510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K123418

B. Purpose for Submission:

To evaluate submitted data to make a substantial equivalence determination for the Gram-Negative *Quick*FISH™ BC Blood Culture Identification Kit to aid in the identification of *Escherichia coli* and/or *Pseudomonas aeruginosa* and/or *Klebsiella pneumonia* from smears from positive blood cultures containing gram-negative bacilli observed on Gram stain.

C. Measurand:

Escherichia coli and/or *Pseudomonas aeruginosa* and/or *Klebsiella pneumonia* species–specific ribosomal ribonucleic acid (rRNA) from smears from positive blood cultures containing gram-negative bacilli observed on Gram stain.

D. Type of Test:

Gram-Negative *Quick*FISH BC is a qualitative fluorescence *in situ* hybridization (FISH) assay using peptide nucleic acid (PNA) probes that hybridize to species – specific rRNA sequences from *Escherichia coli, Pseudomonas aeruginosa* or *Klebsiella pneumoniae*.

E. Applicant:

AdvanDx, Inc.

F. Proprietary and Established Names:

Gram-Negative *Quick*FISH™ BC Blood Culture Identification Kit

G. Regulatory Information:

1. Regulation section:

866.2660

2. Classification:

Class I

3. Product code:

JSS

4. Panel:

83- Microbiology

H. Intended Use:

1. Intended use(s):
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 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.

2. Indication(s) for use:

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 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.

3. Special conditions for use statement(s):

For prescription use only.

3. Special instrument requirements:

AdvanDx Microscope Dual Band Filter (Cat. No. AC007)
AdvanDx *Quick*FISH™ Slides (Cat. No. CS012)
AdvanDx Slide Station 10 Slide Warmer (Cat. No. AC028)
AdvanDx *Quick*FISH™ Mixing Station (Cat. No. AC030)
AdvanDx Filter Vials (Cat. No. AC008)
Fluorescence microscope equipped with a 60x or 100x-oil objective

I. Device Description:

Gram-Negative *Quick*FISH BC is a FISH assay using species-specific PNA probes hybridizing to *Escherichia coli, Pseudomonas aeruginosa Klebsiella pneumoniae* to provide rapid species identification from smears made from positive Gram-negative blood cultures within approximately 20 minutes.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   i. GNR Traffic Light PNA FISH (k101558)

2. Predicate K number(s):

   k101558

3. Comparison with predicate:

Similarities		
Item	**Device**	**Predicate**
	Gram-Negative *Quick*FISH™ BC (k123418)	**GNR Traffic Light PNA FISH (k101558)**
Intended Use	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 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.	GNR Traffic Light PNA FISH is a multicolor, qualitative nucleic acid hybridization assay intended for the identification of *Escherichia coli*, and/or *Klebsiella pneumoniae*, and/or *Pseudomonas aeruginosa* on smears made from positive blood cultures containing Gram- negative rods observed on Gram stain. Subculturing of positive blood cultures is necessary to recover organisms for susceptibility testing, and/or differentiation of mixed growth. GNR Traffic Light PNA FISH is indicated as an aid in the diagnosis of *Escherichia coli*, and/or *Klebsiella pneumoniae*, and/or *Pseudomonas aeruginosa* bacteremia.

Indication for Use	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 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.	GNR Traffic Light PNA FISH is a multicolor, qualitative nucleic acid hybridization assay intended for the identification of *Escherichia coli*, and/or *Klebsiella pneumoniae*, and/or *P. aeruginosa* on smears made from positive blood cultures containing Gram- negative rods observed on Gram stain. Subculturing of positive blood cultures is necessary to recover organisms for susceptibility testing. GNR Traffic Light PNA FISH is indicated as an aid in the diagnosis of *Escherichia coli*, and/or *Klebsiella pneumoniae*, and/or *P. aeruginosa* bacteremia.
Technology	Fluorescence *in situ* hybridization using PNA probes	Same
Probe Sequence	Targeting rRNA sequences for *E. coli*, and/or *P. aeruginosa* and/or *K. pneumoniae*	Same sequences.
Sample Type	Positive blood cultures from standard automated blood culture device	Same
Interpretation of Results	Qualitative fluorescence microscopy	Same
Specimen Preparation	Standard automated blood culture device	Same

Differences		
Item	**Device**	**Predicate**
Device	**Gram-Negative *Quick*FISH™ BC (k123418)**	**GNR Traffic Light PNA FISH (k101558)**
Fixation Reagents	Two solutions: QuickFix 1: Ethanol based QuickFix 2: Methanol based	One solution: 3 mL PBS with detergent
PNA Probe Reagent(s)	1.5 mL PNA Probes in two solutions: Gram-Negative PNA Blue: 6 quenching probes Gram-Negative PNA Yellow: 1 PNA probe each for *E. coli and P. aeruginosa* and 2 PNA probes for *K. pneumoniae*	1.5 mL PNA Probes in a single solution: 1 PNA probe each for *E. coli, P. aeruginosa and K. pneumoniae*
Wash Solution	None	Wash solution with Tris/HCl: 0.3 M, NaCl: 0.9 M, 6% (v/v) Triton X-100
Mounting Solution	None	3 mL photobleaching inhibitor in glycerol
Hybridization	• 1 drop *S. aureus*/CNS PNA directly to sample • Add coverslip • Incubate 30 minutes	• Performed at 55°C • Mix 1 drop each of *Staphylococcus* PNA Blue and • Staphylococcus PNA Yellow on a coverslip • Invert coverslip onto sample • Incubate 15-20 minutes
Washing and Mounting Procedures	None	Rinse, stringent wash, mount cover slip
Time to Result	~20-25 minutes	1.5 hours
Controls	Positive and Negative Controls on same slide as sample	Positive and Negative Controls must be bought or made separately

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

Not applicable.

L. Test Principle:

The *Quick*FISH™ technology uses species-specific Peptide Nucleic Acid (PNA) probes in a fluorescence *in situ* hybridization format. PNA is a synthetic molecule that differs from DNA at the backbone. In PNA, the counterpart of the sugar phosphate backbone of DNA and RNA is a polyamide formed by repetitive units of N-(2-aminoethyl) glycine. Bases (i.e., A, T, C, and G) are attached to this backbone to provide a molecular design that allows PNA to hybridize (i.e., by specific base pairing) to complementary DNA or RNA sequences. The hybridization with PNA probes occurs in accordance with Watson-Crick base-pairing rules. *Quick*FISH™ uses species-specific probes targeting rRNA several thousand rRNA molecules in sufficient concentration to allow individual cells to be detected and identified directly by fluorescent-labeled probes.

A mixture of a fluorescein-labeled *E. coli* specific PNA probe (green), a tetramethylrhodamine labeled *P. aeruginosa* PNA probe (red), and a tetramethylrhodamine and fluorescein-labeled *K. pneumoniae* specific PNA probe (yellow) is added to a smear prepared from a gram-negative bacilli positive blood culture. The probe mixture also includes quencher labeled PNA probes that serve to bind unreacted fluorescent labeled probes to suppress unwanted signal. After the fluorophore-labeled PNA probes and quencher-labeled PNA probes are added to a smear prepared from a culture, hybridization is performed at 55°C for 15-20 minutes. The smear is then ready for examination by fluorescence microscopy. *E. coli*, *P. aeruginosa*, and *K. pneumoniae* cells become fluorescent by specific binding of the fluorophore-labeled PNA probes while maintaining cell morphology.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

   A reproducibility study was performed using the Gram-Negative *Quick*FISH™ BC. The assay was performed on 20 isolates including 5 isolates each of *E. coli* (green positive), *P. aeruginosa* (red positive), and *K. pneumoniae* (yellow positive), and one isolate each of *K. oxytoca, E. aerogenes, S. marcenscens, A. baumannii, P. mirabilis* (negatives) in triplicate on three separate days at three separate sites. Reproducibility was > 95%.

   b. Linearity/assay reportable range:

   Not applicable

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

*Quick*FISH™ slides are provided in individually sealed pouches with nitrogen and a desiccant. Slides must be used immediately after breaking pouch seal and within expiration. Slides are stored s at 2-8°C. Fixed *Quick*FISH™ 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. *Quick*FISH™ 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.

*Quick*FISH™ fixed microscope slides with controls (i.e. CS012) consist of the following strains for the Gram-Negative *Quick*FISH™ BC:

Positive Controls:

Escherichia coli:	
ATCC 11775, 35218, 10536, 11229, 23848:	Green
Pseudomonas aerugniosa:	
ATCC 27853, 10145, 9027, 35032, NCTC 10662:	Red
Klebsiella pneumoniae:	
ATCC 13882, 13883, 10031, 35657, 4352:	Yellow

Negative Controls:

Klebsiella oxytoca ATCC 43086:	No fluorescence
Enterobacter aerogenes ATCC 13048	No fluorescence
Serratia marcescens ATCC 14756	No fluorescence
Acinetobacter baumannii ATCC 19606	No fluorescence
Pseudoonas putida ATCC 49128	No fluorescence

Compatibility Study:

A compatibility study was performed with the same control strains listed above for the following bottle types:

BACTEC:
(Lytic 10 anaerobic, aerobic plus, anaerobic plus, PEDS Plus, Standard 10 aerobic, Standard anaerobic)
BacT/ALERT:
(SA, SN)
VERSA TREK
(REDOX 1 aerobic)

The study demonstrated that these bottle types were compatible. The Gram-Negative *Quick*FISH™ BC is not compatible with bottles supplemented with charcoal and VERSA TREK Redox-2 anaerobic bottles. All results were as

expected. The strains may not represent the range of genetic diversity for each species.

Fixed Smear Stability:

A stability assessment was performed on fixed slides on each of the strains listed above at 55°C for 0 and 5 min at, room temperature 5, 15, 30, 60 min; at 2-8°C for 1, 4, 18 and 24 h. Expected results were attained with each test and demonstrated that the assay was stable in all tested conditions.

d. Detection Limit:

The limit of detection (LoD) for each species was approximated at 2.3×10^5 CFU/ml for *E. coli* (ATCC 11775), 4.0×10^5 CFU/ml for *P. aeruginosa* (ATCC 27853) and 4.5×10^5 CFU/ml for *K. pneumoniae* (ATCC 13882), as an average of 3 tests per species. These LoDs were established by evaluating half-log serial dilutions of aliquots from contrived positive blood cultures containing these species, diluting approximately three orders of magnitude below the limit of detection.

Co-infection Studies:
Co-infection studies were performed for the Gram-Negative *Quick*FISH™ BC assay using growth from BACTEC Standard/10 blood culture bottles with sterile human blood added. To determine the LoD of each species in a mixed infection for dual identification, one species was inoculated at 10^6 CFU/ml while the competing organism was introduced at increasing 10-fold increments.

Co-Infection LoDs		
1st Strain	**2nd Strain**	**LoD of 2nd Strain**
E. coli	***P. aeruginosa***	5.34×10^5
E. coli	***K. pneumoniae***	9.59×10^5
P. aeruginosa	***E. coli***	5.79×10^5
P. aeruginosa	***K. pneumoniae***	8.94×10^5
K. pneumoniae	***E. coli***	6.85×10^5
K. pneumoniae	***P. aeruginosa***	4.31×10^5

Analytical Sensitivity:

The sensitivity of the Gram-Negative *Quick*FISH™ BC assay was established using 16 strains of *E. coli*, 21 strains of *P. aeruginosa* and 12 strains of *K. pneumoniae*. All strains of each species gave the expected green-positive, red-positive and yellow positive results, respectively.

e. Analytical Specificity:

The specificity of the Gram-Negative *Quick*FISH™ BC assay was established using 86 other strains of gram-negative bacilli. 78 of the 86 produced the expected negative results and 8 provided false positive results. The false positive results represent 7 species and 4 genera. The false positive results are summarized in the table below:

Cross-reactivity*:		
Species	**Strain ID**	**False Positive Result**
Acinetobacter radioresistens	ATCC 43998	Red
Pseudomonas fulva	ATCC 31418	Red
Pseudomonas fulva	ATCC 14592	Red
Escherichia albertii	ATCC 17582	Green
Escherichia fergusonii	ATCC 35469	Green
Shigella dysenteriae **(serogroup A)**	ATCC 9361	Green
Shigella flexneri **(serogroup B)**	ATCC 9199	Green
Shigella sonnei **(serogroup D)**	ATCC 9290	Green

*All other strains tested returned the expected negative results.

Wet analytical studies demonstrated that *Brevudimonas diminuta* ATCC 19146, *Escherichia fergusonii* ATCC 33821 and *Herbaspirillum huttiense* ATCC14670 did not cross-react. *In silico* studies and historical evidence indicates that a possibility of cross-reactivity may still remain for these strains.

f. Assay Cut-off:

Not applicable

g. Media Interference Studies

A media compatibility study was performed using the following blood culture bottle types: BacT/Alert (SA and SN), BACTEC (Lytic 10, Aerobic Plus, Anaerobic Plus, PEDS Plus, Standard 10 Aerobic, Standard Anaerobic) and VersaTREK REDOX-1 Aerobic. Organisms used included the 5 positive control strains for each species of *E. coli*, *P. aeruginosa* and *K. pneumoniae* species and the 5 negative control strains listed above listed above. Results showed the Gram-Negative *Quick*FISH™ BC to be compatible with all of the above mentioned blood culture bottle types. All results were as expected.

2. Comparison studies:

   a. *Method comparison with predicate device:*

      The performance of the Gram-Negative *Quick*FISH™ BC was compared to the predicate using conventional culture and bacterial identification methods.

   b. *Matrix comparison:*

      Not applicable.

3. Clinical Studies:

   a. *Clinical Sensitivity:*

      The performance of the Gram-Negative *Quick*FISH™ BC was compared to results obtained from routine identification methods for the identification of Gram-negative bacilli from blood cultures. The clinical studies were performed at 5 clinical laboratory sites in the U.S. A total of 263 patients and 43 spiked samples are included in these analyses. The breakdown of performance by analyte was as follows:

Clinical Performance Data for Gram-Negative *Quick*FISH™ BC vs. Reference Identification Methods by Blood Cultures Positive with Gram-Negative Bacilli (all sites)				
Gram-Negative *Quick*FISH BC	***E. coli***	***P. aeruginosa***	***K. pneumoniae***	**Other**
E. coli	91[1,3]	0	0	0
P. aeruginosa	0	52[2]	0	1
K. pneumoniae	1	0	60[3]	0
Negative	2	1	0	99
Total	**Positive Percent Agreement** 96.8% (91/94) 95% CI (91.0-98.9)	**Positive Percent Agreement** 98.1% (52/53) 95% CI (90.1-99.7)	**Positive Percent Agreement** 100% (60/60) 95% CI (94.0-100)	**Negative Percent Agreement** 99.0% (99/100) 95% CI (94.6-99.8)

[1] Includes 9 blood cultures spiked with clinical strains of *E. coli*.
[2] Includes 34 blood cultures spiked with clinical strains of *P. aeruginosa*.
[3] Includes 1 mixed culture of *E. coli* and *K. pneumoniae*.

In the clinical studies, bottles were stored at room temperature after Gram stain and before Gram-Negative *Quick*FISH™ BC testing. The time between Gram stain and preparation of the Gram-Negative *Quick*FISH™ BC slides varied from less than 1/2 hour to greater than 48 hours. The following were discrepancies in the study:

- There was one false positive result for *P. aeruginosa* in a specimen that was infected with *Pseudomonas fulva*, a known limitation.
- There was one false positive for *K. pneumoniae* that was co-infected with *E. coli*, *Serratia marcenscens* and *Streptococcus mutans* (also accounting for 1 false negative result for *E. coli*).
- There were 2 other false negative results for *E. coli*, whose times between collection and processing were 15h 25 min and 29h 30 min, respectively.
- There was 1 false negative for *P. aeruginosa*, whose time between collection and processing was 9h 4 min.

Other organisms encountered in the clinical study include *Proteus mirabilis, Serratia marcescens, Enterobacter cloacea, Morganella morganii, Bacteroides fragillis, Citrobacter koseri, Proteus mirabilis, Staphylococcus aureus, Klebsiella oxytoca, Moraxella spp, Citrobacter freundii, Haemophilis parainfluenzae, Capnocytophaga spp, Eikenella corodens, Acinetobacter spp, Enterococcus faecium, Bordotella helmesii, Acinetobacter baumannii, Diptheroids, Providencia stuartii, Streptococcus mutans, Pantoea agglomerans, Streptococcus mitis, Lactococcus lactis, Pseudomonas fulva, Staphylococcus epidermidis, Prevotella bivia, Streptococcus gallolyticus, Burkholderia cepacia, Bacillus spp, Ochrobactrum anthropic, Prevotella intermedia, Hafnia alvei, Stenotrophomonas maltophilia, Enterobacter aerogenes, Acinetobacter spp, Pseudomonas fluoresens, Capnocytophaga spp, Providencia rettgeri, Salmonella spp,* and *Pseudomonas putida.*

Performance Data for Gram-Negative *Quick*FISH™ BC **vs. Reference Identification Methods by Blood** Culture Bottle Types (all samples)				
Bottle Type	***E. coli* Positive Percent Agreement**	***P. aeruginosa* Positive Percent Agreement**	***K. pneumoniae* Positive Percent Agreement**	**Negative Percent Agreement**
Total BACTEC (Plus Aerobic and Lytic/10 Anaerobic)	98.6% (69/70) 95% CI (92.3-99.8)	100% (33/33) 95% CI (89.6-100)	100% (41/41) 95% CI (89.6-100)	98.5% (65/66) 95% CI (89.6-99.7)
Total BacT/ALERT (SA Aerobic and SN Anaerobic)	91.7% (22/24) 95% CI (74.2-97.7)	95% (19/20) 95% CI (76.4-99.1)	100% (19/19) 95% CI (83.2-100)	91.6% (34/34) 95% CI (89.9-100)

*b. Clinical specificity**:***

See table 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:

E. coli: multiple bright green fluorescent rods in multiple fields.
K. pneumoniae: multiple bright yellow fluorescent rods in multiple fields.
P. aeruginosa: multiple bright red fluorescent rods in multiple fields.

N. Proposed Labeling:

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

O. Conclusion:

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