SUMMARY AND EXPLANATION OF THE TEST

The *Wound Panel Assay* is a real-time PCR test intended for the qualitative detection of DNA from the wound swab. To collect the specimen, swab the wound by gently rotating a **sterile calcium alginate or rayon swab** between fingers. Swab the wound from margin to margin in a 10-point zigzag fashion. Use enough pressure to express fluid from within the wound tissue

The assay includes the following bacteria targets:

WD1.1	FAM	Enterococcus faecium
WD1.2	ROX	Staphylococcus aureus
WD1.3	JOE	pseudomonas aeruginosa
WD1.4	Cy5	Enterococcus faecalis
WD2.1	ROX	E coli
WD2.2	JOE	citrobacter freundii
WD2.3	Cy5	Streptococcus agalactiae
WD2.4	FAM	Staphylococcus epidermidis (sodA)
WD3.1	ROX	Morganella morganii
WD3.2	FAM	Acinetobacter baumannii
WD3.3	Cy5	Candida albicans
WD3.4	JOE	Candida Auris
WD4.1	JOE	Aerogenes
WD4.2	ROX	Klebsiella pneumoniae
WD4.3	CY5	Streptococcus pyogenes
WD4.4	FAM	RNase P
WD5.1	ROX	Proteus mirabilis
WD5.2	Cy5	Bacteroides
WD5.3	FAM	Enterobacter cloacae
WD5.4	JOE	Klebsiella oxytoca
WD6.1	Cy5	Oxa48
WD6.2	FAM	MecA/MecC
WD6.3	ROX	VanA/Van B
WD6.4	JOE	KPC
WD7.1	JOE	VIM
WD7.2	FAM	BlaSHV
WD7.3	ROX	tetM
WD7.4	Cy5	NDM
WD8.1	JOE	Candida Krusei
WD8.2	FAM	Candida parapsilosis
WD8.3	Cy5	Candia tropicalis
WD8.4	ROX	Candia glabrata

REAQENTS AND MATERIALS PROVIDED

Item	Component	Quantity		Volume /T
		100 Tests / Box	500 Tests / Box	
1	PCR Mix 1 PCR Mix 2 PCR Mix 3 PCR Mix 4 PCR Mix 5 PCR Mix6 PCR Mix7 PCR Mix8	1.6 mL/ tube × 1	1.6 mL/ tube × 5	15 μL
2	Positive Control	150 μL/ tube × 1	500 μL/ tube × 1	5 μL
4	INegative Control	150 μL/ tube × 1	500 μL/ tube × 1	5 μL

MATERIALS MAY REQUIRED BUT NOT PROVIDED

1. Real-Time PCR Thermocycler compatible with JOE (***VIC or HEX is equivalent to JOE***), FAM and Cy5 channels.
2. Specimen Collection Containers
3. DNA extraction reagent
4. PCR tubes compatible with Thermocycler.
5. 1.5 mL centrifuge tubes (RNase-Free)
6. Vortex
7. High-speed centrifuge
8. Micropipette (0.5-2 μL, 1-10 μL)
9. Pipette tips with filters
10. Personal protective material

WARNING AND PRECAUTIONS

1. For research use only. Not for use in diagnostic procedures.
2. Check the expiration date.
3. Follow Good Laboratory Practices: wear appropriate protective clothing and use disposal gloves and protective eyewear. Do not eat, drink, or smoke in designated work areas. Wash hands thoroughly after handling specimens and kit reagents.
4. The testing workflow must be one-directional to minimize contamination risks (allocate segregated areas for each step): it should started from DNA Purification Area, then move to the Reaction Setup Area, followed by Amplification and Detection Area. Do not bring samples, equipment, and reagents to the area in which the previous step was performed and always change gloves when changing areas.
5. Regular decontamination of commonly used equipment is recommended, especially for micropipettes and work surfaces.
6. Specimens must be treated as potentially infectious sources as well as all reagents and materials that have been exposed to the samples and handled in the same manner as an infectious agent. Take appropriate precautions during specimen collection, storage, handling, and disposal in accordance with the guidance from country or region authorities.
7. To ensure optimal performance of the test, always follow appropriate procedures for specimen collection, transport, storage, and processing. Improper procedures may lead to false negative results.
8. Nucleic acids must first be extracted from specimens using a DNA Extraction Kit prior testing. Do not perform a qPCR Assay directly using specimens without extraction.
9. Appropriate precautions should be exercised to monitor contamination and preserve the purity of kit components and reactions. Avoid microbial and nuclease (RNase/DNase) contamination of specimens and kit components. Avoid the spread of aerosols when handling or uncapping specimens.
10. To minimize cross-contamination, open only one tube at a time in the process.
11. Always change pipette tips between liquid transfers. To minimize cross contamination, it's recommended to use aerosol-barrier pipette tips.
12. Always use disposable gloves and regularly check that they are not contaminated with sample materials. Discard gloves if they become contaminated.

TRANSPORT AND STORAGE INSTRUCTIONS

Store the kit frozen at -20±5°C and avoid repeated freeze-thaw cycles (less than 5 frozen-thaw times allowed). The product is valid for 12 months, and the expiry date of the product is shown on the label.

SPECIMEN TYPE and PRESERVATION

Specimen type: Swap, sterile gauze and saline wash.

Specimen preservation: process specimens for DNA extraction and nucleic acid detection as soon as possible. Specimens can be stored at 4°C for 24 hours, or frozen at -20°C for longer storage, if not processed immediately. Avoid repeated freeze-thaw cycles during transport and storage of the specimens.

ASSAY PCR REACTION SETUP (at reagent preparation area)

A. Reagent Preparation

Add 15 μL of each PCR mix into each PCR reaction tube or well.

B. Addition of Bacteria DNA

Add 5 μL of bacteria DNA extracts of the specimen or positive/negative control to each PCR tube according to the reaction preparation table below for testing. Then immediately cap the tube tightly, briefly spin the mixture and move it to the amplification area for PCR detection.

PCR Reaction Preparation:

Component	Volume per test
PCR Mix	15 μL
Negative Control / Positive Control / or RNA of specimen to be tested	5 μL
Total Reaction Volume	20 μL

C. PCR amplification (at Amplification Area, use Applied Biosystems 7500 Real-Time PCR System as an example)

1. Place the complete PCR reaction tubes into the fluorescent quantitative PCR analyzer and label positive control, negative control and testing specimen on the Thermocycler software.
2. Enter the PCR reaction volume: 20 μL.
3. Select fluorescence detection channels: JOE, FAM, Rox and Cy5 channels.
4. Select PCR cycle parameter setting following the instructions below,

save the file and start the PCR run.

5. Analyze the results according to your thermocycler manufacturer's instructions. If the positive and negative control samples do not meet their respective requirements, the PCR run must be repeated.

Note: The positive and negative control can be used directly without purification.

Cycles	Step	Time	Temperature
1	Contamination removal	2 minutes	37 ºC
1	Initial denaturation	2 minutes	95 ºC
40	Denaturation	5 seconds	95 ºC
40	Annealing/Extension	20 seconds	60 ºC

Multiplex qPCR setup 1

Channel	Fungus	Results Interpretation
FAM	Enterococcus faecium	
Rox	Staphylococcus aureus	
JOE	Pseudomonas aeruginosa	
Cy5	Enterococcus faecalis	

Multiplex qPCR setup 2

Channel	Bacteria	Results Interpretation
ROX	E coli	
JOE	Citrobacter freundii	
Cy5	Candida albicans	
FAM	Staphylococcus epidermidis (sodA)	

Multiplex qPCR setup 3

Channel	Bacteria	Results Interpretation
ROX	Morganella morganii	
FAM	Acinetobacter baumannii	
Cy5	Streptococcus agalactiae	
JOE	Candida Auris	

Multiplex qPCR setup 4

Channel	Fungus	Results Interpretation
JOE	Aerogenes	
Rox	Klebsiella pneumoniae	
CY5	Streptococcus pyogenes	
FAM	RNase P	

Multiplex qPCR setup 5

Channel	Fungus	Results Interpretation
ROX	Proteus mirabilis	
Cy5	Bacteriodes	
FAM	Enterobacter cloacae	
JOE	Klebsiella oxytoca	

Multiplex qPCR setup 6

Channel	ABR	Results Interpretation
Cy5	OXA 48	
FAM	MecA/MeC	
ROX	Van A Van B	
JOE	KPC	

Multiplex qPCR setup 7

Channel	ABR	Results Interpretation
JOE	VIM	
FAM	blaSHV	
ROX	tetM	
Cy5	NDM	

Multiplex qPCR setup 8

Channel	Bacteria	Results Interpretation
JOE	Candida krusei	
FAM	Candida parapsilosis	
Cy5	Candida tropicalis	
ROX	Candida glabrata	

COMPATIBILITY OF REAL-TIME PCR THERMOCYCLERS

Manufacturer	Model
Applied Biosystems	Quantstudio 5, 6,7 and 12K
BioRad	BioRad CFX, ATILA

Symbols used

Symbol	Meaning
	Manufacturer
	Authorized representative in the European Community
	Catalogue number
	Batch code
	The number of test
	Caution
	Use by date
	Consult instruction for use
	Date of manufacture
	Storage temperature limit

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Intended Use:
Available for **research use only (RUO)**. Not for use in diagnostic procedures.