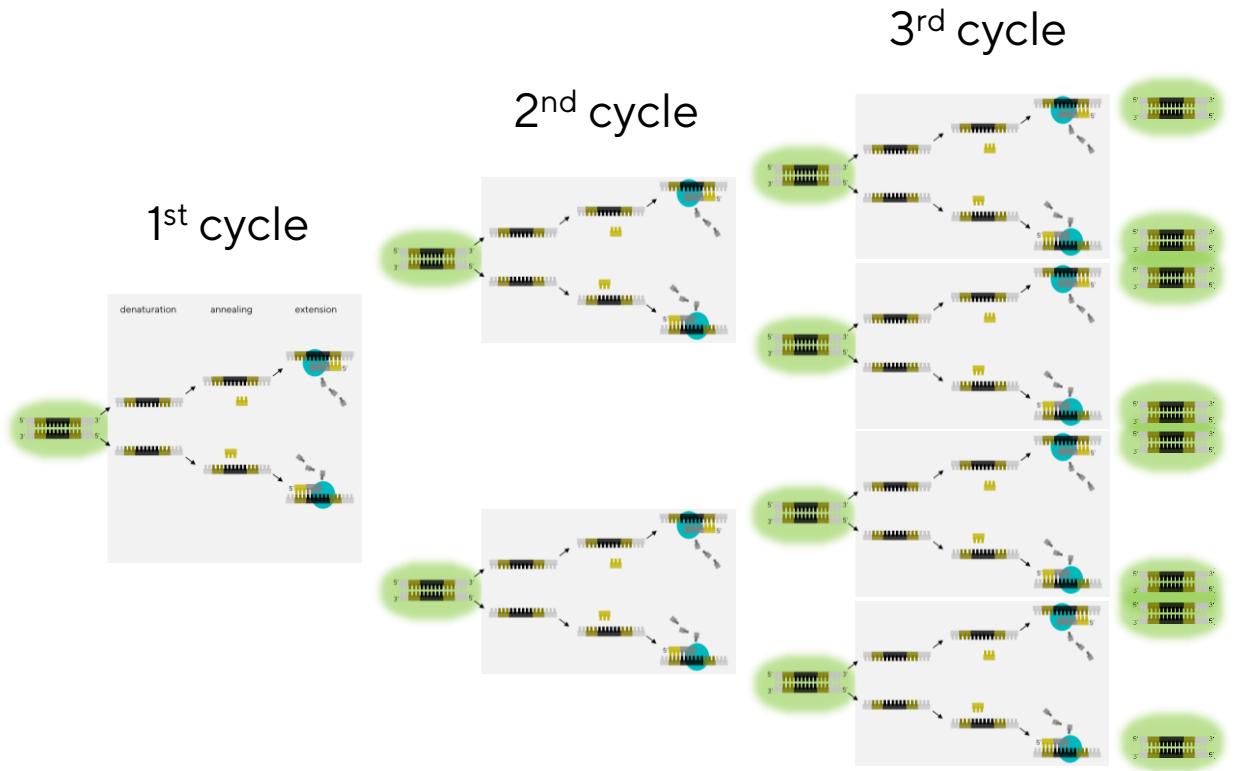
Real-time PCR



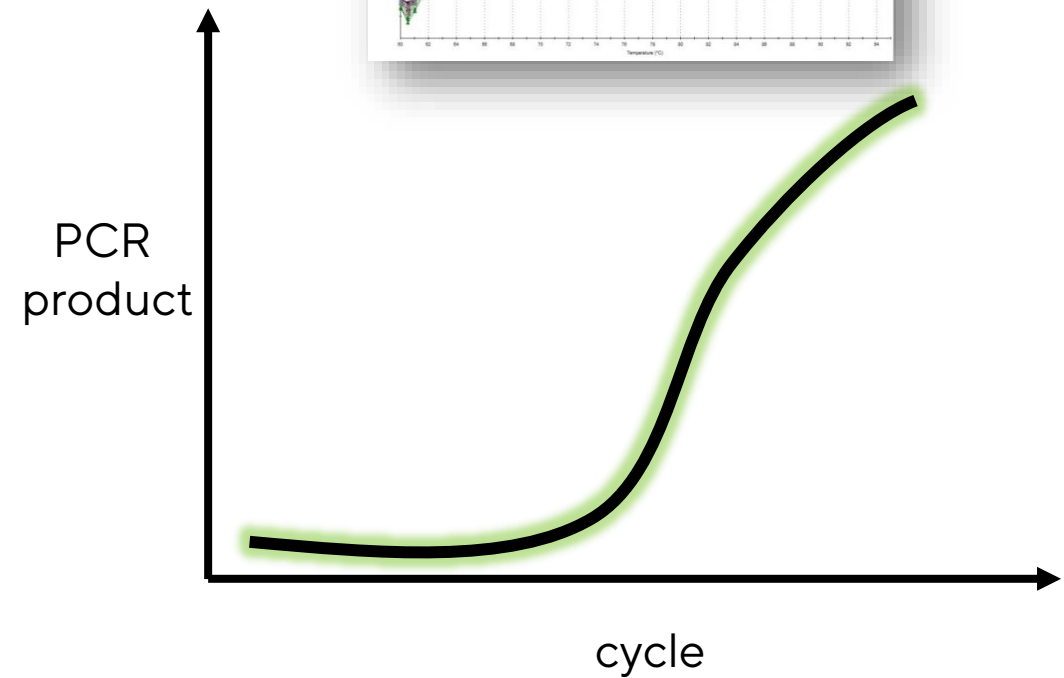
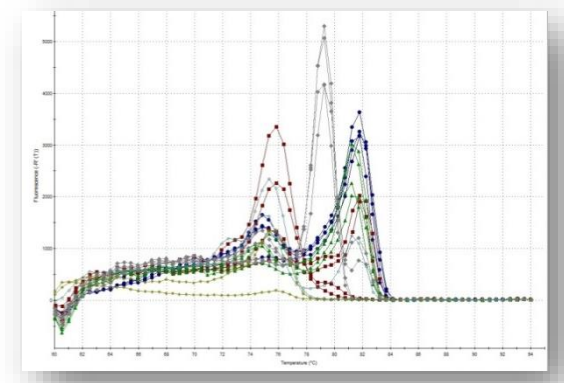
The dye SYBR Green I binds to double stranded DNA!



Real-time PCR

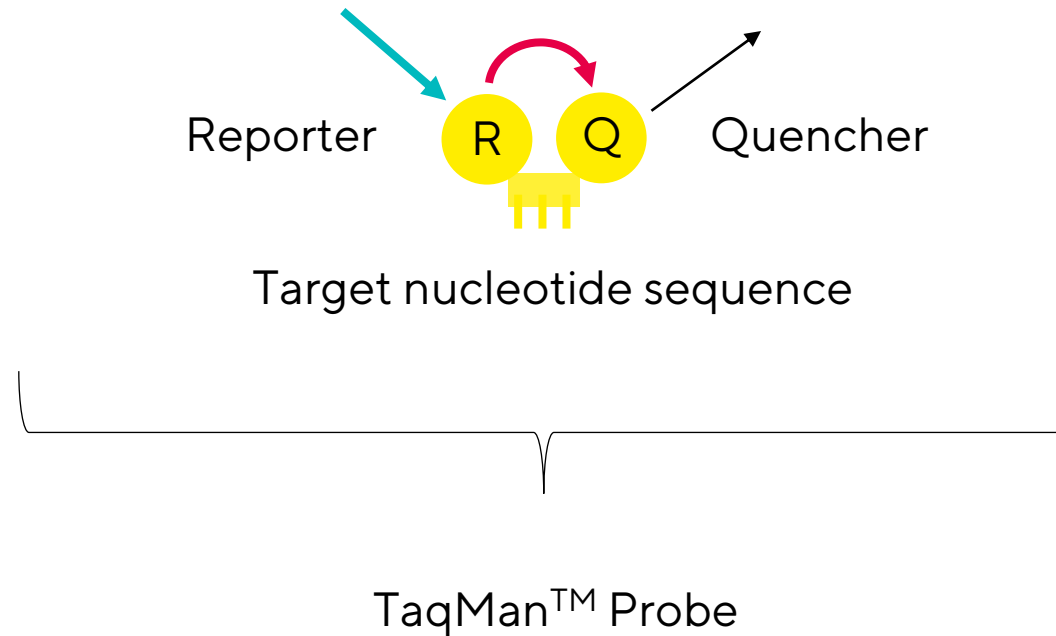


SYBR Green I



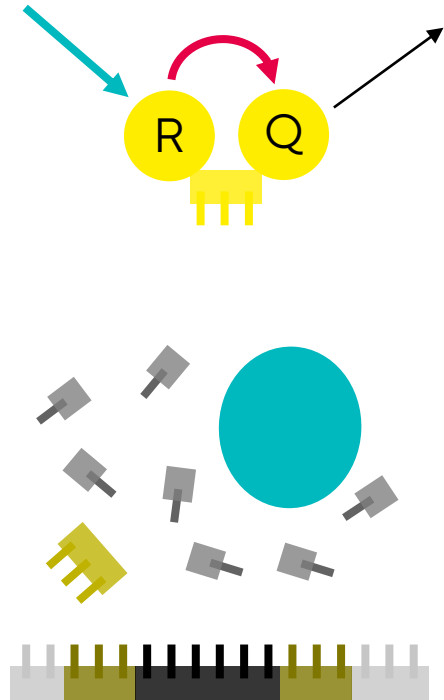
Unspecific binding of SYBR Green I can result in false-positive signals!

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

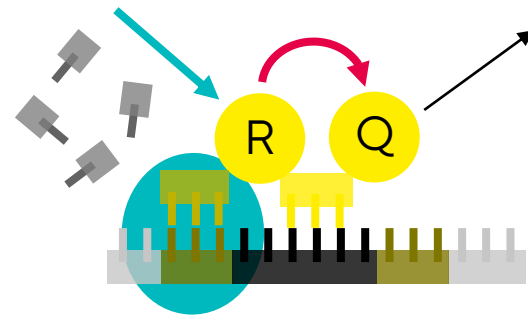


TaqMan™ real-time PCR

TaqMan™ probe is degraded during real-time PCR

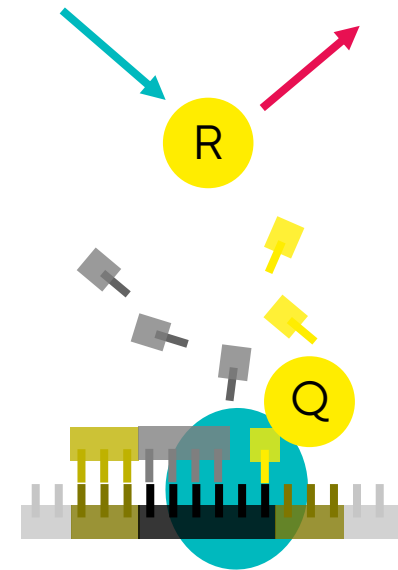


As long as the probe is complete
no light signal can be detected



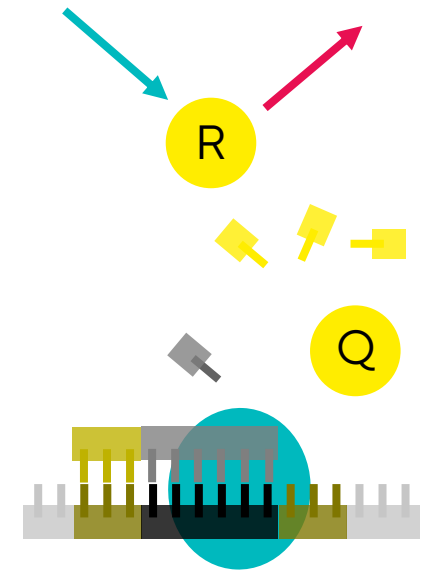
Taq Polymerase functions:

- DNA amplification
- 5'-3' exonuclease activity

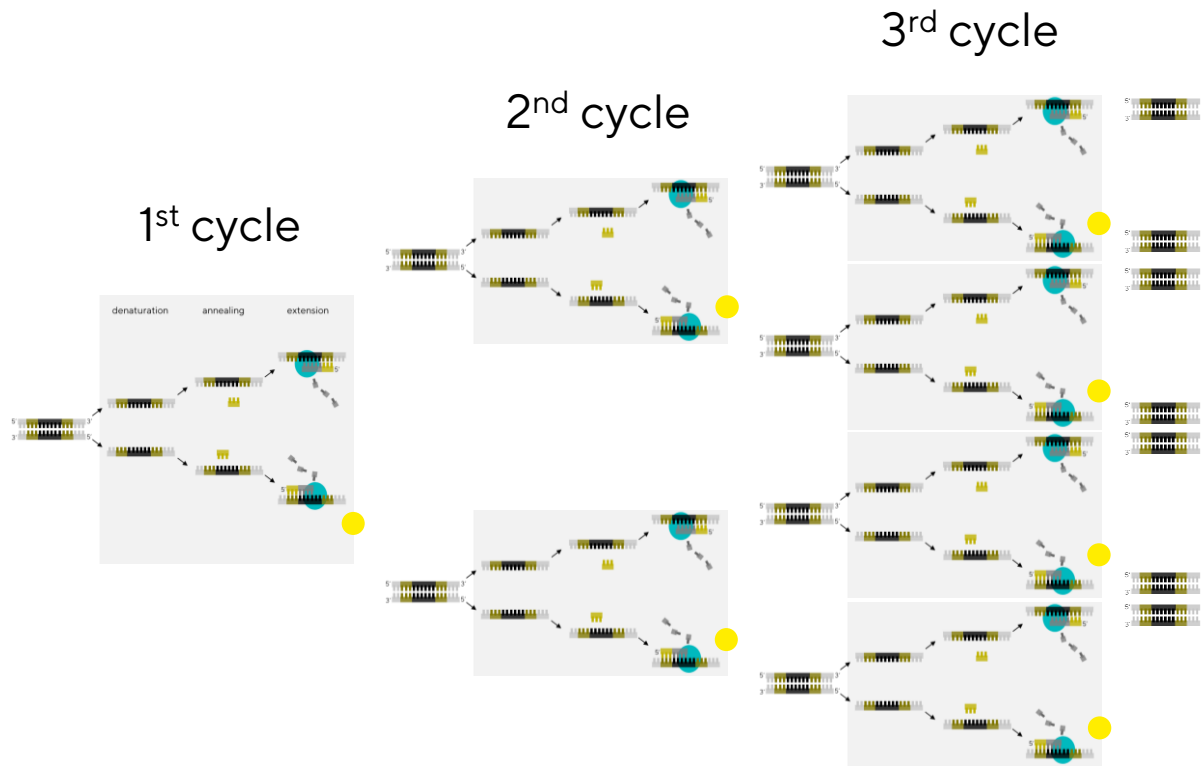


During elongation:

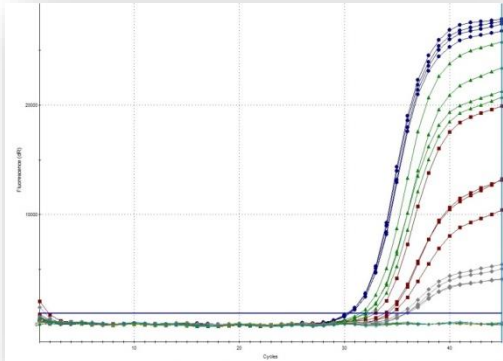
- Polymerase hydrolyses probe
- Dye and quencher are separated
- Reporter dye emits light signal



TaqMan™ real-time PCR



TaqMan® Probe



PCR product

cycle

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

A duplex real-time PCR assay monitors PCR functionality

Problem:

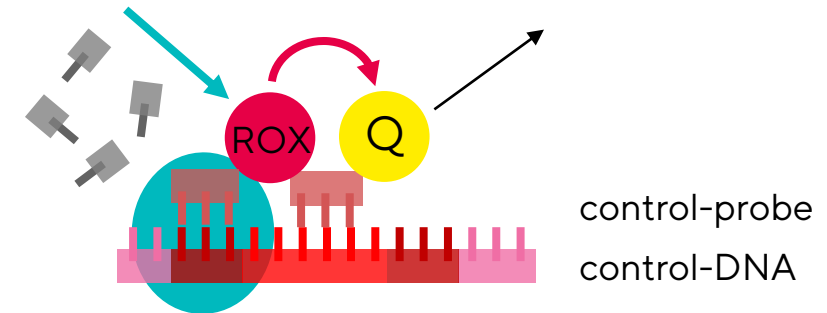
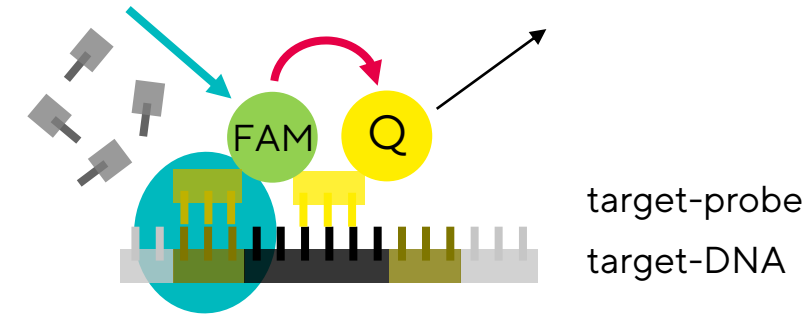
What does **no signal** mean?
No microbial contamination?
PCR inhibition?

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.

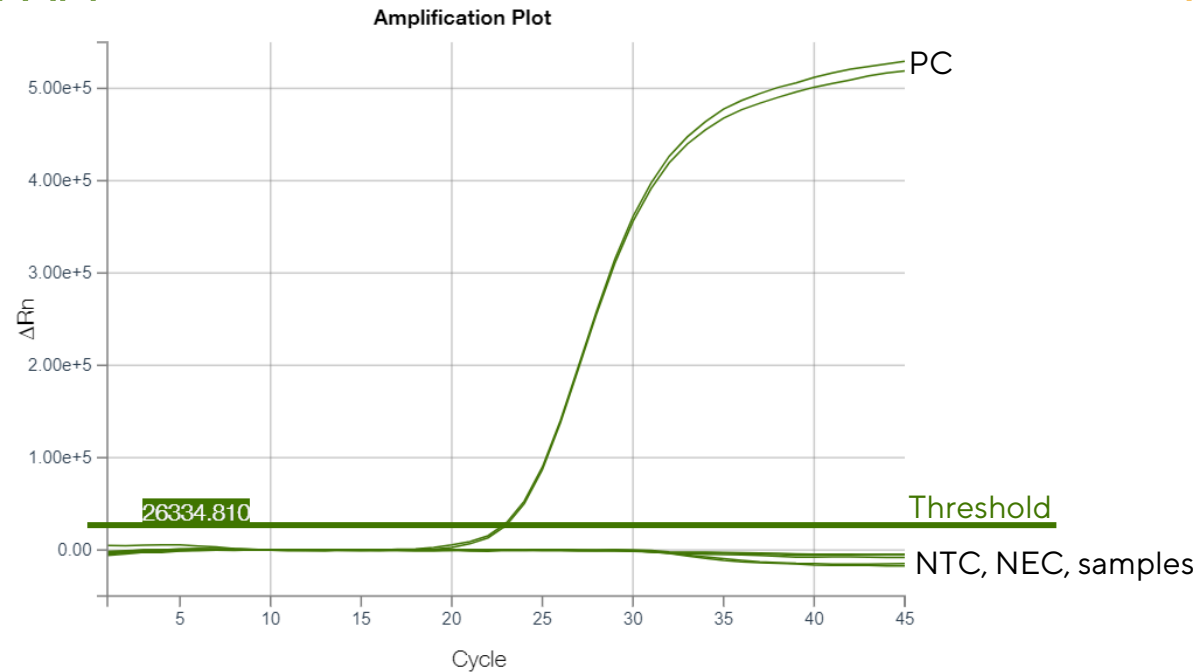
Duplex assay
= two independent real-time PCRs
in one run using different
fluorophores



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

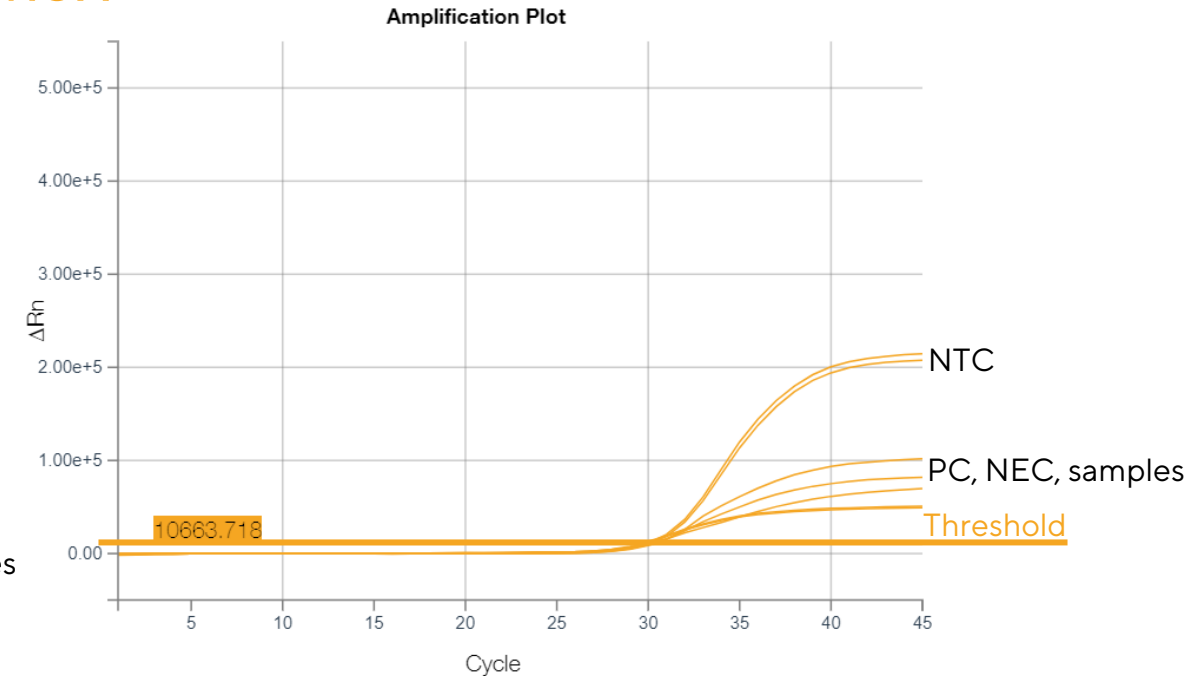
A duplex real-time PCR Analysis

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



Mycoplasma, bacteria & fungi contamination detection



Microsart® ATMP Mycoplasma

- FAM probe:
 - Targets Mycoplasma
 - PCR Mix with red cap
- ROX probe:
 - Targets Internal Control DNA
 - PCR Mix with yellow cap



Microsart® ATMP Bacteria

- FAM probe:
 - Targets Bacteria
 - PCR Mix with red cap
- ROX probe:
 - Targets Internal Control DNA
 - PCR Mix with yellow cap

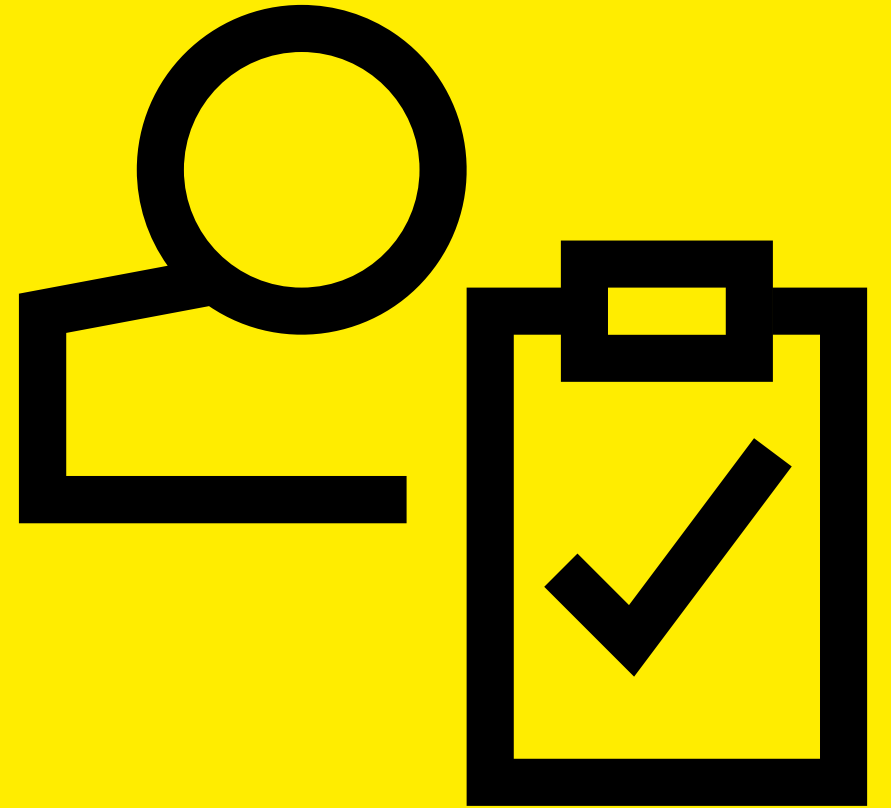


Microsart® ATMP Fungi

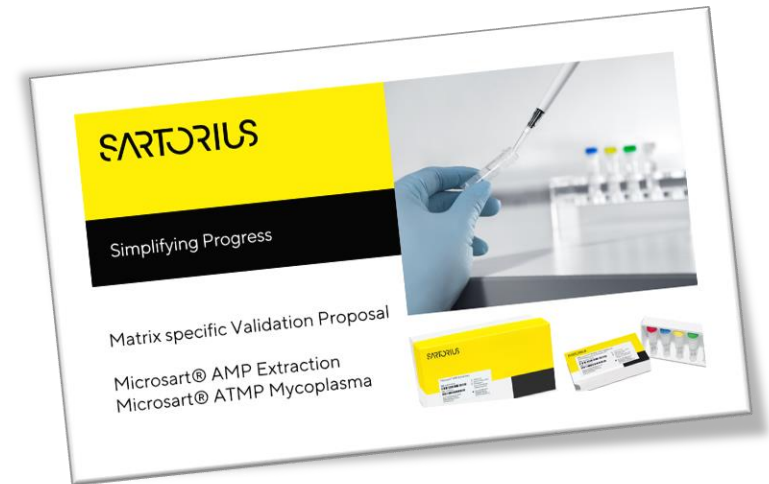
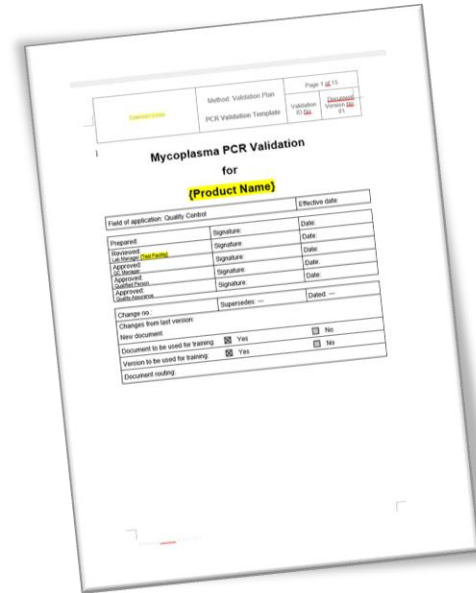
- FAM probe:
 - Targets Fungi
 - PCR Mix with orange cap
- ROX probe:
 - Targets Internal Control DNA
 - PCR Mix with yellow cap

Convenient working: Same principle across all kits

Product Validation



Validation reports, templates & testing proposals



▪ Product Validation Reports

- Microsart® ATMP Bacteria + Microsart® ATMP Extraction
- Microsart® ATMP Fungi + Microsart® ATMP Extraction
- Microsart® ATMP Mycoplasma + Microsart® AMP Extraction

▪ Validation Templates

- Combined validation Template for Microsart® ATMP Bacteria + Microsart® ATMP Fungi + Microsart® ATMP Extraction
- Microsart® ATMP Mycoplasma + Microsart® AMP Extraction

▪ Validation Proposals

- Standard matrix specific validation for all validated kit combinations
- Individual support for all kits

Further support for your validation

- Microsart® Validation Standard (10 CFU/Vial) & Microsart® Calibration Reagents (10⁸ GC/Vial)

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

- Microsart® Validation Standard (99 CFU/Vial) & Microsart® Calibration Reagents (10⁸ GC/Vial for bacteria, 10⁶ GC/Vial for fungi)

- *Bacillus subtilis*
- *Pseudomonas aeruginosa*
- *Kocuria rhizophila* | *Micrococcus luteus*
- *Clostridium sporogenes*
- *Bacteroides vulgatus*
- *Staphylococcus aureus*
- *Candida albicans*
- *Aspergillus brasiliensis*
- *Aspergillus fumigatus*
- *Penicillium chrysogenum*
- *Candida glabrata*
- *Candida krusei*
- *Candida tropicalis*

Non-viable CFU Standards!

Do you miss a species? Let us know!

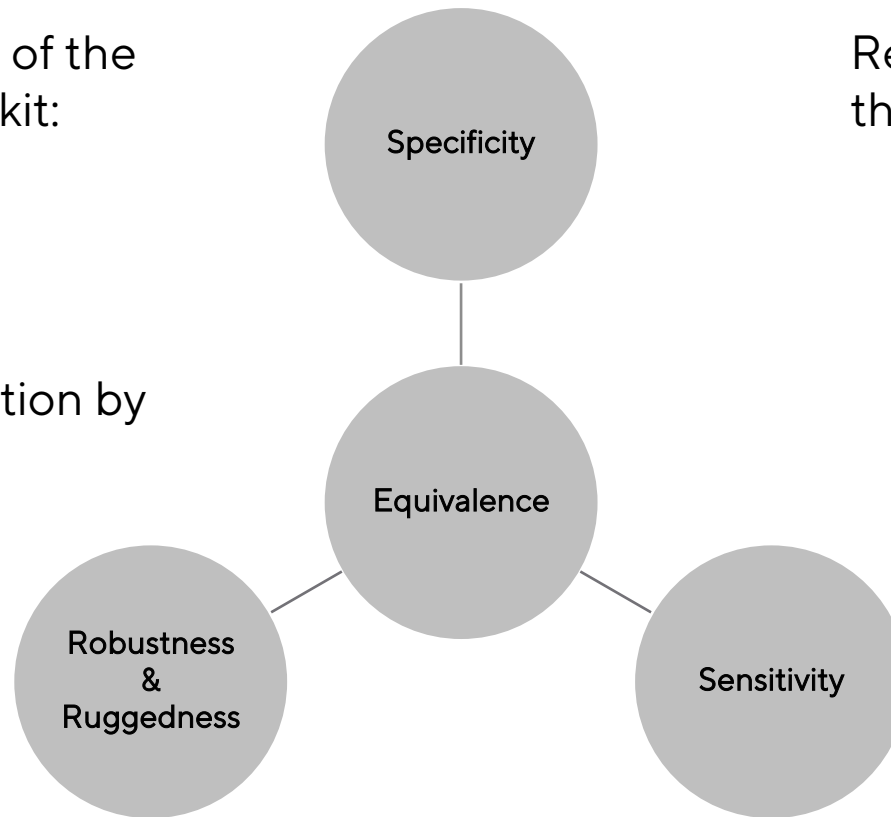
Status quo regulatory landscape

| Microbiological QC-Release testing | | | |
|------------------------------------|----------------------------------|---|-------|
| Method | 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 of the **Microsart® ATMP Bacteria/Fungi** kit:

- PDA, TR 33
- USP <1223>
- USP <1071>
- EP 5.1.6 part 4-1-1 primary validation by supplier
- EP 2.6.27
- (USP<71>)
- (EP2.6.1)



Regulatory guidance for validation of the **Microsart® ATMP Mycoplasma** kit:

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

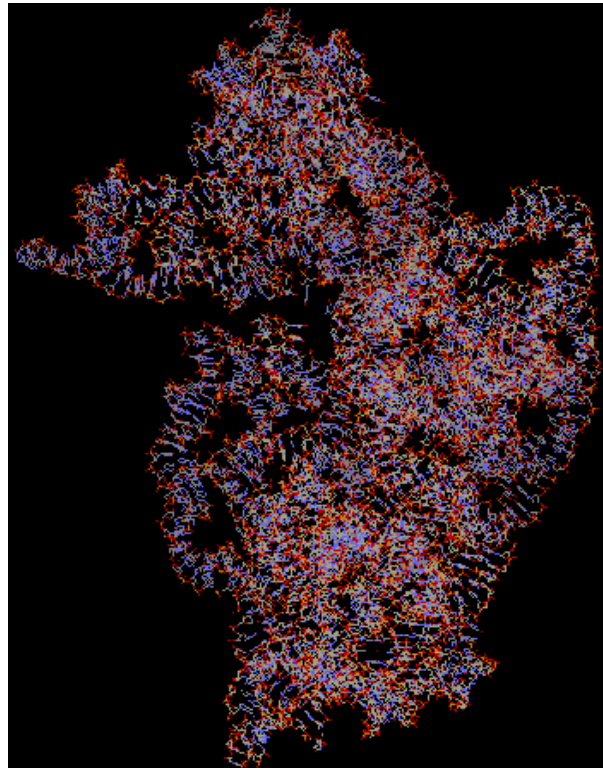
- USP<63>
- USP<1223>
- EP 5.1.6
- PDA, TR 33

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

Paul-Ehrlich-Institut 

In silico prediction by sequence alignment and blast

16S / 18S rDNA



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



NIH U.S. National Library of Medicine

BLAST » blastn suite

blastn blastp blastx tblastn tblastx

Enter Query Sequence

Enter accession number(s), gi(s), or FASTA sequence(s)

AGAGTTTGATCTGGCTCAG

Or, upload file

Job Title

Align two or more sequences

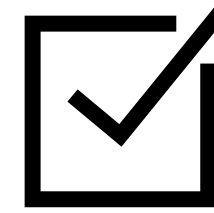
Choose Search Set

Database

Human genomic + transcript M

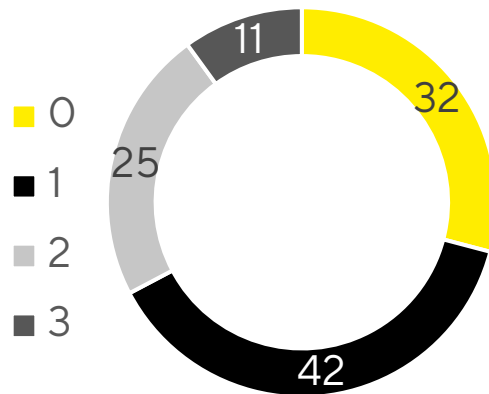
16S ribosomal RNA sequences (Bac

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



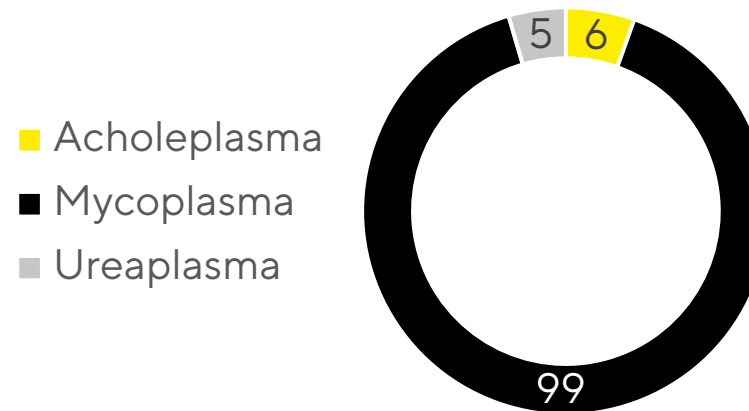
Detection range – Microsart® ATMP Mycoplasma

Primer/Probe Mismatches



Acceptance criterion:
≤ 3 nucleotides mismatch
of primers and probe

Genus



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



Detection range – Microsart® ATMP Bacteria

| | Primer Mismatches | | | |
|------------|-------------------|--------|--------|--------|
| | 0 | 1 | 2 | 3 |
| Bacteria | 48.8 % | 69.4 % | 85.7 % | 94.7 % |
| Archaea | n.a | n.a | 0.1% | 40.4 % |
| Eukaryotes | 0% | 0% | 0.1% | 0.3 % |

Accepting 3 primer mismatches, **94.7 % of the bacteria** are detected



Detection range – Microsart® ATMP Fungi



| Genus | Coverage |
|-----------------------------------|----------|
| <i>Alternaria</i> | 97.7 % |
| <i>Aspergillus</i> | 95.3 % |
| <i>Aureobasidium</i> | 93.5 % |
| <i>Bipolaris</i> | 98 % |
| <i>Candida</i> | 86.3 % |
| <i>Chaetomium</i> | 3.6% |
| <i>Cladosporium</i> | 95.5 % |
| <i>Curvularia</i> | 100 % |
| <i>Epidermophyton</i> | 100 % |
| <i>Exserohilum</i> | 97.4 % |
| <i>Fusarium</i> | 95.9 % |
| <i>Memnoniella (Stachybotrys)</i> | 86,7 % |
| <i>Microsporum</i> | 100 % |
| <i>Myrothecium</i> | 100 % |
| <i>Paecilomyces</i> | 100% |
| <i>Penicillium</i> | 98.2 % |
| <i>Malassezia*</i> | 0.1 % |
| <i>Rhizopus</i> | 4 % |
| <i>Scopulariopsis</i> | 0 % |
| <i>Trichoderma</i> | 98 % |
| <i>Trichophyton</i> | 100 % |

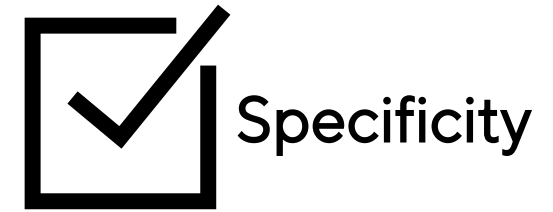
← soil, air, plant debris

← skin microbiome
 ← organic substances
 ← soil, decaying wood

Accepting 2 primer mismatches, already **37 % of the fungi** are detected, including all species of clinical and bioprocess relevance.

Matrix Effects

- Media
 - Typical cell culture media
 - Typical additives for cell culture media
 - Typical buffers
- Cell lines
 - Vero-B4
 - Per. C6
 - RK 13
 - CHO-K1
 - Hela
 - Jurkat
 - ...



FYI:
Application Note
challenging all Microsart®
ATMP Kits with high cell
concentrations

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

Limit of detection – Microsart® ATMP Mycoplasma

EP 2.6.7

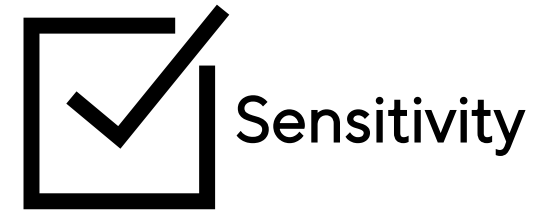
USP/EP required = 9



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

23/24 positive

Limit of detection (LOD₉₅): 80, 40, 20, 10, 5, 2.5 CFU/ml



Limit of detection – Microsart® ATMP Mycoplasma

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 | |

| Species (CFU-based) | Acceptance criterion | LOD ₉₅ (CFU/mL) |
|---------------------------------|----------------------|----------------------------|
| <i>Mycoplasma arginini</i> | 23/24 | 10 |
| <i>Mycoplasma orale</i> | 23/24 | 10 |
| <i>Mycoplasma gallisepticum</i> | 23/24 | 10 |
| <i>Mycoplasma pneumoniae</i> | 23/24 | ≤ 5 |
| <i>Mycoplasma synoviae</i> | 23/24 | 10 |
| <i>Mycoplasma fermentans</i> | 23/24 | 10 |
| <i>Mycoplasma hyorhinitis</i> | 23/24 | ≤ 5 |
| <i>Acholeplasma laidlawii</i> | 23/24 | 10 |
| <i>Spiroplasma citri</i> | 23/24 | ≤ 5 |

Limit of detection – Microsart® ATMP Bacteria/Fungi

EP 2.6.1
USP<71>

regulatory advice;
EP 2.6.27; user feedback

Currently tested = 6



Bacillus subtilis
Clostridium sporogenes
Pseudomonas aeruginosa
Staphylococcus aureus

Candida albicans
Aspergillus brasiliensis

23/24 positive

Recommended Extension = 20

+ Colony Forming Units (CFU)

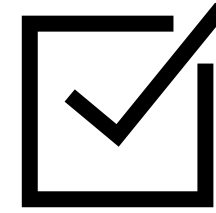
- Streptococcus pyogenes*
- Bacteroides vulgatus*
- Escherichia coli*
- Pseudomonas protegens*
- Bacillus cereus*
- Enterococcus faecalis*
- Kocuria rhizophila*
- Staphylococcus epidermidis*
- Serratia marcescens*
- Cutibacterium acnes*

Genome Copies (GC)

- Candida tropicalis*
- Candida glabrata*
- Candida krusei*
- Aspergillus fumigatus*
- Penicillium chrysogenum*
- Bacteroides fragilis*
- Enterobacter cloacae*
- Klebsiella pneumoniae*
- Clostridium perfringens*
- Yersinia enterocolitica*

8/8 positive

Limit of Detection (LOD₉₅): 99, 50, 25, 10, 5, 2.5, 1.25 CFU/ml



Limit of detection – Microsart® ATMP Bacteria/Fungi

currently tested

recommended extension

| Species | Strain No | Acceptance criterion | LOD ₉₅ (CFU/mL) |
|---------------------------------|------------|----------------------|----------------------------|
| <i>Candida albicans</i> | ATCC 10231 | 23/24 | 50 |
| <i>Aspergillus brasiliensis</i> | ATCC 16404 | 23/24 | 50 |
| <i>Candida tropicalis</i> | ATCC 750 | 8/8 | 10 |
| <i>Candida glabrata</i> | ATCC 90030 | 8/8 | 25 |
| <i>Candida krusei</i> | ATCC 6258 | 8/8 | 50 |
| <i>Aspergillus fumigatus</i> | ATCC 9197 | 8/8 | 99 |
| <i>Penicillium chrysogenum</i> | ATCC 9178 | 8/8 | 99 |

| Species | Strain No | Acceptance criterion | LOD ₉₅ (CFU/mL) |
|-----------------------------------|------------|----------------------|----------------------------|
| <i>Bacillus subtilis</i> | ATCC 6633 | 23/24 | 25 |
| <i>Clostridium sporogenes</i> | ATCC 19404 | 23/24 | 25 |
| <i>Pseudomonas aeruginosa</i> | ATCC 9027 | 23/24 | 5 |
| <i>Staphylococcus aureus</i> | ATCC 6538 | 23/24 | 10 |
| <i>Streptococcus pyogenes</i> | ATCC 19615 | 8/8 | 99 |
| <i>Bacterioides vulgatus</i> | ATCC 8482 | 8/8 | 2,5 |
| <i>Escherichia coli</i> | ATCC 8739 | 8/8 | 10 |
| <i>Pseudomonas protegens</i> | ATCC 17386 | 8/8 | 10 |
| <i>Bacillus cereus</i> | ATCC 10876 | 8/8 | 5 |
| <i>Enterococcus faecalis</i> | ATCC 29212 | 8/8 | 99 |
| <i>Kocuria rhizophila</i> | ATCC 9341 | 8/8 | 10 |
| <i>Staphylococcus epidermidis</i> | ATCC 12228 | 8/8 | 99 |
| <i>Serratia marcescens</i> | ATCC 14756 | 8/8 | 50 |
| <i>Propionibacterium acnes</i> | ATCC 11827 | 8/8 | 25 |
| <i>Bacterioides fragilis</i> * | ATCC 25285 | 8/8 | 10 |
| <i>Enterobacter cloacae</i> * | ATCC 13047 | 8/8 | 10 |
| <i>Klebsiella pneumoniae</i> * | ATCC 13883 | 8/8 | 10 |
| <i>Serratia marcescens</i> * | ATCC 13880 | 8/8 | 10 |
| <i>Clostridium perfringens</i> * | ATCC 13124 | 8/8 | 10 |
| <i>Yersinia enterocolitica</i> * | ATCC 27739 | 8/8 | 10 |

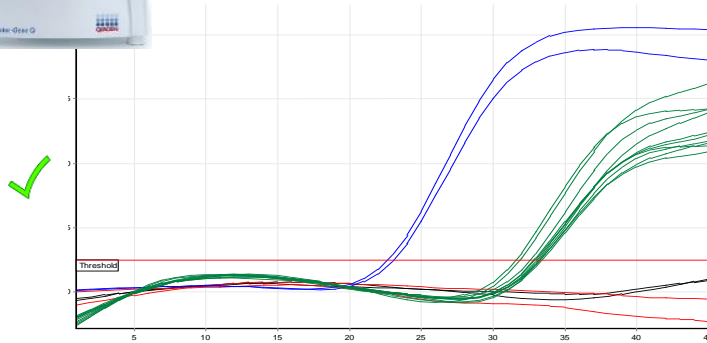
*GC based (GC/PCR)



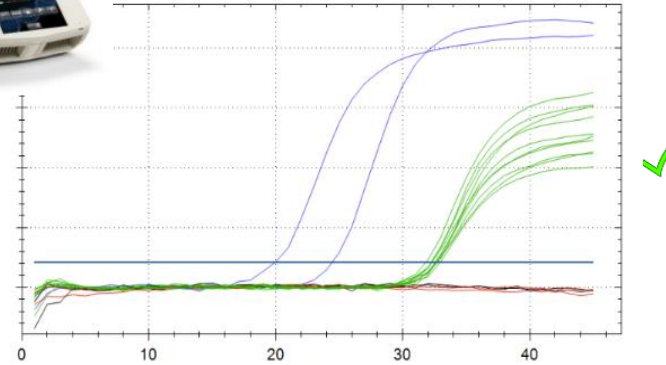
Device comparability



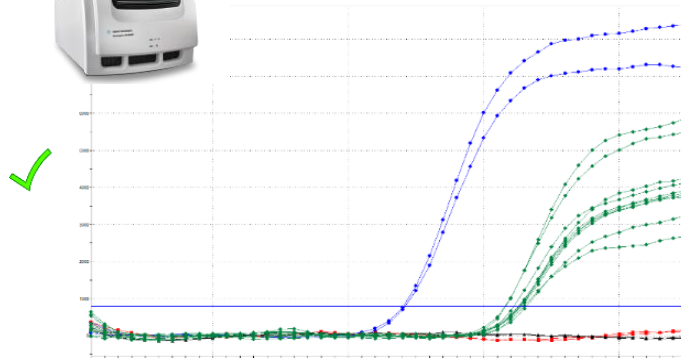
QiaGen Rotor-Gene 6000



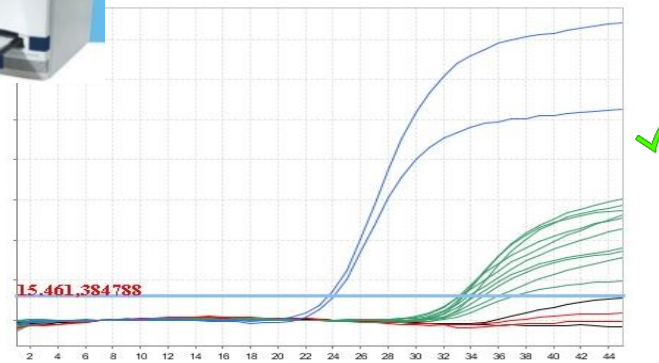
BioRad CFX96 touch



Agilent Mx3005p



Thermo Fischer ABI Prism 7500



Spiking 99 CFU/ml of the species with the highest LOD₉₅
Clostridium sporogenes (LOD₉₅ = 25)
Candida albicans (LOD₉₅ = 50)
Acceptance criterion 8/8 positive

PC
NTC
NEC
Extracts

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



Equivalence with compendial culture method

Sartorius



real-time PCR-based detection

Labor LS

?
=



classical sterility test according to EP 2.6.1. and USP<71>

2x LOD₉₅

all 6 mandatory species

- *Bacillus subtilis*
- *Clostridium sporogenes*
- *Pseudomonas aeruginosa*
- *Staphylococcus aureus*
- *Candida albicans*
- *Aspergillus brasiliensis*

LOD₉₅ ½ LOD₉₅

recommended extension

- *Streptococcus pyogenes*
- *Pseudomonas protegens*





Equivalence with compendial culture method

| | Microsart® ATMP Bacteria | | | Compendial culture method (External) | | |
|-------------------------------|--------------------------|-------------------|----------------------|--------------------------------------|----------------------|----------------------|
| | 2x LOD ₉₅ | LOD ₉₅ | LOD ₉₅ /2 | 2x LOD ₉₅ | LOD ₉₅ | LOD ₉₅ /2 |
| <i>Bacillus subtilis</i> | 33.16 | 34.23 | 35.47 | <i>B. subtilis</i> | <i>B. subtilis</i> | <i>B. subtilis</i> |
| | 33.23 | 34.32 | 34.38 | | | |
| <i>Staphylococcus aureus</i> | 35.42 | 35.77 | 36.56 | <i>S. aureus</i> | <i>S. aureus</i> | <i>S. aureus</i> |
| | 34.13 | 35.67 | 39.90 | | | |
| <i>Clostridium sporogenes</i> | 34.20 | 34.87 | 35.45 | <i>C. sporogenes</i> | <i>C. sporogenes</i> | <i>C. sporogenes</i> |
| | 34.10 | 33.43 | 35.61 | | | |
| <i>Pseudomonas aeruginosa</i> | 36.40 | 36.74 | 37.22 | <i>P. aeruginosa</i> | <i>P. aeruginosa</i> | Negative |
| | 36.22 | 37.96 | No Cd | | | |
| <i>Streptococcus pyogenes</i> | 34.89 | 35.53 | 36.55 | <i>S. pyogenes</i> | <i>S. pyogenes</i> | <i>S. pyogenes</i> |
| | 35.09 | 35.93 | 35.88 | | | |
| <i>Pseudomonas protegens</i> | 34.14 | 34.38 | 36.52 | Gram - Oxidase + | Gram - Oxidase + | Gram - Oxidase + |
| | 33.28 | 34.51 | 35.61 | | | |



Equivalence with compendial culture method

| | Microsart® ATMP Fungi | | | Compendial culture method (External) | | |
|---------------------------------|-----------------------|-------------------|----------------------|--------------------------------------|------------------------|------------------------|
| | 2x LOD ₉₅ | LOD ₉₅ | LOD ₉₅ /2 | 2x LOD ₉₅ | LOD ₉₅ | LOD ₉₅ /2 |
| <i>Candida albicans</i> | 32.25 | 32.27 | 32.96 | <i>C. albicans</i> | <i>C. albicans</i> | Negative |
| | 31.94 | 32.12 | 33.96 | | | |
| <i>Aspergillus brasiliensis</i> | 34.38 | 37.06 | 34.94 | <i>A. brasiliensis</i> | <i>A. brasiliensis</i> | <i>A. brasiliensis</i> |
| | 32.40 | 33.17 | 34.20 | | | |

Validation overview



- Sensitivity
 - LOD₉₅ - limit of detection
- Specificity
 - Sequence alignment
 - Sample matrix effects/cross reactivity
 - Specificity of PCR with genomic DNA
 - Comparison with compendial method
- Robustness
 - Spiked cell culture samples
 - Device compatibility
 - Detection of free-DNA
 - False positive rate

RESEARCH

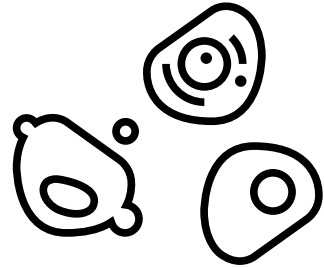


In-process contamination control of Mycoplasma, bacteria & fungi

- 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



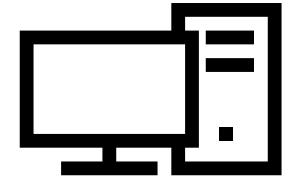
ATMP
(CHO, HEK,...)



2 μ l



23 μ l
RESEARCH
master mix



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

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

In-process contamination control of Mycoplasma, bacteria & fungi



**Microsart® RESEARCH
Mycoplasma**



**Microsart® RESEARCH
Bacteria**



**Microsart® RESEARCH
Fungi**

Thank you.



PCR@Sartorius.com

SARTORIUS