

Enterococcus QuickFISH® BC

Enterococcus faecalis/Selected Other Enterococci Culture Identification Kit



25



QFENTBC1-25

Intended Use

Enterococcus QuickFISH BC is a multicolor, qualitative nucleic acid hybridization assay intended for the identification of *Enterococcus faecalis* and/or the detection of selected other enterococci on smears prepared from positive blood cultures containing gram-positive cocci in pairs and chains observed on Gram stain.

Sub-culturing of positive blood cultures is necessary to recover organisms for susceptibility testing and/or differentiation of mixed growth.

Enterococcus QuickFISH BC is indicated as an aid in the diagnosis of bacteremia caused by enterococci.

IVD For *in vitro* diagnostic use.

Summary and Explanation

In recent years, enterococci have emerged as important causes of nosocomial and community infections.

Identification of enterococci in blood cultures is routinely based on presumptive identification as gram-positive cocci in pairs and chains (GPCPC) followed by final identification after subculture and biochemical analysis (1).

Enterococcus QuickFISH BC is a multicolor fluorescence *in situ* hybridization (FISH) method using PNA probes hybridizing to *E. faecalis*-specific ribosomal RNA sequences and to ribosomal RNA sequences of selected other *Enterococcus* species.

The test provides rapid identification of *E. faecalis* and/or selected other enterococci, on smears made from positive blood cultures.

Principle of the Procedure

A mixture of a fluorescein-labeled *E. faecalis*-specific PNA probe and a Tamra-labeled PNA probe specific for selected other enterococci is added to a smear prepared from a positive blood culture.

Hybridization is performed at 55 ± 1 °C for 15 min. and the smear is examined by fluorescence microscopy.

Reagents

Enterococcus QuickFISH BC is comprised of the following kit components:

Enterococcus PNA Blue

Enterococcus PNA Blue

0.85 mL PNA probes in hybridization solution. Contains 15% formamide.

Enterococcus PNA Yellow

Enterococcus PNA Yellow

0.85 mL PNA probes in hybridization solution. Contains 15% formamide.

Precautions

IVD For *in vitro* diagnostic use.

Caution: Federal Law restricts this device to sale by or on the order of a licensed practitioner.

For professional use only, by personnel trained in laboratory techniques and experienced in fluorescence microscopy.

Safety Precautions

<i>Enterococcus</i> PNA Blue		May cause harm to the unborn child. Harmful to aquatic life with long lasting effects. Avoid exposure - obtain special instructions before use. Safety Data Sheet is available upon request.
<i>Enterococcus</i> PNA Yellow	Danger Contains 15% Formamide	
QuickFix-1	Contains 24% ethanol	Harmful to aquatic life with long lasting effects. Safety Data Sheet is available upon request. Available in the QuickFISH Fixation Kit.
QuickFix-2	 Danger Contains 97% methanol	Highly flammable liquid and vapor. Toxic if swallowed. Tonic in contact with skin. Toxic if inhaled. Causes damage to the central nervous system. Safety Data Sheet is available upon request. Available in the QuickFISH Fixation Kit.

Establish precautions against microbiological hazards.

Do not eat, drink, smoke, apply cosmetics, store or prepare foods within the designated work area.

Dispose of reagents in accordance with federal, state, and local regulations.

Technical Precautions

Reagents must not be used after the expiration dates printed on the labels.

Reagents are provided at fixed concentrations. Assay performance may be affected if the reagents are modified in any way or are not stored under the recommended conditions as detailed in "Storage of Kit Components".

Avoid microbial contamination of reagents.

Avoid any cross-contamination of samples and reagents, as this may give rise to erroneous results.

Do not allow dropper bottle tip to touch the smear as this may cause cross contamination of material between slides, or cause contamination of the reagent.

Be sure to use a new pipette tip and inoculating needle for mixing with each sample.

Do not use microscope filters other than AdvanDx Microscope Filters listed in the section for **Materials Required and Available from AdvanDx**.

Do not use microscope slides other than QuickFISH Slides (CS012).

It is important that the AdvanDx SlideStation 10 is level and equilibrated to 55 ± 1 °C prior to the test procedure.

It is important that the microscope is functioning properly. Make sure that the microscope bulb is correctly adjusted and has not aged beyond its specified lifetime.

Storage and Preparation of Kit Components

To ensure optimal kit performance, it is important that kit components are stored according to the following instructions:

Store kit components at 2-8 °C. Store bottles upright and tighten caps after use. Reagents are supplied ready for use.

QuickFISH slides are provided in individually sealed pouches with nitrogen and a desiccant. Store slides at 2-8 °C. Slides must be used immediately after breaking pouch seal. Do not use slides after the expiration date.

Specimen Collection and Preparation

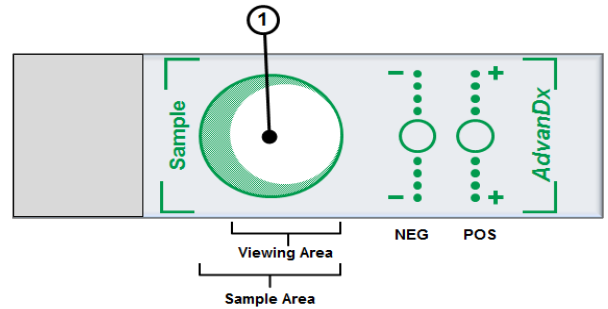
Preparation of *Enterococcus* QuickFISH Smears

The *Enterococcus* QuickFISH BC test should be run only on positive blood cultures which have been Gram stained and found to contain gram-positive cocci in pairs and chains.

Enterococcus QuickFISH BC is not compatible with blood culture media containing charcoal or VersaTREK REDOX 2 blood culture bottles.

- Follow the blood culture system manufacturer's instructions to properly mix the blood culture bottle before smear preparation.
- Place slide on SlideStation at 55 ± 1 °C. When running multiple samples, ensure slides do not come in contact with each other to avoid contamination.
- Do not allow dropper bottle tip to touch the smear as this may cause cross contamination of material between slides, or cause contamination of the reagent.
- Add 1 or more drops of blood culture sample into a secondary vessel (e.g., microcentrifuge tube).
 - For samples containing resin beads (BD BACTEC Plus bottles) – Add 10 or more drops of sample to an AdvanDx Filter Vial. Do not exceed fill line. Insert the filter plunger into the vial and push all the way down to remove the resin beads.
 - Remove cap of AdvanDx Filter Vial to access sample for smear preparation
- Ensure the blood culture sample is well mixed. Using the AdvanDx 10 µL Pipette, transfer 10 µL of sample (if given time to visibly settle, ensure sample is mixed) to the center of the sample area of a QuickFISH slide. Refer to reference ① in the QuickFISH Slide Diagram #1.
- Immediately place one drop of QuickFix-1 onto the sample and spread evenly throughout sample area with a plastic inoculating needle. Avoid air bubbles.
- Allow the smear to dry (1-3 minutes). Smear must be visibly dry.
- Add two drops of QuickFix-2 to the center of the sample area. Refer to reference ① in the QuickFISH Slide Diagram #1.
- Allow the smear to dry (~1 minute). Smear must be visibly dry.
- Fixed QuickFISH smears may be left on the slide warmer at 55 ± 1 °C for up to 5 minutes. Prepared smears which are not used within 5 minutes can be kept at room temperature for 1 hour prior to testing or may be stored at 2-8°C for up to 1 day before testing.

QuickFISH Slide (Diagram #1)



Test Procedure

Material Provided

Enterococcus QuickFISH BC QFENTBC1-25

Each kit contains sufficient material for 25 tests. Reagents are supplied ready for use. The expiration date of the kit is as indicated on the outer box label.

Material Required and Available from AdvanDx.

Large Coverslips	50 x 24 mm No. 1 glass coverslips.	AC027
AdvanDx Microscope Filter	Dual Band Filter for use with high pressure mercury vapor arc lamp light sources or equivalent	AC007
AdvanDx Metal Halide Filter	Dual Band Filter for use with modified mercury vapor arc lamps (metal halide)	AC033
AdvanDx SlideStation-10	Slide warmer (55 ± 1 °C)	AC028
QuickFISH Coverslip Mixing Station		AC030
Holds up to 3 coverslips for mixing <i>Enterococcus</i> PNA Yellow & Blue		
AdvanDx 10 µL Pipette	10 µL fixed volume pipette	AC029
QuickFISH Slide	QuickFISH slide with controls	*CS012
QuickFix-1	Primary fixation solution	*CP0169
QuickFix-2	Secondary fixation solution	*CP0170
AdvanDx Filter Vials	Sample filtration device	AC008

* QuickFISH Slide, QuickFix-1, and QuickFix-2 are available in the QuickFISH Fixation Kit.

Material Required but Not Provided

- Fluorescence microscope equipped with a 60x or 100x oil objective and a high pressure mercury vapor arc lamp, modified mercury vapor arc lamp (metal halide) or light source with equivalent spectral output.
- Immersion oil. Must comply with the microscope objective and be non-fluorescent.
- Pipette tips.
- Plastic inoculating needles.

Assay Procedure:

- QuickFISH smears should be tested immediately following fixation; however, if smears were stored at 2-8 °C or room temperature they must be placed on the slide warmer for approximately 5 minutes at 55 ± 1 °C before adding the hybridization reagents.
- It is important that the AdvanDx SlideStation-10 is level and equilibrated to 55 ± 1 °C prior to the test procedure.
- Use the digital display and the surface thermometer (provided) to verify the SlideStation-10 temperature is 55 ± 1 °C.

Hybridization

- Place a coverslip into one of the *QuickFISH* Coverslip Mixing Station slots. Refer to Diagram #2.
- Invert and hold each bottle and allow a drop to form in the dropper tip before squeezing the bottle to avoid formation of foam in the hybridization mixture.
- Add one drop of *Enterococcus* PNA Blue to the center of the coverslip. Note: the ovoid cutout of the *QuickFISH* Mixing Station slot denotes the center of the coverslip. Place one drop of *Enterococcus* PNA Yellow directly on top of the first drop. Avoid air bubbles. Refer to Diagram #2.
- Thoroughly mix PNA Blue and PNA Yellow together using a plastic inoculating needle until they produce a uniform green color, or no identifiable blue or yellow color remains. Spread lengthwise in order to fill the ovoid template. Refer to Diagram #3.

Diagram # 2

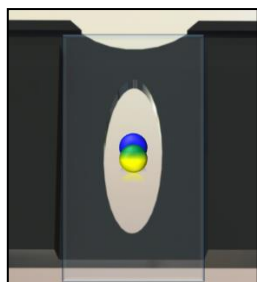
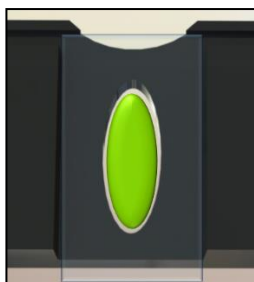


Diagram # 3



- Flip coverslip and apply to slide aligning the edges with the printed border markers on the slide. The coverslip must be placed within the markers. If the coverslip is placed on the white frosted area, the assay may fail due to insufficient flow of reagents.
- Incubate for 15 - 20 min. at 55 ± 1 °C.
- Note: Avoid cross contamination of bottles. Replace dropper caps on appropriate bottles.
- Examine slides as described below.

Do not expose the slides to direct sunlight or strong light sources as this may lead to fluorescence bleaching.

Quality Control

Quality control for fluorescent testing should be performed each time testing is performed.

Control material should be tested in accordance with guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

Use *QuickFISH* Slides with controls (CS012).

QuickFISH slides with controls are provided in individually sealed pouches with nitrogen and a desiccant. Store slides at 2-8 °C. Slides must be used immediately after breaking pouch seal. Do not use slides after expiration date.

The Positive Control will display multiple fluorescent green and red cocci in pairs and chains, the Negative Control will not contain fluorescent red or green cells. Positive (POS, +) and Negative (NEG, -) control wells contain representative organisms for all AdvanDx *QuickFISH* BC kits. Control organisms for other kits may be weakly visible (non-fluorescent) in both the Positive and Negative Control wells.

Cell morphology may vary between samples and controls due to natural variations.

If the Positive and Negative Controls do not perform in accordance with the Interpretation of Results below, results are invalid and patient results should not be reported.

Locating Controls:

Align the center of the microscope objective with the dots of the POS (+) well on the *QuickFISH* Slide (See Diagram #1). Move the slide stage forward or backward until the green outline of the well appears in the field of view. Use the fine focus knob to focus on the green well outline (this is the correct focal plane for reading the slide). Move the objective into the central region of the POS Control to view. To view the NEG

Control, move the objective laterally into the center of the NEG well. Continue moving laterally to find the viewing area of the sample well.

Procedural Notes

Major Blood Culture Systems and Bottle Type Compatibility:

The *QuickFISH* platform is compatible with commercially available continuously monitoring blood culture systems and bottle types except bottle types supplemented with charcoal and the VersaTREK Redox-2 anaerobic bottle. The bottle types tested were:

BacT/Alert (SA, SN)

BACTEC (Lytic 10 anaerobic, Aerobic plus, Anaerobic plus, Peds Plus, Standard 10 aerobic, Standard anaerobic)

VersaTREK (REDOX 1 aerobic)

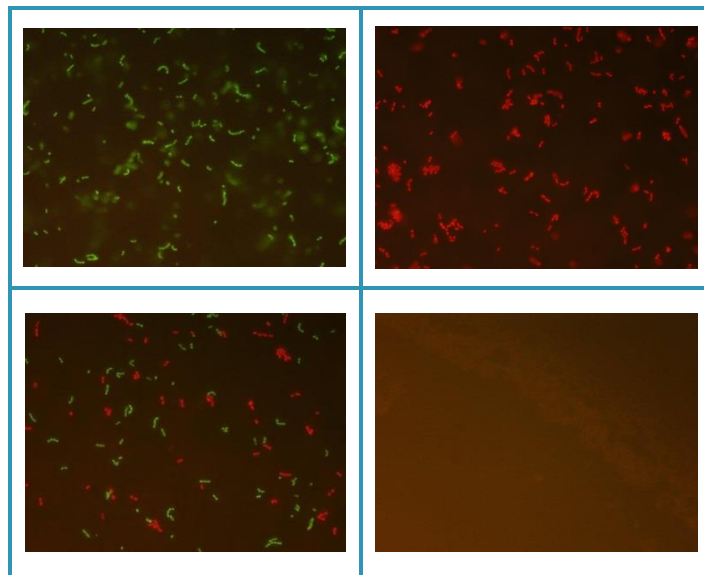
Temperature Control:

It is important that the AdvanDx SlideStation-10 is level and equilibrated to 55 ± 1 °C prior to the test procedure.

Interpretation of Results

Read slides within 2 hours after hybridization.

Examine slides using a fluorescence microscope equipped with a 60X or 100X objective. View the sample in the viewing area within the sample area. The smear background may appear reddish in color. *Enterococcus faecalis* is identified as multiple bright green fluorescent cocci in multiple fields of view, whereas selected other enterococci identified by this assay are seen as multiple bright red fluorescent cocci in multiple fields of view. Non-enterococci and species of enterococci not identified by this assay appear non-fluorescent. Floating organisms or debris should not be interpreted or confused with positive organisms.



Representative examples of green-positive *E. faecalis* (top-left), red-positive *E. faecium* (top-right), mixture of green-positive *E. faecalis* and *E. faecium* (bottom-left) and negative (bottom-right) test results.

Note: In the analytic studies, weak green signals were seen with *Enterobacter cloacae*, *Proteus mirabilis*, *Granulicatella elegans*, *Serratia marcescens*, and 2 strains of *Streptococcus anginosus* (a known limitation) but did not fit the criteria of bright fluorescent cells in multiple fields of view as required for a True Positive signal.

Troubleshooting

False Positive and/or Negative Control and Sample test results may occur if AdvanDx Microscope Filters are not used, or by contamination of the specimens.

False Negative Control or Sample test results may occur if AdvanDx *QuickFISH* Slides (CS012) are not used or if the temperature is not accurately controlled during hybridization.

Please refer to the Precautions and Limitations sections in this product insert or contact AdvanDx.

The lid on the SlideStation is not required to be in place for the kit to perform properly.

The assay may be sensitive to small changes in drop volumes of *Enterococcus* PNA Blue and *Enterococcus* PNA Yellow. If foam is dispensed from bottles, DO NOT USE, discard the coverslip and prepare a new one using fresh hybridization reagents.

Limitations

- The “selected” other enterococci referred to in the Intended Use Statement, refers to *E. faecium* (which was represented in the clinical studies) and 13 other *Enterococcus* species which were tested in analytic studies and/or in limited numbers in the clinical studies. See the Clinical Studies and the Analytical Specificity and Sensitivity sections under Performance Characteristics.
- Some *Enterococcus* species are not detected by either PNA probe: *E. asini*, *E. cecorum*, *E. columbae*, *E. dispar*, *E. pallens*, *E. saccharolyticus*, and *E. sulfureus*.
- E. caccae*, *E. haemoperoxidus*, *E. moraviensis* and some strains of *Streptococcus anginosus* are identified as *E. faecalis* due to sequence similarities.
- Enterococcus QuickFISH BC* is not compatible with blood culture media containing charcoal or VersaTREK REDOX 2 blood culture bottles.
- Clinical studies were conducted using the BACTEC Plus aerobic, BACTEC Plus Anaerobic, BACTEC Lytic/10 Anaerobic, BACTEC Peds Plus and BacT/ALERT SA and SN blood culture bottles. The performance of *Enterococcus QuickFISH BC* with other blood culture bottle types has not been established.
- BACTEC Plus Anaerobic bottles and BACTEC Peds Plus were not extensively evaluated during the clinical investigation, and therefore the performance has not been adequately established.
- The performance of VersaTREK REDOX 1, BACTEC (Anaerobic Plus, Standard 10 Aerobic, Standard Anaerobic/F) blood culture bottles was evaluated in an internal compatibility study only. Therefore, the clinical performance is unknown.
- False Positive green autofluorescence may occur if a standard FITC filter is used instead of the AdvanDx Microscope Filters.
- False Negative results may infrequently occur due to mixed growth or due to error in assay technique.
- The type and condition of the instrumentation used will influence the visual appearance of the image obtained. The fluorescence may vary due to the type of microscope employed, the light source, and the level of rRNA in the cells. Each laboratory should establish its own criteria for reading the results using appropriate controls.
- Isolation on solid media is needed to differentiate mixed growth with other organisms and to identify positive blood cultures yielding a negative result.

The product has not been validated with specimens other than blood cultures.

Expected Results

The *E. faecalis* and selected other enterococci positive result rates from gram-positive cocci in pairs and chains positive blood culture bottles from the clinical studies ranged from 21% to 38% and 9% to 21%, respectively. Non-enterococci species were identified in 49% to 59% of the samples. The study population of gram-positive cocci in pairs and chains-positive blood culture bottles was derived from 5 health care centers in the United States and included 244 blood cultures from 244 patients. Rates presented are a percentage of the number of each target species identified in each blood culture by routine methods as a percent of the total number of all species identified in the studies (refer to the Performance Characteristics section). Actual user rates may vary depending on institution and patient population (2).

Performance Characteristics

The performance of *Enterococcus QuickFISH BC* versus routine laboratory methods has been assessed in five clinical laboratory studies.

A total of 244 routine GPCPC positive blood culture bottles from 244 patients were included in the studies. The studies showed 100% (70/70) positive percent agreement for *E. faecalis* and 97.5% (39/40) positive percent agreement for selected other enterococci. The negative percent agreement was 100% (135/135) from positive blood culture bottles containing GPCPC.

Clinical Studies

		Routine Identification		
		<i>E. faecalis</i>	<i>E. faecium</i> and other enterococci ¹	Other
<i>Enterococcus QuickFISH BC</i>	<i>E. faecalis</i>	70	0	0
	Selected other enterococci	0	39	0
	Negative	0	1 ²	135
Total		Positive Percent Agreement 100% (70/70) ³ 95% CI (94.8-100)	Positive Percent Agreement 97.5% (39/40) ³ 95% CI (87.1-99.6)	Negative Percent Agreement 100% (135/135) 95% CI (97.2-100)

¹In addition to 36 *E. faecium*, 3 *E. gallinarum* and 1 *E. raffinosus* were identified in the clinical studies.

²One false negative sample (tested 1 hour and 15 minutes from the time of Gram stain) was a mixed culture comprised of *E. faecium*, MRSA and *K. pneumoniae*. Repeat testing one week later was weak red-positive.

³Includes 1 mixed culture comprised of *E. faecalis*, *E. gallinarum* and *S. marcescens*.

In the clinical studies, bottles were stored at room temperature after Gram stain and before *QuickFISH* testing. The time between Gram stain and preparation of the *Enterococcus QuickFISH BC* slide varied from less than two hours to greater than 48 hours. There was only one test discrepancy (1/244) in the study. This sample was from a mixed culture and was tested within two hours of Gram stain.

Limit of Detection

The detection limit for *E. faecalis* and selected other enterococci were both determined to be approximately 10⁵ colony-forming units per mL by serial dilutions of positive cultures. This is consistent with the analytical sensitivity of slide-based staining techniques.

Analytical Specificity and Sensitivity

Enterococcus QuickFISH BC was tested on 6 clinical laboratory and 124 reference strains including 16 *Enterococcus faecalis* strains and 32 strains of other *Enterococcus*. All 16 *Enterococcus faecalis* strains tested Green-positive and 22 out of 32 other enterococci were Red-positive. Seven *Enterococcus* species were False Negative. Three *Enterococcus* species gave False Positive green results. See tables below:

Enterococcus QuickFISH BC was also tested on 76 strains of other bacteria as well as 6 yeast strains. All 82 of these organisms displayed the expected negative test results with the exception of *Granulicatella adiacens* which gave a fluorescent orange signal. Weak green signals were seen in *Enterobacter cloacae*, *Proteus mirabilis*, *Granulicatella elegans*, *Serratia marcescens*, and 2 strains of *Streptococcus anginosus* but did not fit the criteria of bright fluorescent cells in multiple fields of view as required for a true positive signal.

The following 22 *Enterococcus* strains representing 14 species were tested in the analytic studies and produced positive red results.

Species	Strain ID	Result
<i>Enterococcus avium</i>	ATCC 49463	Red
<i>Enterococcus casseliflavus</i>	ATCC 25788	Red
<i>Enterococcus durans</i>	ATCC 6056	Red
<i>Enterococcus faecium</i>	ATCC 27270	Red
<i>Enterococcus faecium</i>	ATCC 35667	Red
<i>Enterococcus faecium</i>	ATCC 51559	Red
<i>Enterococcus faecium</i>	ATCC 19434	Red
<i>Enterococcus faecium</i>	ATCC 49224	Red
<i>Enterococcus faecium</i>	BAA 472	Red
<i>Enterococcus faecium</i>	ATCC 51858	Red
<i>Enterococcus faecium</i>	ATCC 6569	Red
<i>Enterococcus flavescens</i>	ATCC 49996	Red
<i>Enterococcus gallinarum</i>	ATCC 49573	Red
<i>Enterococcus gilvus</i>	ATCC BAA-350	Red
<i>Enterococcus hirae</i>	ATCC 8043	Red
<i>Enterococcus hirae</i>	ATCC 49135	Red

Species	Strain ID	Result
<i>Enterococcus malodoratus</i>	ATCC 43197	Red
<i>Enterococcus mundtii</i>	ATCC 43187	Red
<i>Enterococcus phoeniculicola</i>	ATCC BAA-412	Red
<i>Enterococcus raffinosus</i>	ATCC 49464	Red
<i>Enterococcus ratti</i>	ATCC 700914	Red
<i>Enterococcus villorum</i>	ATCC 700913	Red

The following 3 *Enterococcus* species were tested in the analytic studies and produced False Green results and are stated in the Limitations section.

Species	Strain ID	Result
<i>Enterococcus caccae</i>	ATCC BAA-1240	Green
<i>Enterococcus haemoperoxidus</i>	ATCC BAA-382	Green
<i>Enterococcus moraviensis</i>	ATCC BAA-383	Green

The following 7 *Enterococcus* species were tested in the analytic studies and produced negative results and are stated in the Limitations section

Species	Strain ID	Result
<i>Enterococcus asini</i>	ATCC 700915	Negative
<i>Enterococcus cecorum</i>	ATCC BAA-597	Negative
<i>Enterococcus columbae</i>	ATCC 51263	Negative
<i>Enterococcus dispar</i>	ATCC 51266*	Negative
<i>Enterococcus pallens</i>	ATCC BAA-351	Negative
<i>Enterococcus saccharolyticus</i>	ATCC 43076	Negative
<i>Enterococcus sulfureus</i>	ATCC 49903	Negative

*Strain no longer available from ATCC

Reproducibility

A reproducibility study was performed with *Enterococcus QuickFISH BC* and the results are presented below by site across 3 days and by day across 3 sites.

Summary of Reproducibility Results by Site Across 3 Days

	Site 1	Site 2	Site 3	Total
Positive Agreement Green	45/45	45/45	45/45	100% (135/135)
Positive Agreement Red	45/45	39/45	45/45	95.6% (129/135)
Negative Agreement	36/36	36/36	36/36	100% (108/108)
Total Agreement	100% (126/126)	95.2% (120/126)	100% (126/126)	98.4% (372/378)












Summary of Reproducibility Results by Day Across 3 Sites

	Day 1	Day 2	Day 3	Total
Positive Agreement Green	45/45	45/45	45/45	100% (135/135)
Positive Agreement Red	42/45	42/45	45/45	95.6% (129/135)
Negative Agreement	36/36	36/36	36/36	100% (108/108)
Total Agreement	97.6% (123/126)	97.6% (123/126)	100% (126/126)	98.4% (372/378)

Bibliography

1. **Baron, E. J.** 1998. Processing and interpretation of blood cultures, chap. 2.3. In: H.D. Isenberg (Ed.) Essential procedures for clinical microbiology, ASM Press, Washington DC.
2. **Karlowsky JA, Jones ME, Draghi DC, Thornsberry C, Sahm DF, Volturo GA.** 2004. Prevalence and antimicrobial susceptibilities of bacteria isolated from blood cultures of hospitalized patients in the United States in 2002. *Ann Clin Micro and Antibiol.* 3(7).

Definitions

	Product code/catalog number		Batch code
	Consult the instructions for use		Storage temperature limitations
	Contains sufficient for <n> tests		Health Hazard
	Manufacturer		Skull and Crossbones
	Authorized Representative		Flame
	Use by		

Technical Advice and Customer Service

For all inquiries, please contact OpGen or your local distributor.



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The product must not be used for Slide-Based human Cytochemistry, ISH-based Cancer Cytogenetics and Flow Cytometry.

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Purchase of this kit licenses its use under patent numbers: US 5,985,563; US 5,888,733; US 6,664,045; US 6,395,474; US 6,357,163; US 5,539,082; US 7,223,833; US 6,361,942; US 7,816,50; EP 862,650; EP 804,456

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