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

A. 510(k) Number:

K101558

B. Purpose for Submission:

To determine substantial equivalence for the identification of *Escherichia coli* (*E. coli), Klebsiella pneumoniae* (*K. pneumoniae*) and *Pseudomonas aeruginosa* (*P. aeruginosa)* on smears from positive blood cultures containing Gram negative rods.

C. Measurand:

E. coli, K. pneumoniae, and *P. aeruginosa* specific ribosomal RNA sequences

D. Type of Test:

Fluorescence In Situ Hybridization (FISH) using protein nucleic acid (PNA) probes

E. Applicant:

AdvanDx, Inc

F. Proprietary and Established Names:

GNR Traffic Light PNA FISH Culture Identification Kit

G. Regulatory Information:

1. Regulation section:

   866.2660

2. Classification:

   Class I

3. Product code:

   JSS- Kit, Identification, Enterobacteriaceae

4. Panel:

   83- Microbiology

H. Intended Use:

1. Intended use(s):

   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.

2. Indication(s) for use:

   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.

3. Special conditions for use statement(s):

   Prescription use only

4. Special instrument requirements:

   Dual Band Filter (Cat. No. AC003)
   Microscope Slides (Cat. No. AC001)

I. Device Description:

GNR Traffic Light PNA FISH is a is a fluorescence *in situ* hybridization (FISH) method using PNA probes hybridizing to *E. coli, K. pneumoniae* and *P. aeruginosa* specific ribosomal RNA sequences. The test provides rapid (within 90 minutes)

identification of *E. coli*, *K. pneumoniae* and *P. aeruginosa* on smears made from positive blood cultures.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   EK/P. aeruginosa PNA FISH

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

   K092393

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Technology	Fluorescence In Situ Hybridization (FISH) using protein nucleic acid (PNA) probe	Same
Sample	Positive blood culture	Same
Interpretation of results	Qualitative Fluorescence microscope	Same
Time to result	1.5 hours	Same

Differences		
Item	Device	Predicate
Function	Identification of *Escherichia coli, Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* separately	Identification of *Escherichia coli/ Klebsiella pneumoniae* combined, and *Pseudomonas aeruginosa* separately
Control organisms	Positive Control *E. coli,* *K. pneumoniae* *P. aeruginosa* Negative Control *K. oxytoca*	Positive Control *E. coli,* *P. aeruginosa* Negative Control *K. oxytoca*

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

Not applicable

L. Test Principle:

A mixture of fluorescein-labeled *E. coli* specific PNA probe and

tetramethylrhodamine and double fluorescein-labeled *K. pneumoniae* specific PNA probe and Texas Red labeled, *P. aeruginosa* specific PNA probe is added to a smear prepared from a positive blood culture. Hybridization is performed at 55ºC for 30 minutes. The hybridization is followed by a rinse step to remove the cover slip and a post-hybridization wash at 55ºC for 30 min. with a stringent Wash Solution to remove unbound PNA probe. The smear is mounted with Mounting Medium and examined by fluorescence microscopy.

E. coli cells yield green fluorescence by specific binding of the fluorescein-labeled PNA probes whereas *P. aeruginosa* yield red fluorescence by specific binding of the Texas Red-labeled PNA probes; *K. pneumoniae* cells yield yellow fluorescence by specific binding of the tetramethylrhodamine and double fluorescein-labeled PNA probes.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

The assay was performed on 14 slides (19 isolates) in triplicate on three separate days at three separate sites. Each batch was performed independently by at least two different operators at each site. The reproducibility was >95%.

b. Linearity/assay reportable range:

Not applicable

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

The GNR Traffic Control slide set (i.e. CS001) is:

Positive Control:

E. coli ATCC 35218	Green
P. aeruginosa ATCC 10145	Red
K. pneumoniae ATCC 13882	Yellow

Negative Control:

K. oxytoca ATCC 43086	No fluorescence

All results were as expected.

d. Detection limit:

The detection limit was determined to be approximately 10^5 CFU/mL by serial dilutions of *E. coli, K. pneumoniae* and *P. aeruginosa* cultures. The average number of colonies per mL (CFU/mL) was calculated from three plates. The data sets showed a minimum of 10^5 CFU/mL to produce a positive result for the GNR Traffic Light PNA FISH™ assay.

e. Analytical specificity:

The GNR Traffic Light PNA FISH has been evaluated on a total of 163 microorganisms including 144 Gram-negative strains, 13 Gram-positive organisms, and 6 yeasts. The results demonstrated:

All (19/19) *E. coli* were green-positive
All (20/20) *K. pneumoniae* (including the three subspecies: *pneumoniae, ozaenae* and *rhinosclermatis*) were yellow-positive,
All (20/20) *P. aeruginosa* were red-positive

All four *Shigella* spp serogroups (i.e. A, B, C and D), one *Escherichia albertii,* and one *Escherichia fergusonii* cross-reacted, producing a green signal. One *Brevundimonas diminuta,* one *Herbaspirillum huttiense* and one *Acinetobacter radioresistens* cross-reacted, producing a red signal. One *Escherichia vulneris* cross-reacted, producing a yellow signal. The other 75 Gram-negative rods, 13 Gram-positive bacteria and 6 yeast species all yielded negative results.

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison of device to conventional methods, as the reference method:

The performance of the GNR Traffic Light PNA FISH™ assay was compared to the conventional culture methods.

b. Matrix comparison:

Not applicable

3. Clinical studies:

A total of 358 prospective samples, and 32 spiked samples were evaluated at four U.S. sites resulting in 395 results. There were five mixed positive cultures.

a. Clinical Sensitivity:

Performance Data for GNR Traffic Light PNA FISH vs. Routine Identification Methods (Combined) on GNR-positive Blood Culture Bottles

Study	Sensitivity *E. coli*	Sensitivity *K. pneumoniae*	Sensitivity *P. aeruginosa*	Specificity	Blood Culture System
A	35/35	17/17	7/9[3]	35/37[4]	BACTEC
B	31/31	30/30	39/39[6]	36/36	BacT/ALERT
C	48/48	28/28	8/8	29/30[5]	BACTEC
D	21/21	2/3[2]	8/8[1]	15/15	VersaTREK
Total	100% (135/135) 95% CI (97.8-100)	98.7% (77/78) 95% CI (93.1-100)	96.9% (62/64) 95% CI (89.2-99.6)	97.5% (115/118) 95% CI (92.8-99.5)	N=395

[1] Includes 4 samples spiked with *P. aeruginosa* clinical isolates.
[2] One *K. pneumoniae* in a mixed culture of *E. coli* and *K. pneumoniae* was negative by PNA FISH and identified following subculturing.
[3] Two *P. aeruginosa* in mixed cultures of *P. aeruginosa* and *K. pneumoniae* were negative by PNA FISH and identified following subculturing.
[4] One false green positive *E. cloacae* (negative upon re-test) and one false red positive *A. baumannii*
[5] One false red positive *Acinetobacter radioresistens* in a mixed culture with *E. faecalis.*
[6] Includes 28 samples spiked with *P. aeruginosa* isolates

*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

The expected *E. coli*, *K. pneumoniae*, and *P. aeruginosa* positive rates for Gram negative rod positive blood cultures as determined by the clinical studies are approximately 37%, 22%, and 9%, respectively. Rates may vary depending on institution and patient population.

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.