

Proposal of the New Rapid Sterility Test

Shimadzu Diagnostics Corporation



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- 2 BactFinder™/FungiFinder™
- 3 Rapid Test Method
- 4 High Sensitivity Test Method
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About our Products

Kits for quality testing of ATMP and similar products

01

Myco Finder™



- ✓ PCR kit for mycoplasma detection. Compatible with EP, USP, and JP.
- ✓ Tubes come with dried reaction reagents. Produces results in four easy steps.

02

VirFinder™ Type-A/Type-B



- ✓ PCR kit for virus detection testing
- ✓ Two versions available for different target viruses

03

BactFinder™/FungiFinder™



- ✓ PCR kit for rapid microbial detection testing
- ✓ Two test methods for different applications

NEW

Alternative Microbiological Test Methods

EP 2.6.27 and 5.1.6, and USP <1071> list various detection techniques as alternative rapid microbiological test methods.



Examples of alternative microbiological test methods

- ✓ Gas consumption or production
- ✓ Bioluminescence (ATP)
- ✓ Solid phase cytometry
- ✓ Autofluorescence
- ✓ **Genotypic techniques (NAT)**

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We have developed a real-time PCR kit that detects microorganisms by targeting DNA.

Real-time PCR

- High sensitivity and specificity
- Use of commercially available real-time PCR equipment

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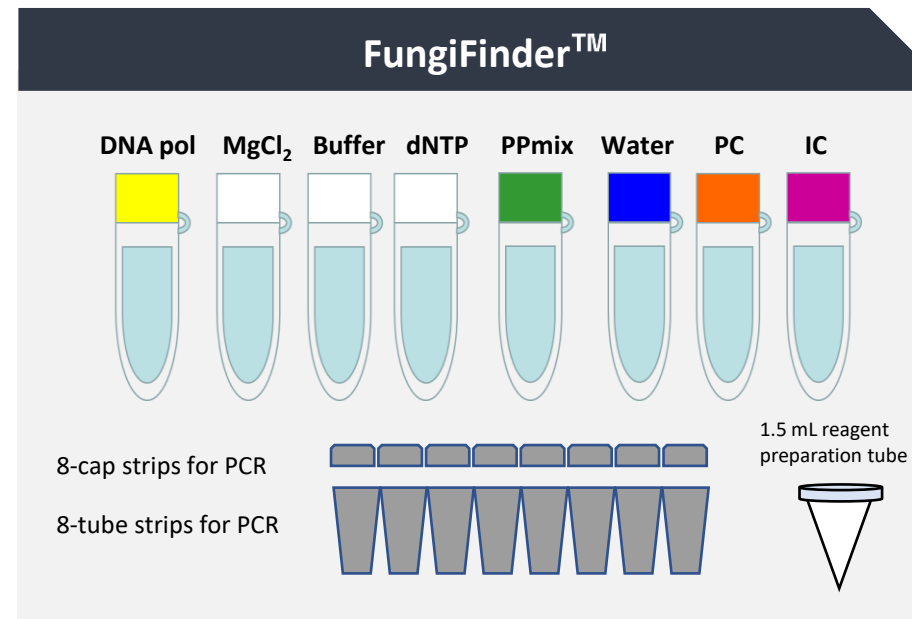
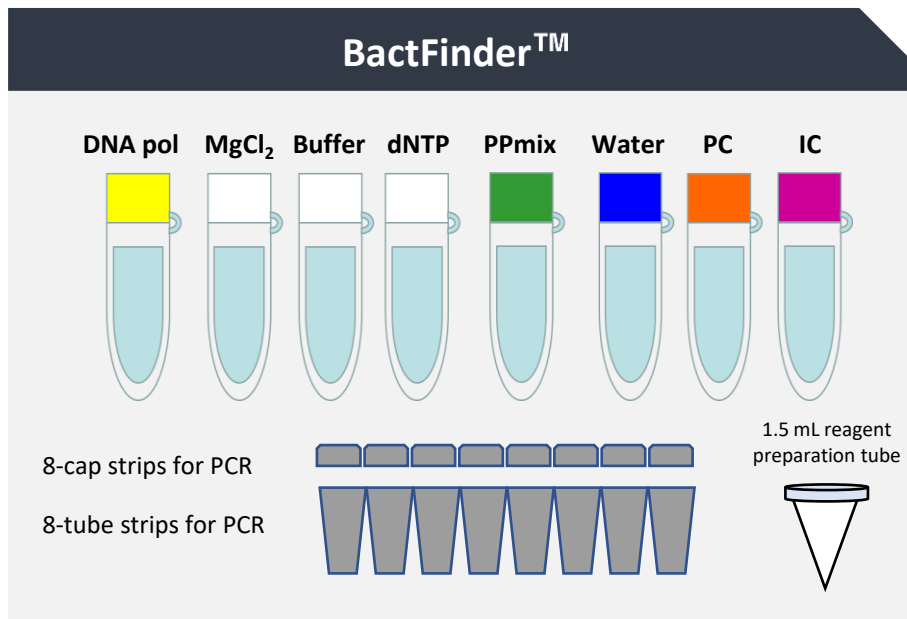
BactFinder™/FungiFinder™



Features

- ✓ PCR kit that detects microbial nucleic acids
- ✓ Clean kit with reduced background nucleic acids
- ✓ Target-specific detection
- ✓ Detects a wide range of environmental and human-derived microorganisms

BactFinder™/FungiFinder™



Capacity: Bacteria PCR reagents: 50 tests; Fungi PCR reagents: 50 tests
Contains enough 1.5 mL tubes and 8-tube strips for at least 50 tests

- ✓ Kit includes tubes for PCR reagent preparation and reactions Prevents contamination from other sources
- ✓ Includes internal control DNA (IC)
Used to verify nucleic acid extraction and monitor for PCR inhibition

BactFinder™ and FungiFinder™ Performance Attributes

Expected Samples	Culture supernatant, cell suspensions
Detection Sensitivity	100 CFU/sample
Kit-derived Background Noise	Very little
Specificity	Target-specific detection
Between-device Variation	Verified compatible with three different real-time PCR equipment
Detection Time	About 4 hours
Microorganism Coverage	Six pharmacopoeia microorganisms plus a wide range of environmental and human-derived microorganisms
Operating Conditions	An environment that allows sterile operation

Offers rapid detection of microorganism-derived nucleic acids

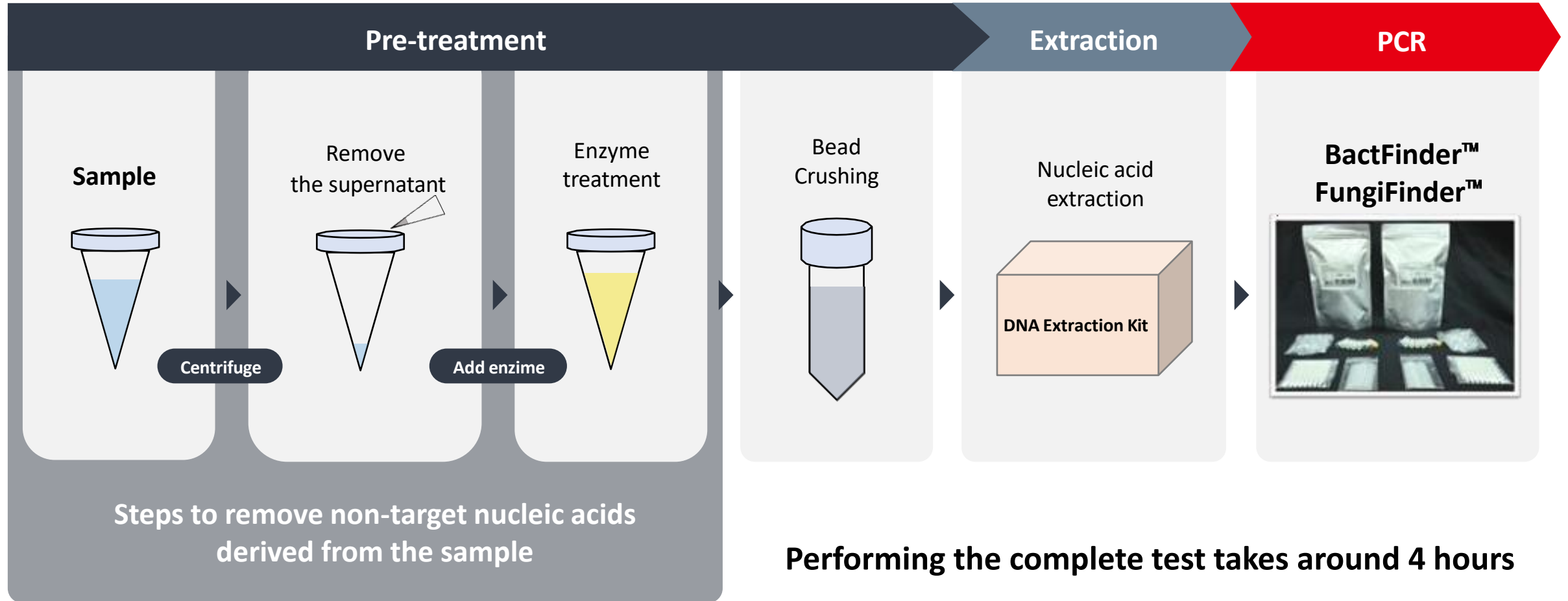
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Rapid Test Method

Developed for reduced microbial background noise in three steps.



Method

Objectives

- Investigate test sensitivity and background noise to verify the suitability of the rapid test method

Sensitivity evaluation with 6 microorganisms

- Tested 1-mL samples of 6 microorganism species at 100 CFU combined with Jurkat cells at 1.0×10^6 cells/mL (N = 8 of each microorganism)
- Cq detected by real-time PCR deemed a positive result; no Cq detected is a negative result

Background noise evaluation

- Tested 1-mL of PBS combined with Jurkat cells at 1.0×10^6 cells/mL (N = 16)
- Performed real-time PCR analysis and showed Cq results

Sensitivity Evaluation

Microorganism strain	Result
<i>Aspergillus brasiliensis</i> ATCC 16404	8/8
<i>Candida albicans</i> ATCC 10231	8/8
<i>Bacillus subtilis</i> subsp. <i>spizizenii</i> ATCC 6633	8/8
<i>Clostridium sporogenes</i> ATCC 11437	8/8
<i>Pseudomonas aeruginosa</i> ATCC 9027	8/8
<i>Staphylococcus aureus</i> ATCC 6538	8/8

Shows number of positive results out of tested samples

Demonstrates the rapid test method can reliably detect microorganisms at 100 CFU in samples containing other cells

Background Noise Evaluation

BactFinder™			
1	35.31	9	N/A
2	N/A	10	N/A
3	34.66	11	N/A
4	35.72	12	37.20
5	N/A	13	N/A
6	N/A	14	N/A
7	N/A	15	N/A
8	N/A	16	36.22

FungiFinder™			
1	N/A	9	N/A
2	N/A	10	N/A
3	N/A	11	N/A
4	N/A	12	N/A
5	N/A	13	N/A
6	N/A	14	N/A
7	N/A	15	N/A
8	N/A	16	N/A

Tables show Cq result for each sample
N/A: No Cq detected

Some background noise detected when using BactFinder™

No background noise detected when using FungiFinder™



Rapid Test Method

- ✓ A rapid test method that detects microorganisms from 100 CFU in around 4 hours
- ✓ A clean method with relatively little background noise
 - Results affected somewhat by sample type and the testing environment

Likely suitable for real-world applications when a cutoff value is used as the judgment criterion and a suitable testing environment is available

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Background to High Sensitivity Test Method



Rapid Test Method

- ✓ Result in around 4 hours → Great!
- ✓ 100 CFU sensitivity → **Would like higher sensitivities**
- ✓ Occasional background noise → **Would like no false positive results**

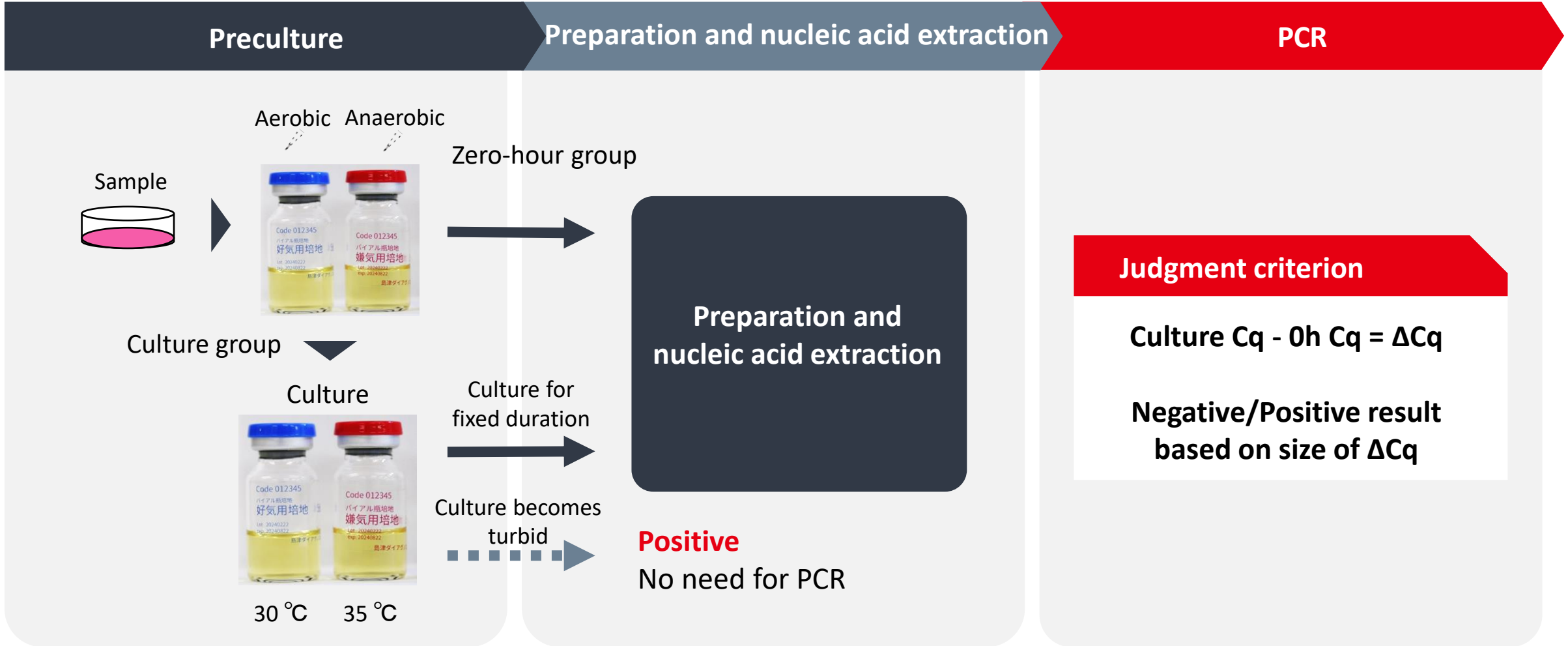


We developed a high sensitivity test method with a higher sensitivity and lower risk of false positives compared to the rapid test method

Features of the High Sensitivity Test Method

	Rapid Test Method	High Sensitivity Test Method
Expected Use	Basic sample screening	Product release testing, in-process testing
Test Method Steps	—	Preculture
	Preparation and nucleic acid extraction	
	Real-time PCR	
Detection Sensitivity	100 CFU	1 CFU
Testing Time	Around 4 hours	2 – 3 days of culture + 4 hours
Test Method Benefits	Same-day results	False positives unlikely Results not influenced by dead microorganisms

High Sensitivity Test Method

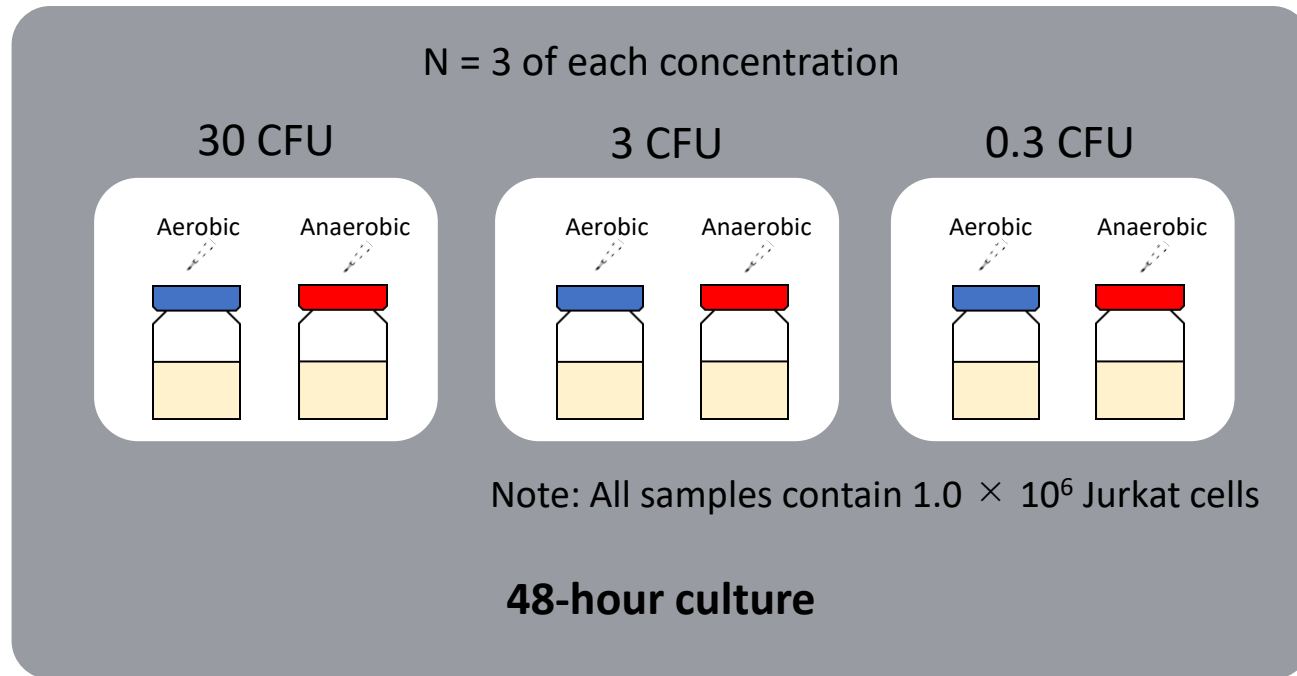


Tested Attributes

We evaluated the performance of the high sensitivity test method by testing the following attributes.

Test items	Definition
Limit of Detection	Lowest concentration of microorganisms that can be detected
Equivalence	Degree of closeness between tests results of rapid and compendial methods
Range of Species	Range of microbial species that can be detected
Specificity	Capacity to not generate false positive results

Method



Culture becomes turbid

Positive
No PCR analysis

No turbidity
in culture

**Preparation → Nucleic acid
extraction → PCR**

**Judgment
criterion**

Culture C_q - 0h C_q = ΔC_q

$\Delta C_q \leq -6$ **Positive**

$\Delta C_q > -6$ Negative

- ✓ TSB and TGC used in the compendial method were cultured for 14 days (N = 1 of each)



Check for turbidity after 14 days

Results compared with high
sensitivity test method

Limit of Detection

Results after 48-hour culture of 30 to 0.3 CFU samples

Microorganism strain	30 CFU			3 CFU			0.3 CFU														
	Turbidity	PCR	Positive	Turbidity	PCR	Positive	Turbidity	PCR	Positive												
<i>Aspergillus brasiliensis</i> ATCC 16404	■	■	■	/	/	/	3/3	■	■	■	/	/	/	3/3	■	■	■	/	/	/	0/3
<i>Candida albicans</i> ATCC 10231	■	■	■	/	/	/	3/3	■	■	■	/	/	/	3/3	■	■	■	/	/	/	2/3
<i>Bacillus subtilis</i> subsp. <i>spizizenii</i> ATCC 6633	■	■	■	/	/	/	3/3	■	■	■	/	/	/	3/3	■	■	■	/	/	/	0/3
<i>Clostridium sporogenes</i> ATCC 11437	■	■	■	/	/	/	3/3	■	■	■	/	/	/	2/3	■	■	■	/	/	/	0/3
<i>Pseudomonas aeruginosa</i> ATCC 9027	■	■	■	/	/	/	3/3	■	■	■	/	/	/	3/3	■	■	■	/	/	/	2/3
<i>Staphylococcus aureus</i> ATCC 6538	■	■	■	/	/	/	3/3	■	■	■	/	/	/	3/3	■	■	■	/	/	/	2/3

/ : No PCR detection due to turbidity
 ■ : Turbidity or ΔCq positive

- ✓ 48-hour preculture gives detection down to 3 CFU
- ✓ Some species also detected to 0.3 CFU
- ✓ At low microbial levels, ΔCq positive results may be identified earlier than turbid cultures

Equivalence

Microorganism strain	30 CFU		3 CFU		0.3 CFU	
	High Sensitivity Test Method	compendial method	High Sensitivity Test Method	compendial method	High Sensitivity Test Method	compendial method
<i>Aspergillus brasiliensis</i> ATCC 16404	3/3	+	3/3	+	0/3	—
<i>Candida albicans</i> ATCC 10231	3/3	+	3/3	+	2/3	+
<i>Bacillus subtilis</i> subsp. <i>spizizenii</i> ATCC 6633	3/3	+	3/3	+	0/3	—
<i>Clostridium sporogenes</i> ATCC 11437	3/3	+	2/3	—	0/3	—
<i>Pseudomonas aeruginosa</i> ATCC 9027	3/3	+	3/3	+	2/3	—
<i>Staphylococcus aureus</i> ATCC 6538	3/3	+	3/3	+	2/3	+

+ : Turbidity of TGC or TSB — : No turbidity in TGC or TSB

- ✓ Demonstrates the high sensitivity test method offers detection accuracy at least equivalent to the compendial method
- ✓ More accurate than the compendial method for some samples

Range of Species

The acne bacterium is a high contamination risk microorganism and detection by the compendial method is time-consuming.

We attempted to detect acne bacteria using the high sensitivity test method with a 48-hour and 72-hour preculture.

	48h						72h					
	29 CFU		5 CFU		0.3 CFU		29 CFU		5 CFU		0.3 CFU	
	Turbidity	PCR	Turbidity	PCR	Turbidity	PCR	Turbidity	PCR	Turbidity	PCR	Turbidity	PCR
<i>Cutibacterium acnes</i> ATCC 11827												
Positive	0/3		1/3		0/3		3/3		3/3		0/3	
compendial method							+		+		-	

: Turbidity or ΔCq positive
 : No turbidity or ΔCq negative
 + : Turbidity of TGC or TSB
 - : No turbidity in TGC or TSB

- ✓ Almost no samples detected after 48-hour preculture
- ✓ 72-hour preculture gives detection down to 5 CFU
- ✓ At low microbial levels, ΔCq positive results may be identified earlier than turbid cultures

Range of Species

Microorganism strain	
<i>Stenotrophomonas maltophilia</i>	<i>Kocuria rhizophila</i>
<i>Bacillus licheniformis</i>	<i>Micrococcus luteus</i>
<i>Brevibacterium casei</i>	<i>Staphylococcus epidermidis</i>
<i>Yersinia enterocolitica</i>	<i>Paenibacillus gluconolyticus</i>
<i>Enterococcus hirae</i>	<i>Corynebacterium jeikeium</i>
<i>Pseudomonas fluorescens</i>	<i>Corynebacterium propinquum</i>
<i>Escherichia coli</i>	<i>Corynebacterium striatum</i>
<i>Salmonella Typhimurium</i>	<i>Corynebacterium resistens</i>
<i>Methylobacterium extorquens</i>	<i>Bacteroides vulgatus</i>
<i>Corynebacterium suicordis</i>	<i>Streptococcus pyogenes</i> ATCC 19615
<i>Rhizopus oryzae</i>	<i>Bacteroides fragilis</i> ATCC 25285
<i>Kocuria rosea</i>	

Strains only mentioned in EP 2.6.27

High sensitivity test method verified to detect microorganisms on the left
 Currently in process of gathering more data

Specificity

Test conditions : Aerobic medium / Anaerobic medium + 1.0×10^6 Jurkat cells

Culture time : 48 h

Samples : N = 12

Sample No.	BactFinder™		FungiFinder™	
	Turbidity	PCR (ΔCq)	Turbidity	PCR (ΔCq)
1	—	—	—	—
2	—	—	—	—
3	—	—	—	—
4	—	—	—	—
5	—	—	—	—
6	—	—	—	—
7	—	—	—	—
8	—	—	—	—
9	—	—	—	—
10	—	—	—	—
11	—	—	—	—
12	—	—	—	—
Positive	0/12	0/12	0/12	0/12

— : Turbidity or ΔCq negative
 + : Turbidity or ΔCq positive

No false positives for bacteria or fungi

High Sensitivity Test Method Summary



High Sensitivity Test Method

- ✓ High sensitivity test method that detects living microorganisms from 1 CFU within 48 hours
 - 72 hours required for some microorganisms
- ✓ No background noise and low risk of false positives

Currently in initial phases of validation

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Conclusion

BactFinder™/FungiFinder™

Rapid Test Method

- Determines if microbial nucleic acids are present in around 4 hours

High Sensitivity Test Method

- Detects living microorganisms within 2-3 days from 1 CFU

Use one PCR kit and choose between two test methods according to sterility test objectives and applications