510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

K150031

B. Purpose for Submission:

To obtain a substantial equivalence determination for the hemoFISH Masterpanel

C. Measurand:

rRNA from the following organisms on slides prepared from positive blood cultures that demonstrate the presence of organisms by Gram stain:

Gram Positive Organisms	Gram Negative Organisms
Staphylococcus species	Enterobacteriaceae
Staphylococcus aureus	*Escherichia coli*
Streptococcus species	*Klebsiella pneumoniae*

D. Type of Test:

A qualitative fluorescence *in situ* hybridization (FISH) assay using fluorescently labeled Beacon probes

E. Applicant:

miacom diagnostics GmbH

F. Proprietary and Established Names:

hemoFISH Masterpanel

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.2660: Microorganism differentiation and identification device

2. Classification:

   Class I

3. Product code:

   JSS: Kit, Identification, Enterobacteriaceae

   MCS: DNA-Probe, *Staphylococcus aureus*
   MDK: DNA-Probe, Reagents, *Streptococcal*

4. Panel:

   83: Microbiology

H. Intended Use:

1. Intended use(s):

   hemoFISH Masterpanel is a qualitative nucleic acid hybridization assay performed directly on blood culture samples identified as positive by a continuous monitoring blood culture system that demonstrates the presence of organisms as determined by Gram stain and is intended for the identification of the following species / genera / families:

Gram Positive Organisms	Gram Negative Organisms
Staphylococcus spp.	Enterobacteriaceae
Staphylococcus aureus	*Escherichia coli*
Streptococcus spp.	*Klebsiella pneumoniae*

   The hemoFISH Masterpanel is indicated as an aid in the diagnosis of specific agents of bacteremia and results should be used in conjunction with other clinical and laboratory findings. Positive hemoFISH Masterpanel results do not rule out co-infection with organisms not included in the hemoFISH Masterpanel. The hemoFISH Masterpanel is not intended to monitor treatment for bacteremia.

   Subculturing of positive blood cultures is necessary to recover organisms for susceptibility testing and epidemiological typing, to identify organisms in the blood culture that are not identified by the hemoFISH Masterpanel, and for species determination of *Staphylococcus* spp., *Streptococcus* spp., and Enterobacteriaceae that are not identified by the hemoFISH Masterpanel.

2. Indication(s) for use:

   hemoFISH Masterpanel is indicated as an aid in the diagnosis of bacteremia.

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

Microscope
Fluorescence microscope equipped with a 100x oil immersion objective and 10x eyepiece (1000x magnification is required).

- Filterset A for ATTO550-label: absorption maximum 554nm/emission maximum 576nm
- Filterset B for FAM-label: absorption maximum 494nm/emission maximum 520nm
- Illumination device comparable to 100W HBO lamp

Hotplate
Hotplate that can maintain an operating temperature of 52±1° C

I. Device Description:

The hemoFISH Masterpanel is a Fluorescence *In Situ* Hybridization (FISH)-based assay that uses DNA Beacon probes to detect specific bacterial rRNA sequences in intact cells that are fixed on microscope slides prepared from positive blood cultures. The assay identifies 3 groups each of Gram-positive and Gram-negative bacteria to family, genus or species level within 30 minutes.

J. Substantial Equivalence Information: (Provided by sponsor)

1. Predicate device name(s):

   AdvanDx, Inc., Gram-Negative QuickFISH

2. Predicate 510(k) number(s):

   K123418

3. Comparison with predicate:

Similarities		
Item	**Device K150031**	**Predicate K123418**
Intended Use	hemoFISH Masterpanel is a qualitative nucleic acid hybridization assay performed directly on blood culture samples identified as positive by a continuous monitoring blood culture system that demonstrates	Gram-Negative QuickFISH BC is a multicolor, qualitative nucleic acid hybridization assay intended for the identification of *Escherichia coli* and/or *Pseudomonas aeruginosa* and/or *Klebsiella pneumoniae* on smears from

Similarities		
Item	**Device K150031**	**Predicate K123418**
	the presence of organisms as determined by Gram stain and is intended for the identification of the following species / genera / families: **Gram Positive Organisms** *Staphylococcus* spp. *Staphylococcus aureus* *Streptococcus* spp. **Gram Negative Organisms** Enterobacteriaceae *Escherichia coli* *Klebsiella pneumoniae* The hemoFISH Masterpanel is indicated as an aid in the diagnosis of specific agents of bacteremia and results should be used in conjunction with other clinical and laboratory findings. Positive hemoFISH Masterpanel results do not rule out co-infection with organisms not included in the hemoFISH Masterpanel. The hemoFISH Masterpanel is not intended to monitor treatment for bacteremia. Subculturing of positive blood cultures is necessary to recover organisms for susceptibility testing and epidemiological typing, to identify organisms in the blood culture that are not identified by the hemoFISH Masterpanel, and for species	positive blood cultures containing gram-negative bacilli observed on Gram stain. Subculturing of positive blood cultures is necessary to recover organisms for susceptibility testing and/or differentiation of mixed growth. The Gram-Negative QuickFISH BC assay is indicated for use as an aid in the diagnosis of *E. coli*, and/or *K. pneumoniae*, and/or *P. aeruginosa* bacteremia.

Similarities		
Item	**Device K150031**	**Predicate K123418**
	determination of *Staphylococcus* spp., *Streptococcus* spp., and Enterobacteriaceae that are not identified by the hemoFISH Masterpanel.	
Technology	Fluorescence *In Situ* Hybridization (rRNA)	Same
Sample	Positive blood cultures from standard automated blood culture device, confirmed by Gram stain	Same
Interpretation	Visual by qualitative fluorescence microscopy	Same

Differences		
Item	**Device K150031**	**Predicate K123418**
Organisms Detected	Identifies the following to species/genus/family level: *Staphylococcus* species *Staphylococcus aureus* *Streptococcus* species Enterobacteriaceae *Escherichia coli* *Klebsiella pneumoniae*	Identifies the following to species level: *Escherichia coli* *Pseudomonas aeruginosa* *Klebsiella pneumoniae*
Probe type	Self-reporting DNA probes labeled with green (FAM) or red (ATTO550) fluorophores	Self-reporting Peptide Nucleic Acid (PNA) probes labeled with green (FAM) or red (tetramethylrhodamine) fluorophores
Probe Hybridization Mixtures	Four (4) i. Eubacteria ii. *Staphylococcus* spp.- Enterobacteriaceae iii. *S. aureus*-*E. coli* iv. *Streptococcus* spp.- *K. pneumoniae*	One (1) *E. coli* *P. aeruginosa* *K. pneumoniae*
Positive Control	Integral Eubacterial probe on each slide	Pre-fixed QuickFISH Control Slides

K. Standard/Guidance Document Referenced (if applicable):

Not applicable

L. Test Principle:

The hemoFISH Masterpanel is a Fluorescence *In Situ* Hybridization (FISH)-based assay that uses DNA Beacon probes to detect specific bacterial rRNA sequences on microscope slides prepared from positive blood cultures. The assay identifies the targeted Gram-positive and Gram-negative panel members to family, genus or species level within 30 minutes. Other "off-panel" species are also detected using a universal Eubacterial probe.

The method is based on classical fluorescence *in situ* hybridization (FISH) using fluorescently labeled molecular beacon probes. The molecular beacons consist of a DNA sequence folded into a hairpin-like structure that is linked to a fluorophore on the 5´end and to a quencher on the 3´end. A part of the DNA sequence on both ends forms a stem region through complementary base-pairing. This structure keeps the fluorophore and quencher in close proximity, suppressing the generation of fluorescent signal. The DNA sequence between the stem regions of the probe is complementary to an rRNA region that is specific to the targeted group of organisms. Because each bacterial cell includes more than 10,000 copies of rRNA, no amplification step is necessary. The DNA sequences of the beacons that are specific to the target region form a loop-like structure and are able to bind to their rRNA targets after passing through the bacterial cell wall and membrane. In a bound state, the fluorophore of the beacon is spatially separated from its quencher and fluoresces when excited with an appropriate light source.

Every rRNA copy with a bound beacon contributes to the overall fluorescent signal and cells containing hybridized probe can be detected under a fluorescence microscope. Unbound beacons do not fluoresce, so that no washing step is required.

To conduct the assay, solutions of fluorescently labeled molecular DNA beacons are dispensed onto fixed, permeablized cells that are prepared from a positive blood culture. Different wells of the microscope slide are used for each probe combination. Hybridization is carried out at 52±1°C for 10 minutes. Slides are immersed in a bath containing a stop solution and rinsed in alcohol to end the reaction. Mounting Medium is then added to each well to prevent fading of fluorescence. After applying a cover slip, the slide is ready for examination by fluorescence microscopy.

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

   a. Precision/Reproducibility:

   The reproducibility of the hemoFISH Masterpanel was assessed by testing slides prepared from positive blood cultures at 3 sites over a period 10 days. Two sites (A

and B) used cultures grown in VersaTREK REDOX 1 medium and one site (C) used BD BACTEC Plus Aerobic/F Culture Vials. Cultures that were positive for 9 different bacterial species were included in the study. All slides were prepared at "bottle ring", dried and stored until testing. Three replicate slides for each strain were tested on each day. There were two operators per site and each operator tested 45 slides (total = 90 slides per strain). The results are summarized in **Tables 1** and **2**. The study showed that the results of the hemoFISH Masterpanel were reproducible, with no important differences in performance between sites, type of culture medium, operators or days of testing.

Table 1. Summary of results from the Reproducibility Study stratified by site and overall

Species	Expected hemoFISH Result	Number Correct/Number Tested (%)			
		Site A	Site B	Site C	Total
S. aureus	*S. aureus*	30/30 (100)	30/30 (100)	30/30 (100)	**90/90 (100)**
S. pneumoniae	Streptococci	30/30 (100)	30/30 (100)	30/30 (100)	**90/90 (100)**
S. agalactiae	Streptococci	30/30 (100)	29/30 [2] (96.7)	30/30 (100)	**89/90 (98.9)**
E. faecalis	Other [1]	30/30 (100)	30/30 (100)	30/30 (100)	**90/90 (100)**
E. coli	*E. coli*	29/29 [3] (100)	30/30 (100)	30/30 (100)	**89/89 (100)**
K. pneumoniae	*K. pneumoniae*	29/30 [4] (96.7)	30/30 (100)	30/30 (100)	**89/90 (98.9)**
P. aeruginosa	Other	30/30 (100)	30/30 (100)	30/30 (100)	**90/90 (100)**
S. maltophilia	Other	30/30 (100)	29/29 [2] (100)	30/30 (100)	**89/89 (100)**
A. baumannii	Other	30/30 (100)	30/30 (100)	30/30 (100)	**90/90 (100)**
	Total	**268/269 (99.6)**	**268/269 (99.6)**	**270/270 (100)**	**806/808 (99.8)**

[1] Other: Other (not Staphylocci, Streptococci or Enterobacteriaceae)
[2] 1 sample reported as Other (not Staphylocci, Streptococci or Enterobacteriaceae) due to failure to obtain signal from *Streptococcus* Beacon
[3] 1 sample reported as Fail due to green fluorescence in Field 1 (Negative Control) was excluded from calculation of percent agreement
[4] 1 sample reported positive for Other Enterobacteriaceae (not *E. coli* or *K. pneumoniae*)

Table 2. Summary of results from the Reproducibility Study by hemoFISH probe

Beacon Probe	Positive Agreement (%)
E. coli	89/89 (100)
K. pneumoniae	89/90 (98.9)
S. aureus	90/90 (100)
Enterobacteriaceae	178/179 (99.4)
Staphylococcus	90/90 (100)
Streptococcus	179/180 (99.4)
Eubacteria [1]	359/359 (100)

[1] Samples that are positive by the Eubacterial probe and not by any other probe(s) in the hemoFISH panel are reported as "Other (not Staphylococci, Streptococci or Enterobacteriaceae)"

b. Linearity/assay reportable range:

Not applicable.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Assay Controls
One well of each hemoFISH Masterpanel microscope slide is used for quality control. This well is used for hybridization of an ATTO550-labeled Eubacterial probe. In order for the results from hybridization of the hemoFISH probes in the other wells of the slide to be interpreted, cells in the control well with a morphology that is consistent with that observed under Gram stain should fluoresce red (Positive Control) and there should be no green fluorescent cells (Negative Control). In addition, in order to interpret positive results from *E. coli*, *K. pneumoniae* and *S. aureus* species-specific probes, the appropriate family or genus probe must also produce a positive result.

Table 3 summarizes the observed failure modes associated with "Invalid" or "Fail" test results during the Clinical Study described in **Section M 3**.

Table 3. Summary of failure modes associated with Invalid/Fail test results

Failure Mode	Assay Result	Site A	Site B	Site C	In-house
Positive Control	Fail	6	2	4	5
Negative Control	Fail	0	5	0	0
Beacon Probe [1]	Invalid	4	0	2	0
Sub-Total	Fail/Invalid	10	7	6	5
Total Samples Tested [2]		590	347	90	69
Percent Invalid/Fail		**1.7**	**2.0**	**6.7**	**7.2**
		2.6			

[1] Genus/family probe failed when the corresponding species probe produced a positive result
[2] Sum of all samples tested in the study prior to exclusion of duplicates, etc. (refer to **Section M 3(a)**, **Table 16**)

Additional Positive and Negative External Controls comprised of cultured isolates may be tested as appropriate. Instructions for preparation of External Positive and Negative Controls are included in the Package Insert. The recommended bacterial strains for use as External Controls and expected results are shown in **Table 4**.

Table 4. Recommended strains for use as External Controls and expected results

Species	ATCC No.	Expected Result by hemoFISH Probe						
		EUB	STA	ENT	SA	EC	STR	KP
S. aureus	9144	+	+	-	+	-	-	-
E. coli	14948	+	-	+	-	+	-	-
P. aeruginosa	10145	+	-	-	-	-	-	-
S. agalactiae	12403	+	-	-	-	-	+	-
K. pneumoniae	13883	+	-	+	-	-	-	+

EUB: Eubacteria; STA: *Staphylococcus*; ENT: Enterobacteriaceae; SA: *S. aureus*; EC: *E. coli*; STR: *Streptococcus*; KP: *K. pneumoniae*

During the clinical study, a total of 244 External Controls were tested (88 *E. coli*, 75 *S. aureus* and 81 *P. aeruginosa*), all (100%) of which produced the expected results.

Note: No External Controls were tested during the Clinical Study to monitor the performance of the *Streptococcus* or *K. pneumoniae* probes. However, the Eubacterial Control was included on every slide and served to monitor hybridization conditions and clinical performance for the detection of these analytes was determined to be acceptable.

Specimen Stability

Studies were performed to evaluate the ability of the hemoFISH Masterpanel to produce the expected results with blood culture bottles that were held under different conditions after turning positive. Testing was conducted with BD BACTEC Plus Aerobic/F blood culture bottles inoculated with different species of bacteria representing each of the targeted analytes. After turning positive, the bottles were either tested with the hemoFISH Masterpanel immediately or held for 8 or 12 hours at either room temperature or 35°C prior to testing. The appropriate results were obtained for each organism at each time point. The instructions in the Package Insert therefore indicate that testing with the hemoFISH Masterpanel may be conducted up to 12 hours after bottle ring.

Fixed Slide Stability

The stability of dried microscope slides after heat fixation and prior to permeablization and hybridization was established by storing fixed slides in the dark for up to 14 days at ~25°C prior to testing with the hemoFISH Masterpanel. The slides were prepared from BD BACTEC Plus Aerobic/F Culture Vials using the strains listed in **Table 5**. All results were as expected. The Package Insert indicates that fixed slides may be stored for up to 14 days in the dark prior to testing.

Post Hybridization Slide Stability

The stability of microscope slides after hybridization and mounting was determined by reading slides prepared from positive blood cultures of different organisms after storage for up to 24 hours in the dark at ~25°C. The bacterial strains and culture media used in the study are shown in **Table 5**. All results at each time point (0, 2, 4 and 24 hours) were as expected. The Package Insert indicates that slides may be stored for up to 12 hours in the dark after hybridization prior to reading.

Table 5. Bacterial strains and culture media used in determination of slide stability

Species	ATTC No.	Fixed Slide Stability	Post Hybridization Stability		Expected hemoFISH Result
		BD BACTEC Plus Aerobic/F	VersaTREK REDOX 1 [1]	BacT/ALERT PF Pediatric FAN	
S. agalactiae	12403	X	X	X	STR
S. aureus	9144	X	X	X	SA
A. baumannii	19606	X	X	X	Other
E. coli	14948	X	X	X	EC
E. faecalis	29212	X	X	X	Other
E. faecium	35667	X	X	X	Other
K. pneumoniae	13883	X	X	X	KP
P. aeruginosa	10145	X	X	X	Other
P. mirabilis	29245	X	X	X	ENT
S. maltophilia	51331	X	X	X	Other
S. pneumoniae	6303	X	X	X	STR
S. aureus / *K. pneumoniae*	9144 13883	Not Done	X	X	SA/KP
P. aeruginosa / *S. maltophila*	10145 51331	Not Done	X	X	Other

X: Condition tested; STR: Streptococci; SA. *S. aureus*; EC: *E. coli*; KP: *K. pneumoniae*; ENT: Other Enterobacteriaceae (not *E. coli* or *K. pneumoniae*); Other: Other (not Staphylococci, Streptococci or Enterobacteriaceae)

[1] VersaTREK REDOX 1 was the most common culture medium in the Clinical Study used to evaluate the performance of the hemoFISH Masterpanel (refer to **Section M 3(a)**, **Table 28**)

d. Detection limit:

The limit of detection (LOD) of each probe in the hemoFISH Masterpanel was estimated by testing serial dilutions of different bacterial species prepared from positive BD BACTEC Plus Aerobic/F blood culture vials. The LOD was confirmed by testing 20 replicates at the estimated LOD target level. Target levels were determined by colony counts. The LOD was considered confirmed if ≥19/20 replicates produced a positive result for the hemoFISH probe(s) under evaluation (**Table 6**).

Table 6. Analytical sensitivity of the hemoFISH Masterpanel

Species	ATCC Strain	hemoFISH Probe		LOD (CFU/mL)	hemoFISH % Positive (n = 20)
		Family/Genus	Species		
E. coli	14948	ENT	EC	10^5	100
K. pneumoniae	13883	ENT	KP	10^5	100
P. mirabilis	29245	ENT	--	10^5	95
S. agalactiae	27956	STR	--	10^5	100
S. aureus	9144	STA	SA	10^5	100
S. epidermidis	14990	STA	--	10^5	100
S. pneumoniae	6303	STR	--	10^5	100

EC: *E. coli*; KP: *K. pneumoniae*; SA: *S. aureus*; ENT: Enterobacteriaceae; STA: *Staphylococcus*; STR: *Streptococcus*

e. Analytical specificity:

The analytical specificity of the hemoFISH Masterpanel was evaluated using suspensions of different organisms prepared from colonies grown on solid media that were suspended in hemoFISH Clinical Sample Buffer. The results are summarized in **Tables 7** to **11**. The observed false positive and false negative results are noted in the Limitations Section of the Package Insert.

Table 7. Specificity of the hemoFISH Masterpanel for *E. coli*, *K. pneumoniae* and *S. aureus*

hemoFISH Masterpanel Results for *E. coli*, *K. pneumoniae* and *S. aureus*				
Strains Correctly Identified to Species Level		**False Positive for *E. coli*, *K. pneumoniae* or *S. aureus*** [1]		
Species	**Strain**	**Species**	**Strain**	**hemoFISH Result**
Escherichia coli	ATCC 14948	*Escherichia albertii*	DSM 17582	*E. coli*
Escherichia coli	ATCC 35218	*Escherichia fergusonii*	ATCC 35469	*E. coli*
Klebsiella pneumoniae	ATCC 700603	*Klebsiella variicola*	ATCC BAA-830	*K. pneumoniae*
Klebsiella pneumoniae subsp. *ozaenae*	ATCC 11296	*Macrococcus caseolyticus*	ATCC 35662	*S. aureus*
Klebsiella pneumoniae subsp. *pneumoniae*	ATCC 13883	*Shigella boydii*	ATCC 9207	*E. coli*
Klebsiella pneumoniae subsp. *rhinoscleromatis*	ATCC 13884	*Shigella flexneri*	ATCC 9199	*E. coli*
Staphylococcus aureus	ATCC 11822	*Staphylococcus schleiferi* subsp. *coagulans*	ATCC 49545	*S. aureus*
Staphylococcus aureus	ATCC 17091	*Staphylococcus schleiferi* subsp. *schleiferi*	ATCC 43808	*S. aureus*
Staphylococcus aureus	ATCC 9144			
Staphylococcus aureus	ATCC 43300			
Staphylococcus aureus	ATCC 29213			

[1] As noted in the Limitations Section of the Package Insert

Table 8. Specificity of the hemoFISH Masterpanel for "Other Staphylococci (not *S. aureus*)"

Species Correctly Identified as "Other Staphylococci (not *S. aureus*)" by hemoFISH Masterpanel			
Species	**Strain**	**Species**	**Strain**
Staphylococcus arlettae	ATCC 43957	*Staphylococcus lutrae*	DSM 10244
Staphylococcus capitis	ATCC 35661	*Staphylococcus microti*	DSM 22147
Staphylococcus chromogenes	ATCC 43764	*Staphylococcus pasteuri*	ATCC 51129
Staphylococcus condimenti	DSM 11674	*Staphylococcus piscifermentans*	ATCC 51136
Staphylococcus epidermidis	ATCC 14990	*Staphylococcus pseudointermedius*	ATCC 49444
Staphylococcus equorum subsp. *equorum*	ATCC 43958	*Staphylococcus pulvereri (vitulinus)*	ATCC 51145
Staphylococcus gallinarum	ATCC 700401	*Staphylococcus rostri*	DSM 21968
Staphylococcus heamolyticus	ATCC 29970	*Staphylococcus saprophyticus*	ATCC 15305
Staphylococcus hominis subsp. *hominis*	ATCC 27844	*Staphylococcus sciuri*	ATCC 29061
Staphylococcus intermedius	ATCC 29663	*Staphylococcus simiae*	DSM 17636
Staphylococcus kloosii	ATCC 43959	*Staphylococcus succinus* subsp. *succinus*	ATCC 700337
Staphylococcus lentus	ATCC 29070	*Staphylococcus xylosus*	ATCC 35033
Staphylococcus lugdunensis	ATCC 49576		
Species False-Negative for "Other Staphylococci (not *S. aureus*)" by hemoFISH Masterpanel [1]			
Species	**Strain**	**hemoFISH Result**	
Staphylococcus schleiferi subsp. *coagulans*	ATCC 49545	*S. aureus*	
Staphylococcus schleiferi subsp. *schleiferi*	ATCC 43808	*S. aureus*	

[1] As noted in the Limitations Section of the Package Insert

Table 9. Specificity of hemoFISH Masterpanel for "Other Enterobacteriaceae (not *E. coli* or *K. pneumoniae*)"

Species Correctly Identified as "Other Enterobacteriaceae (not *E. coli* or *K. pneumoniae*)" by hemoFISH Masterpanel			
Species	**Strain**	**Species**	**Strain**
Buttiauxella gaviniae	ATCC 51604	*Proteus hauseri*	ATCC 13315
Cedeceae davisiae	ATCC 33431	*Proteus mirabilis*	ATCC 29245
Citrobacter freundii	ATCC 43864	*Proteus penneri*	ATCC 33519
Citrobacter koseri	ATCC 27156	*Proteus vulgaris*	ATCC 8427
Cronobacter (Enterobacter) sakazakii	ATCC 29544	*Proteus vulgaris*	ATCC 6896
Cronobacter muytjensi	ATCC 51329	*Providencia (Proteus) acalifaciens*	ATCC 9886
Edwardsiella tarda	ATCC 15947	*Providencia (Proteus) rettgeri*	ATCC 9250
Enterobacter aerogenes	ATCC 13048	*Providencia rettgeri*	ATCC 9259
Enterobacter asburiae	ATCC 35953	*Providencia stuartii*	ATCC 33672
Enterobacter cancerogenus	ATCC 33241	*Rahnella aquatilis*	ATCC 33071
Enterobacter cloacae subsp. *cloacae*	ATCC 13047	*Raoultella planticola*	ATCC 33531
Enterobacter cloacae subsp. *dissolvens*	ATCC 23373	*Raoultella terrigena*	ATCC 33257
Enterobacter gergoviae	ATCC 33028	*Salmonella abony*	NCTC 6017
Enterobacter hormaechei	ATCC 700323	*Salmonella bongori*	ATCC 43975
Enterobacter (Pluribacter) pyrinus	ATCC 49851	*Salmonella choleraesuis*	ATCC 7001

Species Correctly Identified as "Other Enterobacteriaceae (not *E. coli* or *K. pneumoniae*)" by hemoFISH Masterpanel			
Species	**Strain**	**Species**	**Strain**
Escherichia (Shimwellia) blattae	DSM 4481/ATCC 29907	*Salmonella enterica-heidelberg*	ATCC 8326
Escherichia hermanii	ATCC 33650	*Salmonella enterica-paratyphi*	ATCC 9150
Escherichia vulneris	ATCC 33821	*Salmonella typhimurium*	ATCC 25241
Ewingella americana	ATCC 33852	*Salmonella vellore*	ATCC 15611
Hafnia alvei	ATCC 51815	*Serratia fonticola*	ATCC 29844
Klebsiella oxytoca	ATCC 8724	*Serratia liquefaciens*	ATCC 27592
Kluyvera ascorbata	ATCC 33433	*Serratia marcescens*	ATCC 43862
Leclercia adecarboxylata	ATCC 23216	*Serratia plymuthica*	ATCC 183
Morganella morganii	ATCC 25829	*Serratia proteamaculans*	ATCC 19323
Pantoea (Enterobacter) agglomerans	ATCC 27155	*Yokenella regensburgei*	ATCC 35313
Plesiomonas shigelloides	ATCC 14029		
Species False-Negative for "Other Enterobacteriaceae (not *E. coli* or *K. pneumoniae*)" by hemoFISH Masterpanel [1]			
Species	**Strain**	**hemoFISH Result**	
Tatumella ptyseos	ATCC 33301	Other (not Staphylococci, Streptococci or Enterobacteriaceae)	

[1] As noted in the Limitations Section of the Package Insert

Table 10. Specificity of the hemoFISH Masterpanel for "Streptococci"

Species Correctly Identified as "Streptococci" by hemoFISH Masterpanel			
Species	**Strain**	**Species**	**Strain**
Streptococcus agalactiae	ATCC 12403	*Streptococcus mitis*	ATCC 13770
Streptococcus agalactiae	ATCC 27956	*Streptococcus mutans*	ATCC 25175
Streptococcus anginosus	ATCC 33397	*Streptococcus oralis*	ATCC 20627
Streptococcus australis	ATCC 700641	*Streptococcus parasanguinis*	ATCC 15909
Streptococcus bovis	ATCC 33317	*Streptococcus pasteurianus*	ATCC 49133
Streptococcus constellatus	ATCC 27823	*Streptococcus pneumoniae*	ATCC 6305
Streptococcus dysgalactiae	ATCC 12394	*Streptococcus pneumoniae*	ATCC 10015
Streptococcus equi	ATCC 9528	*Streptococcus porcinus*	ATCC 43138
Streptococcus equinis	ATCC 9812	*Streptococcus pseudopneumoniae*	ATCC BAA-960
Streptococcus gallolyticus	ATCC 9809	*Streptococcus pyogenes*	ATCC 12344
Streptococcus gordonii	ATCC 10558	*Streptococcus sanguinis*	ATCC 10556
Streptococcus infantarius	ATCC BAA-102	*Streptococcus suis*	ATCC 43765
Streptococcus infantis	DSM 12492	*Streptococcus urinalis*	DSM 16830
Streptococcus mitis	ATCC 6249	*Streptococcus vestibularis*	ATCC 49124
Species False-Positive for "Streptococci" by hemoFISH Masterpanel [1]			
Species	**Strain**		
Lactococcus lactis	ATCC 11454		
Leuconostoc carnosum	DSM 5576 / ATCC 49367		
Leuconostoc mesenteroides	DSM 20343 / ATCC 8293		

[1] As noted in the Limitations Section of the Package Insert

Table 11. Specificity of the hemoFISH Masterpanel for "Other (not Staphylococci, Streptococci or Enterobacteriaceae)"

Species Correctly Identified as "Other (not Staphylococci, Streptococci or Enterobacteriaceae)" by hemoFISH Masterpanel			
Species	**Strain**	**Species**	**Strain**
Acinetobacter baumannii	NCIMB 12457	*Enterococcus hirae*	ATCC 10541
Acinetobacter baumannii	BAA-747	*Fusobacterium nucleatum*	ATCC 25586
Acinetobacter baylyi	DSM 14961	*Gemella morbillorum*	DSM 20772 / ATCC 27824
Acinetobacter bereziniae	ATCC 17924	*Granulicatella adiacens*	ATCC 43205
Acinetobacter calcoaceticus	ATCC 23055	*Haemophilus influenzae*	ATCC 43065
Acinetobacter guillouiae	ATCC 11171	*Haemophilus parahaemolyticus*	ATCC 10014
Acinetobacter haemolyticus	ATCC 19002	*Haemophilus parainfluenzae*	DSM 8978 / ATCC 33392
Acinetobacter johnsonii	ATCC 17909	*Kingella kingae*	ATCC 7536
Acinetobacter lwofii	ATCC 15309	*Lactobacillus crispatus*	DSM 20584 / ATCC 33820
Acinetobacter radioresistens	ATCC 43998	*Legionella pneumophila*	ATCC 33152
Acinetobacter schindleri	DSM 16038	*Listeria welshimeri*	ATCC 35897
Acinetobacter ursingii	DSM 16037	*Listeria innocua*	ATCC 33090
Aeromonas caviae	ATCC 15468	*Listeria monocytogenes*	ATCC 13932
Aeromonas hydrophila	ATCC 7966	*Micrococcus luteus*	ATCC 4698
Aggregatibacter aphrophilus	ATCC 7901	*Moraxella catarrhalis*	ATCC 25238
Alcaligenes faecalis	ATCC 8750	*Neisseria elongata*	DSM 17712 / ATCC 25295
Arcanobacterium (Trueperella) bernardiae	DSM 9152	*Neisseria lactamica*	ATCC 23970
Bacillus atropheus	ATCC 9372	*Neisseria meningitidis*	ATCC 13077
Bacillus cereus	ATCC 10872	*Neisseria sicca*	ATCC 9913
Bacillus licheniformis	ATCC 12759	*Pandorea apista*	ATCC 16535
Bacillus pumilus	ATCC 14884	*Pandorea pnomenusa*	ATCC 16536
Bacillus spizizenii	ATCC 6633	*Parabacteroides distasonis*	ATCC-BAA 1295
Bacillus subtilis	ATCC 11774	*Pasteurella aerogenes*	ATCC 27883
Bacillus subtilis	ATCC 6051	*Pediococcus pentosaceus*	ATCC 33316
Bacteroides uniformis	ATCC 8492	*Peptostreptococcus anaerobius*	ATCC 27337

Species Correctly Identified as "Other (not Staphylococci, Streptococci or Enterobacteriaceae)" by hemoFISH Masterpanel			
Species	**Strain**	**Species**	**Strain**
Burkholderia multivorans	ATCC 13243	*Planococcus citreus*	DSM 20549 / ATCC 25536
Burkholderia vietnamiensis	ATCC 11319	*Prevotella melaninogenica*	DSM 7089 / ATCC 25845
Buttiauxella gaviniae	ATCC 51604	*Propionibacterium acnes*	ATCC 11827
Cardiobacterium hominis	DSM 8339 / ATCC 15826	*Pseudomonas aeruginosa*	ATCC 9027
Cellumonas turbata (Oerskovia turbata)	DSM 20577 / ATCC 25835	*Pseudomonas aeruginosa*	ATCC 15442
Clostridium difficile	ATCC 9698	*Pseudomonas aeruginosa*	ATCC 10145
Comamonas testosteroni	DSM 50244 / ATCC 11996	*Pseudomonas fluorescens*	ATCC 13525
Eikenella corrodens	ATCC 23834	*Pseudomonas fulva*	DSM 17717
Elizabethkingia meningoseptica	ATCC 13253	*Pseudomonas luteola*	DSM 6975 / ATCC 43273
Enterococcus faecium	ATCC 700221	*Pseudomonas mendocina*	DSM 50017 / ATCC 25411
Enterococcus faecium	ATCC 35667	*Pseudomonas mucidolens*	DSM 19186
Enterococcus faecalis	ATCC 19433	*Pseudomonas nitroreducens*	DSM 14399 / ATCC 33634
Enterococcus faecalis	ATCC 29212	*Pseudomonas pertucinogena*	DSM 18268 / ATCC 190
Enterococcus casseliflavis	ATCC 25788	*Pseudomonas putida*	ATCC 49128
Enterococcus mundtii	DSM 4338 / ATCC 43186	*Rhodococcus equi*	ATCC 6939
Enterococcus pseudoavium	DSM 5632 / ATCC 49372	*Rothia dentocariosa*	DSM 43762 / ATCC 17931
Enterococcus saccharolyticus	ATCC 43076	*Stenotrophomonas maltophila*	ATCC 49130
Enterococcus avium	ATCC 14025	*Uruburuella suis*	ATCC 17474
Enterococcus durans	ATCC 6056	*Vibrio parahaemolyticus*	ATCC 17802
Species False-Positive for "Other (not Staphylococci, Streptococci or Enterobacteriaceae)" by hemoFISH Masterpanel [1]			
Species	**Strain**		
Tatumella ptyseos [2]	ATCC 33301		

[1] As noted in the Limitations Section of the Package Insert
[2] False Negative for "Other Enterobacteriaceae" (refer to **Table 9)**

Laboratory testing using suspecnsions of different organsims was supported by *in silico* analysis performed on bacterial 16S and 23rRNA sequences available through databases maintained at the National Center for Biotechnology Information (NCBI, US National Library of Medicine) and ARB/Silva (Max Plank Institute, Bremen).

Organisms whose potential reactivity with the hemoFISH Masterpanel was evaluated

exclusively through *in silico* analysis are listed in **Table 12**, which shows the expected (taxonomic) and predicted (*in silico*) hemoFISH result for each species/strain. The potential for false results with *Shigella* spp. is noted in the device labeling (refer also to **Table 13**).

Table 12. Summary of *in silico* analysis of hemoFISH Masterpanel inclusivity/exclusivity

Species/Strain	hemoFISH Result		Species/Strain	hemoFISH Result	
	Expected	*In silico* Predicted		Expected	*In silico* Predicted
Abiotrophia defectiva	Other	Other	*Escherichia coli* O157-H7	*E.coli*	*E.coli*
Acinetobacter tartarogenes	Other	Other	*Escherichia coli* O26-H11	*E.coli*	*E.coli*
Actinomyces odontolyticus	Other	Other	*Escherichia coli* O55-H7	*E.coli*	*E.coli*
Aeromonas taiwanensis	Other	Other	*Fusobacterium necrophorum*	Other	Other
Aeromonas veronii	Other	Other	*Haemophilus parasuis*	Other	Other
Arcanobacterium haemolyticum	Other	Other	*Haemophilus somnus*	Other	Other
Bacteroides ovatus	Other	Other	*Herbaspirillum huttiense*	Other	Other
Bacteroides thetaiotamicron	Other	Other	*Kluyvera (Enterobacter) intermedius*	ENT	ENT
Brevundimonas diminuta	Other	Other	*Kluyvera cryocrescens*	ENT	ENT
Brevundimonas vesicularis	Other	Other	*Kluyvera georgiana*	ENT	ENT
Campylobacter fetus	Other	Other	*Kytococcus sedentarius*	Other	Other
Candida glabrata	Fail	Fail	*Lactobacillus acidophilus*	Other	Other
Candida krusei	Fail	Fail	*Lactobacillus rhamnosus*	Other	Other
Candida parapsilosis	Fail	Fail	*Leminorella grimontii*	ENT	ENT
Candida tropicalis	Fail	Fail	*Leminorella richardii*	ENT	ENT
Capnocytophaga ochracea	Other	Other	*Mycobacterium tuberculosis*	Other	Other
Cellulosimicrobium cellulans	Other	Other	*Mycoplasma hominis*	Other	Other
Citrobacter sedlakii	ENT	ENT	*Mycoplasma pneumoniae*	Other	Other
Corynebacterium bovis	Other	Other	*Neisseria mucosa*	Other	Other
Corynebacterium flavescens	Other	Other	*Neisseria perflava*	Other	Other
Corynebacterium genitalium	Other	Other	*Parabacteroides merdae*	Other	Other
Corynebacterium glutamicum	Other	Other	*Pediococcus acidilactici*	Other	Other
Corynebacterium jeikeium	Other	Other	*Planococcus kocurri*	Other	Other

Species/Strain	hemoFISH Result		Species/Strain	hemoFISH Result	
	Expected	*In silico* Predicted		Expected	*In silico* Predicted
Corynebacterium renale	Other	Other	*Prevotella bivia*	Other	Other
Corynebacterium striatum	Other	Other	*Prevotella buccae*	Other	Other
Corynebacterium urealyticum	Other	Other	*Prevotella denticola*	Other	Other
Cryptococcus neoformans	Fail	Fail	*Pseudomonas chloraphis*	Other	Other
Delftia acidovorans	Other	Other	*Pseudomonas fragi*	Other	Other
Enteric group 137	ENT	ENT	*Pseudomonas oryzihabitans*	Other	Other
Enterobacter amnigenus (Lelliottia amnigena)	ENT	ENT	*Pseudomonas pseudoalcaligenes*	Other	Other
Enterobacter kobei	ENT	ENT	*Pseudomonas stutzeri*	Other	Other
Enterobacter ludwigii	ENT	ENT	*Pseudomonas veronii*	Other	Other
Enterobacter nimipressuralis (Lelliottia nimipressuralis)	ENT	ENT	*Raoultella ornithinolytica*	ENT	ENT
Enterobacter oryzae (Kosakonia oryzae)	ENT	ENT	*Rothia mucilaginosa*	Other	Other
Enterococcus cecorum	Other	Other	*Serratia entomophila*	ENT	ENT
Enterococcus dispar	Other	Other	*Serratia ficaria*	ENT	ENT
Enterococcus flavescens	Other	Other	*Serratia grimesii*	ENT	ENT
Escherichia coli ETEC	*E.coli*	*E.coli*	*Serratia odorifera*	ENT	ENT
Escherichia coli O103-H2	*E.coli*	*E.coli*	*Serratia rubidaea*	ENT	ENT
Escherichia coli O104-H4	*E.coli*	*E.coli*	*Shigella dysenteriae*	ENT	*E. coli*
Escherichia coli O111-H11	*E.coli*	*E.coli*	*Shigella sonnei*	ENT	*E. coli*
Escherichia coli O121-H19	*E.coli*	*E.coli*	*Vagococcus fluvialis*	Other	Other
Escherichia coli O124	*E.coli*	*E.coli*	*Veillonella parvula*	Other	Other

Expected: Expected result based on species taxonomy
In silico predicted: Predicted result based on sequence alignment
ENT: Other Enterobacteriaceae (not *E. coli* or *K. pneumoniae*)
Other: Other (not Staphylococci, Streptococci or Enterobacteriaceae)
Fail: Negative test result with Eubacterial probe

Bacterial species for which false-positive or -negative hemoFISH results were predicted by *in silico* analysis and which also underwent confirmatory laboratory testing are listed in **Table 13**. Those species for which the potential for false results was confirmed are listed in the device labeling.

Table 13. Summary of *in silico*-predicted false-positive and false-negative results for the hemoFISH Masterpanel

Species	Taxonomic Classification	hemoFISH *In silico* Prediction	Functional hemoFISH Result [1]
Tatumella ptyseos	ENT	ENT	Other
Macrococcus caseolyticus	Other	*S. aureus*	*S. aureus*
Lactococcus lactis	Other	STR	STR
Leuconostoc carnosum	Other	STR	STR
Leuconostoc mesenteroides	Other	STR	STR
Escherichia albertii	ENT	*E. coli*	*E. coli*
Escherichia fergusonii	ENT	*E. coli*	*E. coli*
Shigella boydii	ENT	*E. coli*	*E. coli*
Shigella flexneri	ENT	*E. coli*	*E. coli*
S. schleiferi subsp. *coagulans*	STA	*S. aureus*	*S. aureus*
S. schleiferi subsp. *schleiferi*	STA	*S. aureus*	*S. aureus*
S. succinus subsp. *succinus* [2]	STA	*S. aureus*	STA

ENT: Other Enterobacteriaceae (not *E. coli* or *K. pneumoniae*)
Other: Other (not Staphylococci, Streptococci or Enterobacteriaceae)
STA: Other Staphylococci (not *S. aureus*)
STR: Streptococci
[1] Obtained from functional testing
[2] Same mismatches in rRNA target region with the *S. aureus* Beacon probe as *S. schleiferi* subsp. *schleiferi* and subsp. *coagulans* (both hemoFISH false-positive for *S. aureus*)

f. Assay cut-off:

Not applicable.

g. Assay Interference:

A study was conducted to determine the ability of the hemoFISH Masterpanel to report the correct results from mixed cultures of two species that are identified in the same microscope field. Testing was performed with BD BACTEC Plus Aerobic/F culture vials containing whole blood that was inoculated with different ratios of *S. aureus* (10^3-10^5 CFU/mL) and *E. coli* (10^6-10^8 CFU/mL) or *S. pneumoniae* (10^3-10^5 CFU/mL) and *K. pneumoniae* (10^6-10^9 CFU/mL). The blood culture vials were incubated until they turned positive and were then tested with the hemoFISH Masterpanel. All assay results were as expected, indicating that the hemoFISH Masterpanel is capable of correctly identifying mixed cultures of specific organisms that are detected in the same field when the target levels are above the limit of detection of the assay.

Note: Based on the design of the hemoFISH Masterpanel and the performance observed with mixed cultures observed in the Clinical Study (**Section M 3**), the following specific Limitations are included in the device labeling.

- The hemoFISH assay does not differentiate species of *Staphylococcus* other than *Staphylococcus aureus*. Therefore, whether a sample contains one or multiple species of *Staphylococcus* (other than *S. aureus*), the result will be

reported as "Other staphylococci." Samples that are reported as positive for *S. aureus* may also contain other *Staphyloccocus* species. The blood culture bottle should be plated on solid medium to obtain isolated colonies for *Staphyloccus* species identification and/or susceptibility testing.

- The hemoFISH assay does not differentiate species of *Streptococcus*. Therefore, whether a sample contains one or multiple species of *Streptococcus*, the result will be reported as "Streptococci." The blood culture bottle should be plated on solid medium to obtain isolated colonies for *Streptococcus* species identification and/or susceptibility testing.
- The hemoFISH assay does not differentiate species of Enterobacteriaceae other than *E. coli*, and *K. pneumoniae*. Therefore, whether a sample contains one or multiple species of Enterobacteriaceae (other than *E. coli*, or *K. pneumoniae*), the result will be reported as "Other Enterobacteriaceae." Samples that are reported as positive for *E. coli*, or *K. pneumoniae* may also contain other species of Enterobacteriaceae. The blood culture bottle should be plated on solid medium to obtain isolated colonies for species identification and/or susceptibility testing.
- The hemoFISH assay may produce false-negative results with mixed cultures that contain one or more organisms below the limit of detection.
- If a mixed culture is suspected on the basis of Gram stain or if the hemoFISH Masterpanel result is positive for "Other" (not Staphylococci, Streptococci or Enterobacteriaceae), the blood culture bottle should be plated on solid medium to obtain isolated colonies for further identification.

2. Comparison studies:

a. Method comparison with predicate device:

Not applicable.

b. Matrix comparison:

An analytical study was performed to evaluate the compatibility of the hemoFISH Masterpanel with 13 different types of culture media (**Table 14)**. Testing was performed with blood cultures containing representative isolates of each species, genus or family in the hemoFISH Masterpanel. Bottles were inoculated at low levels and incubated at 35°C for 20-23 hours prior to testing depending on the culture medium. The expected results were obtained with each of the culture medium in which growth occurred. No growth was obtained when *E. coli* was inoculated into in VersaTREK REDOX 2 vials, although the ability of the hemoFISH Masterpanel to detect *E. coli* in this culture medium was demonstrated in the Clinical Study (**Section M 3**, **Table 28**).

Table 14. Culture media shown to be compatible with the hemoFISH Masterpanel

Blood Culture System	Culture Medium	
	Aerobic	**Anaerobic**
BD BACTEC	Plus Aerobic/F	Plus Anaerobic/F
	Standard 10 Aerobic/F	Standard Anaerobic/F
	Pediatric PEDS/Plus/F	Lytic/10 Anaerobic/F
VersaTREK	REDOX 1	REDOX 2
BacT/ALERT	SA Standard Aerobic	SN Standard Anaerobic
	FA Aerobic FAN	FN Anaerobic FAN
	PF Pediatric FAN	

An additional analytical study was conducted to verify the tolerance of the hemoFISH Masterpanel to differences in the volume of blood inoculated into the culture medium. Testing was performed with a representative panel of organisms grown in BD BACTEC Plus Aerobic/F and Plus Anaerobic/F culture vials containing volumes of blood ranging from 5 to 10mL per vial. All results were as expected, with no difference in performance according to blood volume.

3. Clinical studies:

a. Clinical Sensitivity:

The performance of the hemoFISH Masterpanel was evaluated in a prospective Clinical Study that was conducted at three testing sites (two in the US and one ex-US). Samples included in the study were residual blood culture vials that were called positive by an automated blood culture system and which were confirmed to contain bacteria by Gram stain.

The prospective study at the clinical sites was supplemented with in-house testing of additional contrived samples prepared by inoculating blood culture vials with whole human blood containing organisms and incubating until "bottle ring." **Table 15** shows the total number of samples included in the study.

Table 15. Sources of positive blood cultures included in determination of performance for the hemoFISH Masterpanel

Source	Total	Contrived	Routine	Culture	Excluded	Included in Performance Determination		
						Sub-total	Mono-microbial	Poly-microbial
Site A	590	0	590	VersaTREK	228	362	344	18
Site B	347	0	347	VersaTREK	115	232	199	33
Site C [1]	90	0	90	BACTEC	20	70	66	4
In-house	69	69	0	BACTEC	8	61	61	0
Total	**1096**	**69**	**1027**		**371**	**725**	**670**	**55**

[1] Ex-US

The reasons for exclusion of samples from the analysis of performance are summarized in **Table 16**.

Table 16. Reasons for exclusion of samples from performance calculations

Excluded Monomicrobial Cultures									
Source	**Duplicate**	**Failure**			**ID**	**Not Tested**	**Cont.**	**Sample Stability**	**Total**
		Negative Control	**Positive Control**	**Genus / Family Probe**					
Site A	89	0	6	1	111	0	0	0	207
Site B	66	4	2	0	1	1	0	23	97
Site C	0	0	4	2	7	0	0	0	13
In-house	2	0	5	0	0	0	1	0	8
Total	**159**	**4**	**17**	**3**	**119**	**1**	**1**	**0**	**325**
Excluded Polymicrobial Cultures									
Source	**Duplicate**	**Failure**			**ID**	**Not Tested**	**Cont.**	**Sample Stability**	**Total**
		Negative Control	**Positive Control**	**Genus / Family Probe**					
Site A	5	0	0	3	13	0	0	0	21
Site B	13	1	0	0	0	0	0	4	18
Site C	0	0	0	0	1	0	0	0	1
In-house	0	0	0	0	0	0	0	0	0
Total	**18**	**1**	**0**	**3**	**14**	**0**	**0**	**4**	**40**

Duplicate: repeat sample from previously enrolled subject (or repeat culture of the same strain for contrived samples)
Negative Control Failure: Green fluorescence in control well
Positive Control Failure: Failure of Eubacterial probe; absence of red fluorescence from control well
Genus/Family Probe Failure: hemoFISH species probe positive but genus/family probe negative
ID: Reference identification not performed or method not FDA-cleared
Not Tested: hemoFISH assay not performed
Cont.: Sample contaminated
Sample Stability: >12 hours between bottle ring and hemoFISH testing (protocol deviation)
Note: An additional 6 samples at Site C were excluded because the result from the reference identification method was not available (5 samples) or subculture yielded no growth (1 sample)

Monomicrobial Cultures

The performance of the hemoFISH Masterpanel with prospectively collected monomicrobial samples, contrived monomicrobial samples and both sample types combined is shown in **Tables 17** to **22.**

Table 17. Performance of the hemoFISH Masterpanel with prospective monomicrobial clinical samples (n = 609)

Identification of *E. coli*				
		Reference		
		Positive	**Negative**	**Total**
hemoFISH Masterpanel	**Positive**	90	1	**91**
	Negative	4	514	**518**
	Total	**94**	**515**	**609**
Sensitivity		90/94 = 95.7% (89.6, 98.3%)		
Specificity		514/515 = 99.8% (98.9, 100%)		
Positive Predictive Value		90/91 = 98.9%		
Negative Predictive Value		514/518 = 99.2%		
Identification of *K. pneumoniae*				
		Reference		
		Positive	**Negative**	**Total**
hemoFISH Masterpanel	**Positive**	36	0	**36**
	Negative	0	573	**573**
	Total	**36**	**573**	**609**
Sensitivity		36/36 = 100% (90.4, 100%)		
Specificity		573/573 = 100% (99.3, 100%)		
Positive Predictive Value		36/36 = 100%		
Negative Predictive Value		573/573 = 100%		
Identification of *S. aureus*				
		Reference		
		Positive	**Negative**	**Total**
hemoFISH Masterpanel	**Positive**	69	2	**71**
	Negative	0	538	**558**
	Total	**69**	**540**	**609**
Sensitivity		69/69 = 100% (94.7, 100%)		
Specificity		538/540 = 99.6% (98.7, 99.9%)		
Positive Predictive Value		69/71 = 97.2%		
Negative Predictive Value		538/538 = 100%		
Identification of Other Enterobacteriaceae (species other than *E. coli* and *K. pneumoniae*)				
		Reference		
		Positive	**Negative**	**Total**
hemoFISH Masterpanel	**Positive**	41	2	**43**
	Negative	0	566	**566**
	Total	**41**	**568**	**609**
Sensitivity		41/41 = 100% (91.4, 100%)		
Specificity		566/568 = 99.6% (98.7, 99.9%)		
Positive Predictive Value		41/43 = 95.3%		
Negative Predictive Value		566/566 = 100%		

Identification of Other Staphylococci (species other than *S. aureus*)				
		Reference		
		Positive	**Negative**	**Total**
hemoFISH Masterpanel	**Positive**	160	1	**161**
	Negative	7	441	**448**
	Total	**167**	**442**	**609**
Sensitivity		160/167 = 95.8% (91.6, 98.0%)		
Specificity		441/442 = 99.8% (98.7, 100%)		
Positive Predictive Value		160/161 = 99.4%		
Negative Predictive Value		441/448 = 98.4%		
Identification of Streptococci				
		Reference		
		Positive	**Negative**	**Total**
hemoFISH Masterpanel	**Positive**	67	2	**69**
	Negative	0	540	**540**
	Total	**67**	**542**	**609**
Sensitivity		67/67 = 100% (94.6, 100%)		
Specificity		540/542 = 99.6% (98.7, 99.9%)		
Positive Predictive Value		67/69 = 97.1%		
Negative Predictive Value		540/540 = 100%		
Identification of Other Species (not Staphylococci, Streptococci or Enterobacteriaceae)				
		Reference		
		Positive	**Negative**	**Total**
hemoFISH Masterpanel	**Positive**	131 [1]	8	**139**
	Negative	4	466	**470**
	Total	**135**	**474**	**609**
Sensitivity		131/135 = 97.0% (92.6, 98.8%)		
Specificity		466/474 = 98.3% (96.7, 99.1%)		
Positive Predictive Value		131/139 = 94.2%		
Negative Predictive Value		466/470 = 99.1%		

[1] Includes 1 sample that was positive by the reference method for yeast. Based on *in silico* analysis, the expected hemoFISH result with cultures that are positive for yeast when no bacteria are present is "Fail" due to the absence of signal with the Eubacterial probe. As noted in the Package Insert, cultures identified as positive for "Other" (not Staphylococci, Streptococci or Enterobacteriaceae) require additional follow-up for definitive identification of the species present.

Table 18. Summary of performance of the hemoFISH Masterpanel with prospective clinical monomicrobial samples (n = 609)

		Reference Method							Total
		EC	**KP**	**SA**	**ENT**	**STA**	**STR**	**Other**	
hemoFISH	**EC**	90 [1]	0	0	0	0	0	1	**91**
	KP	0	36	0	0	0	0	0	**36**
	SA	0	0	69	0	2	0	0	**71**
	ENT	1	0	0	41	0	0	1	**43**
	STA	0	0	0	0	160	0	1	**161**
	STR	1 [1]	0	0	0	0	67	1	**69**
	Other	3	0	0	0	5	0	131 [2]	**139**
	Total	**94**	**36**	**69**	**41**	**167**	**67**	**135**	**609** [1]
Sensitivity		90/94	36/36	69/69	41/41	160/167	67/67	131/135	
		95.7% (89.6, 98.3%)	100% (90.4, 100%)	100% (94.7, 100%)	100% (91.4, 100%)	95.8% (91.6, 98.0%)	100% (94.6, 100%)	97.0% (92.6, 98.8%)	
Specificity		514/515	573/573	538/540	566/568	441/442	540/542	466/474	
		99.8% (98.9, 100%)	100% (99.3, 100%)	99.6% (98.7, 99.9%)	99.6% (98.7, 99.9%)	99.8% (98.7, 100%)	99.6% (98.7, 99.9%)	98.3% (96.7, 99.1%)	
Positive Predictive Value		90/91	36/36	69/71	41/43	160/161	67/69	131/139	
		98.9%	100%	97.2%	95.3%	99.4%	97.1%	94.2%	
Negative Predictive Value		514/518	573/573	538/538	566/566	441/448	540/540	466/470	
		99.2%	100%	100%	100%	98.4%	100%	99.1%	

EC: *E. coli*; KP: *K. pneumoniae*; SA: *S. aureus*; ENT: Other Enterobacteriaceae (not *E. coli* or *K. pneumoniae*); STA: Other Staphylococci (not *S. aureus*); STR: Streptococci; Other (not Staphylococci, Streptococci or Enterobacteriaceae)

[1] 1 sample reference positive for EC; hemoFISH positive for EC and STR

[2] Includes 1 sample that was positive by the reference method for *C. albicans*. Based on *in silico* analysis, the expected hemoFISH result with blood cultures that are positive for *Candida* spp. is "Fail" due to the absence of signal from the Eubacterial probe (refer to **Table 12**)

Table 19. Performance of the hemoFISH Masterpanel with contrived monomicrobial samples (n = 61)

Identification of *E. coli*				
		Reference		
		Positive	**Negative**	**Total**
hemoFISH Masterpanel	**Positive**	2	0	**2**
	Negative	0	59	**59**
	Total	**2**	**59**	**61**
Positive Percent Agreement		2/2 = 100% (34.2, 100%)		
Negative Percent Agreement		59/59 = 100% (93.9, 100%)		
Positive Predictive Value		2/2 = 100%		
Negative Predictive Value		59/59 = 100%		
Identification of *K. pneumoniae*				
		Reference		
		Positive	**Negative**	**Total**
hemoFISH Masterpanel	**Positive**	2	0	**2**
	Negative	0	61	**61**
	Total	**2**	**61**	**63**
Positive Percent Agreement		2/2 = 100% (34.2, 100%)		
Negative Percent Agreement		59/59 = 100% (93.9, 100%)		
Positive Predictive Value		2/2 = 100%		
Negative Predictive Value		59/59 = 100%		
Identification of *S. aureus*				
		Reference		
		Positive	**Negative**	**Total**
hemoFISH Masterpanel	**Positive**	5	0	**5**
	Negative	0	56	**56**
	Total	**5**	**56**	**61**
Positive Percent Agreement		5/5 = 100% (56.6, 100%)		
Negative Percent Agreement		56/56 = 100% (93.6, 100%)		
Positive Predictive Value		5/5 = 100%		
Negative Predictive Value		56/56 = 100%		
Identification of Other Enterobacteriaceae (species other than *E. coli* and *K. pneumoniae*)				
		Reference		
		Positive	**Negative**	**Total**
hemoFISH Masterpanel	**Positive**	21	0	**21**
	Negative	2	38	**40**
	Total	**23**	**38**	**61**
Positive Percent Agreement		21/23 = 91.3% (73.2, 97.6%)		
Negative Percent Agreement		38/38 = 100% (90.8, 100%)		
Positive Predictive Value		21/21 = 100%		
Negative Predictive Value		38/40 = 95.0%		

Identification of Other Staphylococci (species other than *S. aureus*)				
		Reference		
		Positive	Negative	Total
hemoFISH Masterpanel	Positive	7	0	**7**
	Negative	0	54	**54**
	Total	**7**	**54**	**61**
Positive Percent Agreement		7/7 = 100% (64.6, 100%)		
Negative Percent Agreement		54/54 = 100% (93.4, 100%)		
Positive Predictive Value		7/7 = 100%		
Negative Predictive Value		54/54= 100%		
Identification of Streptococci				
		Reference		
		Positive	Negative	Total
hemoFISH Masterpanel	Positive	9	0	**9**
	Negative	0	52	**52**
	Total	**9**	**52**	**61**
Positive Percent Agreement		9/9 = 100% (77.1, 100%)		
Negative Percent Agreement		52/52 = 100% (93.1, 100%)		
Positive Predictive Value		9/9 = 100%		
Negative Predictive Value		52/52 = 100%		
Identification of Other Species (not Staphylococci, Streptococci or Enterobacteriaceae)				
		Reference		
		Positive	Negative	Total
hemoFISH Masterpanel	Positive	13	2	**15**
	Negative	0	46	**48**
	Total	**13**	**48**	**61**
Positive Percent Agreement		13/13 = 100% (77.2, 100%)		
Negative Percent Agreement		46/48 = 95.8% (86.0, 98.9%)		
Positive Predictive Value		13/15 = 86.7%		
Negative Predictive Value		46/46 = 100%		

Table 20. Summary of performance of the hemoFISH Masterpanel with contrived monomicrobial samples (n = 61)

		Reference Method							Total
		EC	**KP**	**SA**	**ENT**	**STA**	**STR**	**Other**	
hemoFISH	**EC**	2	0	0	0	0	0	0	**2**
	KP	0	2	0	0	0	0	0	**2**
	SA	0	0	5	0	0	0	0	**5**
	ENT	0	0	0	21	0	0	0	**21**
	STA	0	0	0	0	7	0	0	**7**
	STR	0	0	0	0	0	9	0	**9**
	Other	0	0	0	2	0	0	13	**15**
	Total	**2**	**2**	**5**	**23**	**7**	**9**	**13**	**61**
Positive Agreement		2/2	2/2	5/5	21/23	7/7	9/9	13/13	
		100% (34.2, 100%)	100% (34.2, 100%)	100% (56.6, 100%)	91.3% (73.2, 97.6%)	100% (64.6, 100%)	100% (77.1, 100%)	100% (77.2, 100%)	
Negative Agreement		59/59	59/59	59/59	38/38	54/54	52/52	46/48	
		100% (93.9, 100%)	100% (93.9, 100%)	100% (93.6, 100%)	100% (90.8, 100%)	100% (93.4, 100%)	100% (93.1, 100%)	95.8% (86.0, 98.9%)	
Positive Predictive Value		2/2	2/2	5/5	21/21	7/7	9/9	13/15	
		100%	100%	100%	100%	100%	100%	86.7%	
Negative Predictive Value		59/59	59/59	56/56	38/40	54/54	52/52	46/46	
		100%	100%	100%	95.0%	100%	100%	100%	

EC: *E. coli*; KP: *K. pneumoniae*; SA: *S. aureus*; ENT: Other Enterobacteriaceae (not *E. coli* or *K. pneumoniae*); STA: Other Staphylococci (not *S. aureus*); STR: Streptococci; Other (not Staphylococci, Streptococci or Enterobacteriaceae)

Table 21. Overall performance of the hemoFISH Masterpanel with prospective and contrived monomicrobial samples combined (n = 670)

Identification of *E. coli*				
		Reference		
		Positive	**Negative**	**Total**
hemoFISH Masterpanel	**Positive**	92	1	**93**
	Negative	4	573	**577**
	Total	**96**	**574**	**670**
Positive Percent Agreement		92/96 = 95.8% (89.8, 98.4%)		
Negative Percent Agreement		573/574 = 99.8% (99.0, 100%)		
Positive Predictive Value		92/93 = 98.9%		
Negative Predictive Value		573/577 = 99.3%		
Identification of *K. pneumoniae*				
		Reference		
		Positive	**Negative**	**Total**
hemoFISH Masterpanel	**Positive**	38	0	**38**
	Negative	0	632	**632**
	Total	**38**	**632**	**670**
Positive Percent Agreement		38/38 = 100% (90.8, 100%)		
Negative Percent Agreement		632/632 = 100% (99.4, 100%)		
Positive Predictive Value		38/38 = 100%		
Negative Predictive Value		632/632 = 100%		
Identification of *S. aureus*				
		Reference		
		Positive	**Negative**	**Total**
hemoFISH Masterpanel	**Positive**	74	2	**76**
	Negative	0	594	**594**
	Total	**74**	**596**	**670**
Positive Percent Agreement		74/74 = 100% (95.1, 100%)		
Negative Percent Agreement		594/596 = 99.7% (98.8, 99.9%)		
Positive Predictive Value		74/76 = 97.4%		
Negative Predictive Value		594/594 = 100%		
Identification of Other Enterobacteriaceae (species other than *E. coli* and *K. pneumoniae*)				
		Reference		
		Positive	**Negative**	**Total**
hemoFISH Masterpanel	**Positive**	62	2	**64**
	Negative	2	604	**606**
	Total	**64**	**606**	**670**
Positive Percent Agreement		62/64 = 96.9% (89.3, 99.1%)		
Negative Percent Agreement		604/606 = 99.7% (98.8, 99.1%)		
Positive Predictive Value		62/64 = 97.0%		
Negative Predictive Value		604/606 = 99.7%		

Identification of Other Staphylococci (species other than *S. aureus*)				
		Reference		
		Positive	**Negative**	**Total**
hemoFISH Masterpanel	**Positive**	167	1	**168**
	Negative	7	495	**502**
	Total	**174**	**496**	**670**
Positive Percent Agreement		167/174 = 96.0% (91.9, 98.0%)		
Negative Percent Agreement		495/496 = 99.8% (98.9, 100%)		
Positive Predictive Value		167/168 = 99.4%		
Negative Predictive Value		495/502 = 98.6%		
Identification of Streptococci				
		Reference		
		Positive	**Negative**	**Total**
hemoFISH Masterpanel	**Positive**	76	2	**78**
	Negative	0	592	**592**
	Total	**76**	**594**	**670**
Positive Percent Agreement		76/76 = 100% (95.2, 100%)		
Negative Percent Agreement		592/594 = 99.7% (98.8, 99.9%)		
Positive Predictive Value		76/78 = 97.4%		
Negative Predictive Value		592/592 = 100%		
Identification of Other Species (not Staphylococci, Streptococci or Enterobacteriaceae)				
		Reference		
		Positive	**Negative**	**Total**
hemoFISH Masterpanel	**Positive**	144 [1]	10	**154**
	Negative	4	512	**516**
	Total	**148**	**522**	**670**
Positive Percent Agreement		144/148 = 97.3% (93.3, 98.9%)		
Negative Percent Agreement		512/522 = 98.1% (96.5, 99.0%)		
Positive Predictive Value		144/154 = 93.5%		
Negative Predictive Value		512/516 = 99.2%		

[1] Includes 1 sample that was positive by the reference method for yeast. Based on *in silico* analysis, the expected hemoFISH result with cultures that are positive for yeast when no bacteria are present is "Fail" due to the absence of signal with the Eubacterial probe. As noted in the Package Insert, cultures identified as positive for "Other" (not Staphylococci, Streptococci or Enterobacteriaceae) require additional follow-up for definitive identification of the species present.

Note:

Based on analysis of the prospectively clinical samples and contrived monomicrobial samples combined (**Table 21**):

1) The lower 95% confidence interval for identification of "Other Enterobacteriaceae (not *E. coli* or *K. pneumoniae*)" was 89.3%, which is below the acceptance criterion of ≥90%. However, with the prospective samples alone, the point estimate and lower 95% confidence interval for identification of "Other Enterobacteriaceae" were 100% and 91.4%, respectively (**Table 18**). In addition, according to the design of the hemoFISH Masterpanel, samples that are negative for "Other Enterobacteriaceae" and not positive for any of the other individual species or genera identified by the panel are reported as positive for "Other (not Staphylococci, Streptococci or Enterobacteriaceae)." A Limitation in the Package

Insert stipulates that samples reported positive for "Other (not Staphylococci, Streptococci or Enterobacteriaceae)" should be subcultured for further identification. The risk of reporting a false-negative result for "Other Enterobacteriaceae" is therefore mitigated.

2) The lower 95% confidence interval for identification of *E. coli* was 89.8%. With rounding to the nearest integer, this meets the acceptance criterion of a lower 95% confidence interval ≥90%. All 4 samples that were reported by the hemoFISH Masterpanel as false-negative for *E. coli* were from the ex-US clinical site. Three of the 4 were reported as positive for "Other (not Staphylococci, Streptococci or Enterobacteriaceae)" and 1 was reported as positive for Other Enterobacteriaceae (not *E.coli* or *K. pneumoniae*)."

Table 22. Summary of performance of the hemoFISH Masterpanel with prospective clinical and contrived samples combined (n = 670)

		Reference Method							Total
		EC	**KP**	**SA**	**ENT**	**STA**	**STR**	**Other**	
hemoFISH	**EC**	92 [1]	0	0	0	0	0	1	**93**
	KP	0	38	0	0	0	0	0	**38**
	SA	0	0	74	0	2	0	0	**76**
	ENT	1	0	0	62	0	0	1	**64**
	STA	0	0	0	0	167	0	1	**168**
	STR	1 [1]	0	0	0	0	76	1	**78**
	Other	3	0	0	2	5	0	144	**154**
	Total	**96**	**38**	**74**	**64**	**174**	**76**	**148**	**670**
Positive Agreement		92/96	38/38	74/74	62/64	167/174	76/76	144/148	
		95.8% (89.8, 98.4%)	100% (90.8, 100%)	100% (95.1, 100%)	96.9% (89.3, 99.1%)	96.0% (91.9, 98.0%)	100% (95.2, 100%)	97.3% (93.3, 98.9%)	
Negative Agreement		573/574	632/632	594/596	604/606	495/496	592/594	512/522	
		99.8% (99.0, 100%)	100% (99.4, 100%)	99.7% (98.8, 99.9%)	99.7% (98.8, 99.1%)	99.8% (98.9, 100%)	99.7% (98.8, 99.9%)	98.1% (96.5, 99.0%)	
Positive Predictive Value		92/93	38/38	74/76	62/64	167/168	76/78	144/154	
		98.9%	100%	97.4%	97.0%	99.4%	97.4%	93.5%	
Negative Predictive Value		573/577	632/632	594/594	604/606	495/502	592/592	512/516	
		99.3%	100%	100%	99.7%	98.6%	100%	99.2%	

EC: *E. coli*; KP: *K. pneumoniae*; SA: *S. aureus*; ENT: Other Enterobacteriaceae (not *E. coli* or *K. pneumoniae*); STA: Other Staphylococci (not *S. aureus*); STR: Streptococci; Other (not Staphylococci, Streptococci or Enterobacteriaceae)

[1] 1 sample reference positive for EC; hemoFISH positive for EC and STR

[2] Includes 1 sample that was positive by the reference method for *C. albicans*. Based on *in silico* analysis, the expected hemoFISH result with blood cultures that are positive for *Candida* spp. is "Fail" due to the absence of signal from the Eubacterial probe (refer to **Table 12**)

The performance of the individual probes within the hemoFISH Masterpanel with monomicrobial samples (prospective clinical and contrived, combined) is summarized in **Tables 23** to **26**.

Table 23. Performance of individual probes within the hemoFISH Masterpanel for detection of Enterobacteriaceae in monomicrobial samples (n = 670)

Reference Identification Result			hemoFISH Result							
Genus	**Species**	**Number**	**EC**	**KP**	**SA**	**ENT**	**STA**	**STR**	**OTHER**	**% Agmt**
Citrobacter	*amalonaticus*	1				1				**100**
Citrobacter	*braakii*	1				1				**100**
Citrobacter	*farmeri*	1				1				**100**
Citrobacter	*freundii*	1				1				**100**
Citrobacter	*gillenii*	1				1				**100**
Citrobacter	*koseri*	1				1				**100**
Citrobacter	*murliniae*	1				1				**100**
Citrobacter	*rodentium*	1				1				**100**
Citrobacter	*werkmanii*	1				1				**100**
Citrobacter	*youngae*	1				1				**100**
Cronobacter	*muytjensi*	1				1				**100**
Enterobacter	*cloacae*	6				6				**100**
Escherichia	*coli*	96	92			1		1 [1]	3	**95.8**
Klebsiella	*oxytoca*	7				7				**100**
Klebsiella	*pneumoniae*	38		38						**100**
Morganella	*morganii*	1				1				**100**
Proteus	*mirabilis*	21				21				**100**
Proteus	*vulgaris*	3				2			1	**66.7**
Providencia	*stuartii*	1				1				**100**
Salmonella	*abony*	1				1				**100**
Salmonella	*enterica*	3				3				**100**
Salmonella	Group B	2				2				**100**
Salmonella	*typhimurium*	1				1				**100**
Salmonella	*vellore*	1				1				**100**
Serratia	*marcescens*	5				5				**100**
Yersinia	*enterocolitica*	1							1	**0**
	Total	**198**	**92**	**38**	**0**	**63**	**0**	**1**	**5**	**97.0**

EC: *E. coli*; KP: *K. pneumoniae*; SA: *S. aureus*; ENT; Other Enterobacteriaceae (not *E. coli* or *K. pneumoniae*); STA: Other Staphylococci (not *S. aureus*); STR: Streptococci; OTHER: Other (not Staphylococci, Streptococci or Enterobacteriaceae); % Agmt: Percent Agreement

[1] Sample also positive by hemoFISH assay for *E. coli*

Table 24. Performance of individual probes within the hemoFISH Masterpanel for detection of *Staphylococcus* spp. in monomicrobial samples (n = 670)

Reference Identification Result		hemoFISH Result							
Species	**Number**	**EC**	**KP**	**SA**	**ENT**	**STA**	**STR**	**OTHER**	**% Agmt**
S. aureus	74			74					**100**
S. auricularis	1					1			**100**
S. capitis	16			1		15			**93.8**
S. caprae	1					1			**100**
S. carnosus	1							1	**0**
S. epidermidis	97					94		3	**96.9**
S. gallinarum	1					1			**100**
S. haemolyticus	17					17			**100**
S. hominis	29			1		27		1	**93.1**
S. lugdunensis	3					3			**100**
S. pettenkoferi	1					1			**100**
S. saccharolyticus	2					2			**100**
S. simulans	1					1			**100**
S. warneri	3					3			**100**
S. xylosus	1					1			**100**
Total	**248**	**0**	**0**	**76**	**0**	**167**	**0**	**5**	**97.2**

EC: *E. coli*; KP: *K. pneumoniae*; SA: *S. aureus*; ENT; Other Enterobacteriaceae (not *E. coli* or *K. pneumoniae*); STA: Other Staphylococci (not *S. aureus*); STR: Streptococci: OTHER: Other (not Staphylococci, Streptococci or Enterobacteriaceae); % Agmt: Percent Agreement

Table 25. Performance of the hemoFISH Masterpanel for detection of *Streptococcus* spp. in monomicrobial samples (n = 670)

Reference Identification Result		hemoFISH Result							
Species	**Number**	**EC**	**KP**	**SA**	**ENT**	**STA**	**STR**	**OTHER**	**% Agmt**
S. agalactiae	8						8		**100**
S. alactolyticus	1						1		**100**
S. anginosus	3						3		**100**
S. bovis	1						1		**100**
S. dysgalactiae	4						4		**100**
S. gallolyticus	3						3		**100**
S. gordonii	1						1		**100**
Streptococcus Group G	1						1		**100**
S. intermedius	1						1		**100**
S. lutentiensis	1						1		**100**
S. mitis	6						6		**100**
S. mutans	1						1		**100**
S. oralis	10						10		**100**

Reference Identification Result		hemoFISH Result							
Species	**Number**	**EC**	**KP**	**SA**	**ENT**	**STA**	**STR**	**OTHER**	**% Agmt**
S. parasanguinis	1						1		**100**
S. pneumoniae	18						18		**100**
S. pyogenes	5						5		**100**
S. salivarius	5						5		**100**
S. sanguinis	4						4		**100**
S. thermophilus	1						1		**100**
S. vestibularis	1						1		**100**
Total	**76**	**0**	**0**	**0**	**0**	**0**	**76**	**0**	**100**

EC: *E. coli*; KP: *K. pneumoniae*; SA: *S. aureus*; ENT; Other Enterobacteriaceae (not *E. coli* or *K. pneumoniae*); STA: Other Staphylococci (not *S. aureus*); STR: Streptococci: OTHER: Other (not Staphylococci, Streptococci or Enterobacteriaceae); % Agmt: Percent Agreement

Table 26. Performance of the hemoFISH Masterpanel for detection of bacteria other than *Staphylococcus* spp., *Streptococcus* spp. or Enterobacteriaceae in monomicrobial samples (n = 670)

Reference Identification Result			hemoFISH Result							
Genus	**Species**	**Number**	**EC**	**KP**	**SA**	**ENT**	**STA**	**STR**	**OTHER**	**% Agmt**
Abiotrophia	*defectiva*	1							1	**100**
Acinetobacter	*baumannii*	4							4	**100**
Acinetobacter	*hemolyticus*	1							1	**100**
Acinetobacter	*junii*	1							1	**100**
Acinetobacter	*lwoffi*	1							1	**100**
Acinetobacter	*sp.*	1				1				**0**
Actinomyces	*meyeri*	1							1	**100**
Actinomyces	*naeslundi*	1							1	**100**
Aerococcus	*sp.*	1							1	**100**
Bacillus	*cereus*	3							3	**100**
Bacillus	*circulans*	4							4	**100**
Bacillus	*coagulans*	2							2	**100**
Bacillus	*megaterium*	2							2	**100**
Bacillus	*sp.*	2							2	**100**
Bacillus	*subtilis*	1							1	**100**
Bacillus	*thuringiensis*	3							3	**100**
Bacteroides	*caccae*	1							1	**100**
Bacteroides	*distasonis*	1	1							**0**
Bacteroides	*fragilis*	3							3	**100**
Bacteroides	*ovatus*	1							1	**100**
Bacteroides	*thetaiotaomicron*	1							1	**100**
Bacteroides	*vulgatus*	1							1	**100**
Candida	*albicans*	1							1 [1]	**0**
Clostridium	*sp.*	1							1	**100**

Reference Identification Result			hemoFISH Result							
Genus	**Species**	**Number**	**EC**	**KP**	**SA**	**ENT**	**STA**	**STR**	**OTHER**	**% Agmt**
Clostridium	*bifermentas*	2							2	**100**
Corynebacterium	*amycolatum*	1							1	**100**
Corynebacterium	*matruchoti*	1							1	**100**
Corynebacterium	*sp.*	1							1	**100**
Corynebacterium	*xerosis*	1							1	**100**
Elizabethkingia	*meningoseptica*	1							1	**100**
Enterococcus	*faecalis*	27						1	26	**96.3**
Enterococcus	*faecium*	22							22	**100**
Enterococcus	*gallinarum*	1							1	**100**
Enterococcus	*raffinosus*	1							1	**100**
Fusobacterium	*nucleatum*	2							2	**100**
Fusobacterium	*sp.*	1							1	**100**
Granulicatella	*adjacens*	1							1	**100**
Kocuria	*kristinae*	4							4	**100**
Kocuria	*varians*	2							2	**100**
Kroppenstedtia	*eburnea*	1							1	**100**
Leuconostoc	*sp.*	1							1	**100**
Micrococcus	*luteus*	5					1		4	**80.0**
Moraxella	*catarrhalis*	1							1	**100**
Pasteurella	*multocida*	1							1	**100**
Pediococcus	*pentosaceus*	1							1	**100**
Peptostreptococcus	*asaccharolyticus*	3							3	**100**
Prevotella	*disiens*	1							1	**100**
Propionibacterium	*acnes*	3							3	**100**
Propionibacterium	*sp.*	1							1	**100**
Pseudomonas	*aeruginosa*	18							18	**100**
Rotha	*dentocariosa*	1							1	**100**
Stenotrophomonas	*acidominophila*	1							1	**100**
Stenotrophomonas	*maltophilia*	3							3	**100**
	Total	**152**	**1**	**0**	**0**	**1**	**1**	**1**	**148**	**96.7**

EC: *E. coli*; KP: *K. pneumoniae*; SA: *S. aureus*; ENT; Other Enterobacteriaceae (not *E. coli* or *K. pneumoniae*); STA: Other Staphylococci; STR: *Streptococcus*: OTHER: Other (not Staphylococci, Streptococci or Enterobacteriaceae); % Agmt: Percent Agreement

[1] False-positive with Eubacterial probe

A summary of the performance of each probe in the hemoFISH Masterpanel with prospective clinical and contrived monomicrobial samples combined is shown in **Table 27**.

Table 27. Performance of hemoFISH Masterpanel Beacon probes with monomicrobial samples (prospective clinical and contrived, combined)

	hemoFISH Probe					
	EC	**KP**	**SA**	**ENT**	**STA**	**STR**
True Positive	92	38	74	193	243	76
False Positive	1	0	2	2	1	2
False Negative	4	0	0	5	5	0
True Negative	573	632	594	470	421	592
Total	670	670	670	670	670	670
Positive Agreement (95% CI)	92/96 95.8% (89.8, 98.4%)	38/38 100% (90.8, 100%)	74/74 100% (95.1, 100%)	193/198 97.5% (94.4, 98.9%)	243/248 98.0% (95.4, 99.1%)	76/76 100% (95.2-100%)
Negative Agreement (95% CI)	573/574 99.8% (99.0, 100%)	632/632 100% (99.4, 100%)	594/596 99.7% (98.8, 100%)	470/472 99.6% (98.5-99.9%)	421/422 99.8% (98.7, 100%)	592/594 99.7% (98.8, 99.9%)

EC: *E. coli*; KP: *K. pneumoniae*; SA: *S. aureus*; ENT; Enterobacteriaceae; STA: *Staphylococcus*; STR: *Streptococcus*; CI: Confidence Interval

The Clinical Study was conducted using a combination of different blood culture media according to each site's standard laboratory practice. The performance of the hemoFISH Masterpanel with monomicrobial cultures in each type of medium is summarized in **Table 28**.

Table 28. Comparison of results with monomicrobial cultures in different media used in the hemoFISH Masterpanel Clinical Study

Culture Medium	Number of Samples	Agreement	hemoFISH Result (%)						
			EC	KP	SA	ENT	STA	STR	Other
VersaTREK REDOX 1	370	Positive	56/56 (100)	29/29 (100)	36/36 (100)	30/30 (100)	87/88 (98.9)	48/48 (100)	79/82 (96.3)
		Negative	314/314 (100)	341/341 (100)	333/334 (99.7)	339/340 (99.7)	281/282 (99.6)	321/322 (99.7)	287/288 (99.7)
VersaTREK REDOX 2	173	Positive	23/23 (100)	5/5 (100)	26/26 (100)	7/7 (100)	50/50 (100)	19/19 (100)	41/42 (97.6)
		Negative	149/150 (99.3)	168/168 (100)	147/147 (100)	166/166 (100)	123/123 (100)	154/154 (100)	131/131 (100)
BD BACTEC Plus Aerobic/F	104 [1]	Positive	10/13 (76.9) [2]	3/3 (100)	11/11 (100)	23/25 (92.0)	18/22 (81.8)	9/9 (100)	21/21 (100)
		Negative	91/91 (100)	101/101 (100)	92/93 (98.9)	79/79 (100)	82/82 (100)	95/95 (100)	75/83 (90.4)
BD BACTEC Plus Anaerobic/F	20	Positive	2/3 (66.7)	1/1 (100)	1/1 (100)	2/2 (100)	10/12 (83.3)	0/0 (N/A)	1/1 (100)
		Negative	17/17 (100)	19/19 (100)	19/19 (100)	17/18 (94.4)	8/8 (100)	20/20 (100)	17/19 (89.5)
BD BACTEC Peds Plus/F	2	Positive	0/0 (N/A)	0/0 (N/A)	0/0 (N/A)	0/0 (N/A)	1/1 (100)	0/0 (N/A)	1/1 (100)
		Negative	2/2 (100)	2/2 (100)	2/2 (100)	2/2 (100)	1/1 (100)	2/2 (100)	1/1 (100)

EC: *E. coli*; KP: *K. pneumoniae*; SA: *S. aureus*; ENT: Other Enterobacteriaceae (not *E. coli* or *K. pneumoniae*); STA: Other Staphylococci (not *S. aureus*); STR: Streptococci; Other: Other (not Staphylococci, Streptococci or Enterobacteriaceae); N/A: Not Applicable

[1] Includes 61 contrived samples

[2] All 3 false-negatiive results for *E. coli* were obtained at a single ex-US clinical site

Note: The total number of samples included in the table is 670. There was on (1) monomicrobial sample from the Clinical Study for which no information was available regarding the type of medium used.

Polymicrobial Cultures

The performance of the hemoFISH Master panel for identification of organisms in polymicrobial blood cultures is summarized in **Tables 29** and **30**. Results from 55 prospectively collected polymicrobial samples were included in the analysis. The hemoFISH Masterpanel correctly identified each of the organisms present in only 5/55 (9.1%) polymicrobial samples, all of which contained 2 bacterial species. For 49/50 (98.0%) of the remaining samples, the hemoFISH Masterpanel correctly identified at least one of the organisms present.

The performance of the hemoFISH Masterpanel with mixed cultures in part reflects the inability to discriminate between co-infecting species of the same genus (for Staphylococci and Streptococci) or family (Enterobacteriaceae). As noted in **Section M 1(g)**, Limitations with respect to correct identification of organisms in polymicrobial cultures are included in the device labeling.

Table 29. Summary of hemoFISH results from 55 prospectively collected polymicrobial samples

Reference Result					Number of Samples	hemoFISH Result											
								EC	EC	KP		SA			STA		
ID-1	ID-2	ID-3	ID-4	ID-5		EC	KP	KP	STR	STA	ENT	ENT	SA	STA	STR	STR	OTHER
EC	ENT				1	***1***											
EC	KP				2	***1***		1									
EC	OTHER	ENT	ENT	OTHER	1	***1***											
EC	OTHER	ENT	ENT		1	***1***											
EC	OTHER	ENT			1	***1***											
EC	OTHER	OTHER			1	***1***											
EC	OTHER				5	***4***											***1***
EC	STA				2	***2***											
EC	STR				1											***1***	
ENT	ENT	ENT			1						***1***						
ENT	OTHER				2						***2***						
KP	ENT	OTHER			1		***1***										
KP	ENT				4		***4***										
KP	OTHER				4		***4***										
OTHER	OTHER	ENT			1												***1***
OTHER	OTHER	STA			1												***1***
OTHER	OTHER				4	1 *											***3***
OTHER	STA	YEAST			1										***1***		
OTHER	STA				3									***2***			***1***
SA	ENT				1							1					
SA	OTHER				2								***1***				***1***
STA	KP				1					1							
STA	STA	OTHER			1									***1***			
STA	STR	OTHER			1				***1***								
STA	STA				6									***6***			
STA	STR				5										2	***3***	
STR	STR				1											***1***	

ID: Identification; EC: *E. coli*; KP: *K. pneumoniae*; SA: *S. aureus*; ENT; Other Enterobacteriaceae (not *E. coli* or *K. pneumoniae*); STA: Other Staphylococci (not *S. aureus*); STR: Streptococci; Other (Other (not Staphylococci, Streptococci or Enterobacteriaceae)

Samples for which the hemoFISH assay agreed with the reference method are shaded

Samples for which at least one organism identified by hemoFISH matched at least one of those identified by the reference method are shown in ***bold italics***

The one sample for which there was no agreement between the hemoFISH assay and reference method is denoted by *

Table 30. Summary of hemoFISH results from polymicrobial samples by analyte

		Reference Result						
		EC	**KP**	**SA**	**ENT**	**STA**	**STR**	**Other**
hemoFISH	**EC**	13	0	0	0	0	0	2 [1]
	KP	0	11	0	0	0	0	0
	SA	0	0	2	0	0	0	0
	ENT	0	0	0	4	0	0	[0]
	STA	0	0	0	0	14	0	0
	STR	0	0	0	0	0	7	0
	Other	0	0	0	0	0	0	8
	Negative	2	1	1	10	7	1	20 [1]
	Total	**15**	**12**	**3**	**14**	**21**	**8**	**30**

EC: *E. coli*; KP: *K. pneumoniae*; SA: *S. aureus*; ENT; Other Enterobacteriaceae (not *E. coli* or *K. pneumoniae)*; STA: Staphylococci (not *S. aureus*); STR: Streptococci; Other: Other (not Staphylococci, Streptococci or Enterobacteriaceae); Negative: hemoFISH negative for the species or group identified by the reference method

[1] 2 samples that were positive by the reference method for "Other (no Staphylococci, Streptococci or Enterobacteriaceae) were hemoFISH false-positive for *E. coli*

b. Clinical specificity:

Refer to **Section M 3(a)**, above.

c. Other clinical supportive data (when a. and b. are not applicable):

Not applicable

4. Clinical cut-off**:**

Not applicable

5. Expected values/Reference range:

The clinical study included 664 prospectively collected monomicrobial and polymicrobial samples from three (3) sites. The prevalence at each clinical site for each analyte as determined by the reference culture method and by the hemoFISH Masterpanel is shown in **Table 31.** The prevelance of each analyte in monomicrobial and polymicrobial cultures is shown in **Table 32**.

Table 31. Prevalence of hemoFISH analytes in prospective blood cultures stratified by site

Analyte	Percent Prevalence (n)							
	Site A (332)		Site B (232)		Site C (70)		Overall (664)	
	Reference	hemoFISH	Reference	hemoFISH	Reference	hemoFISH	Reference	hemoFISH
E. coli	14.6 (53)	14.4 (52)	17.2 (40)	17.7 (41)	22.9 (16)	18.6 (13)	16.4 (109)	16.0 (106)
K. pneumoniae	6.4 (23)	6.4 (23)	9.9 (23)	9.5 (22)	2.9 (2)	2.9 (2)	7.2 (48)	7.1 (47)
S. aureus	14.1 (51)	14.1 (51)	6.0 (14)	6.0 (14)	10.0 (7)	11.4 (8)	10.8 (72)	11.0 (73)
Other Enterobacteriaceae [1]	6.1 (22)	5.5 (20)	11.6 (27)	9.5 (22)	8.6 (6)	7.1 (5)	8.3 (55)	7.1 (47)
Other Staphylococci [2]	27.1 (98)	27.1 (98)	25.4 (59)	22.8 (53)	44.3 (31)	34.3 (24)	28.3 (188)	26.4 (175)
Streptococci	9.7 (35)	9.9 (36)	16.4 (38)	16.8 (39)	2.9 (2)	1.4 (1)	11.3 (75)	11.4 (76)
Other [3]	25.4 (92)	23.5 (85)	25.9 (60)	19.0 (44)	18.6 (13)	25.7 (18)	24.8 (165)	22.1 (147)

[1] Not *E. coli* or *K. pneumoniae*
[2] Not *S. aureus*
[3] Not Staphylococci, Streptococci or Enterobacteriaceae

Table 32. Prevalence of hemoFISH analytes in prospective monomicrobial and polymicrobial blood cultures

Analyte	Percent Prevalence (n)					
	Monomicrobial (609)		Polymicrobial (55)		Overall (664)	
	Reference	hemoFISH	Reference	hemoFISH	Reference	hemoFISH
E. coli	15.4 (94)	14.9 (91)	27.3 (15)	27.3 (15)	16.4 (109)	16.0 (106)
K. pneumoniae	5.9 (36)	5.9 (36)	21.8 (12)	20.0 (11)	7.2 (48)	7.1 (47)
S. aureus	11.3 (69)	11.7 (71)	5.5 (3)	3.6 (2)	10.8 (72)	11.0 (73)
Other Enterobacteriaceae [1]	6.7 (41)	7.1 (43)	25.5 (14)	7.3 (4)	8.3 (55)	7.1 (47)
Other Staphylococci [2]	27.4 (167)	26.4 (161)	38.2 (21)	25.5 (14)	28.3 (188)	26.4 (175)
Streptococci	11.0 (67)	11.3 (69)	14.5 (8)	12.7 (7)	11.3 (75)	11.4 (76)
Other [3]	22.2 (135)	22.8 (139)	54.5 (30)	14.5 (8)	24.8 (165)	22.1 (147)

[1] Not *E. coli* or *K. pneumoniae*
[2] Not *S. aureus*
[3] Not Staphylococci, Streptococci or Enterobacteriaceae

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted in this premarket notification is complete and supports a substantial equivalence decision.