

## Amended Abstract

**Background:** Identification of Gram-negative rods from positive blood cultures currently requires growth on solid media followed by biochemical analysis requiring 18 to 36 hours. Identification of the organism allows for initiation of appropriate therapy; therefore, the time to identification is critical for implementation of appropriate therapy. Three Gram-negative rods commonly found in blood are *E. coli*, *K. pneumoniae* and *P. aeruginosa*. Recently, an increase in infections caused by multidrug resistant Gram-negative organisms, *K. pneumoniae* in particular, has been reported in major medical centers. This fact highlights the importance of rapid identification of these organisms. GNR Traffic Light PNA FISH, a 3-color multiplex assay, allows for identification of these 3 organisms within 90 minutes of a positive blood culture.

**Method:** 75 blood cultures containing Gram-negative rods were tested. Smears of blood cultures were heat fixed onto microscope slides, a drop of GNR Traffic Light PNA probe was added to the smear and then hybridized at 55°C for 30 minutes. The slides were then washed for 30 minutes at 55°C. Next, the slides were mounted and visualized with a fluorescence microscope with a FITC/Texas Red filter and 60x oil objective. *E. coli* was identified as green fluorescent cells, *K. pneumoniae* as yellow and *P. aeruginosa* as red.

**Results:** All *E. coli* (34/34) produced a green positive result, the majority of *K. pneumoniae* (10/11) produced a yellow positive result and all *P. aeruginosa* (8/8) produced a red positive result. GNR Traffic Light PNA FISH provided an accuracy of 98.6% (74/75) with 75 positive blood cultures as compared to standard methods.

**Conclusion:** GNR Traffic Light PNA FISH provides a highly accurate identification of 3 major pathogens in under 90 minutes. This assay improves upon currently available PNA FISH assays by providing specific identification of *K. pneumoniae* from positive blood culture bottles.

## Introduction

Identification of Gram-negative rods from positive blood cultures currently necessitates growth on solid media followed by biochemical analysis requiring 18 to 36 hours. Identification of the organism allows for initiation of appropriate antibiotic therapy; therefore, the time to identification is critical. Three Gram-negative rods commonly found in blood are *E. coli*, *K. pneumoniae* and *P. aeruginosa*.

PNA FISH is a platform that allows for rapid identification of organisms in blood cultures. Currently, there are two commercially available PNA FISH kits that identify gram negative rods, *E. coli*/*P. aeruginosa* and EK/*P. aeruginosa* PNA FISH. However, it is not possible to differentiate *K. pneumoniae*. GNR Traffic Light PNA FISH uses the same platform as the currently available PNA FISH kits but allows for simultaneous detection of *E. coli*, *P. aeruginosa* and *K. pneumoniae*.

Recently, an increase in infections caused by multidrug resistant Gram-negative organisms, *K. pneumoniae* in particular, has been reported in

major medical centers. A resistant strain known as KPC (*Klebsiella pneumoniae*- Carbapenemase) is steadily emerging; which is a hazard to patient safety since carbapenem antibiotics are usually the last line of defense against multi-drug resistant bacteria<sup>1</sup>. These facts highlight the importance of rapid identification of these organisms.

GNR Traffic Light PNA FISH, a 3-color multiplex assay, allows for identification of these three organisms within 90 minutes of a positive blood culture. This assay generates a green fluorescent signal for *E. coli*, a red fluorescent signal for *P. aeruginosa* and a yellow fluorescent signal for *K. pneumoniae*.

## Materials and Methods

75 unknown blood cultures, from the clinical microbiology laboratory Lahey Clinic (Burlington, MA), containing Gram-negative rods were tested. This study compared GNR Traffic Light PNA FISH results with standard culture identification (Microscan, Siemens and API 20, bioMérieux). See Figure 1 for PNA FISH procedure. *E. coli* was identified as green fluorescent cells, *K. pneumoniae* as yellow and *P. aeruginosa* as red (Figure 2).

**Figure 1:** GNR Traffic Light PNA FISH Procedure



### Prepare Smear

- Add drop of GN Fixation Solution
- Add drop from BC+
- Heat Fix GNR onto slide for 20 min. at 55°C

### Hybridize

- Add PNA probe
- Add coverslip
- Hybridize for 30 min. at 55°C

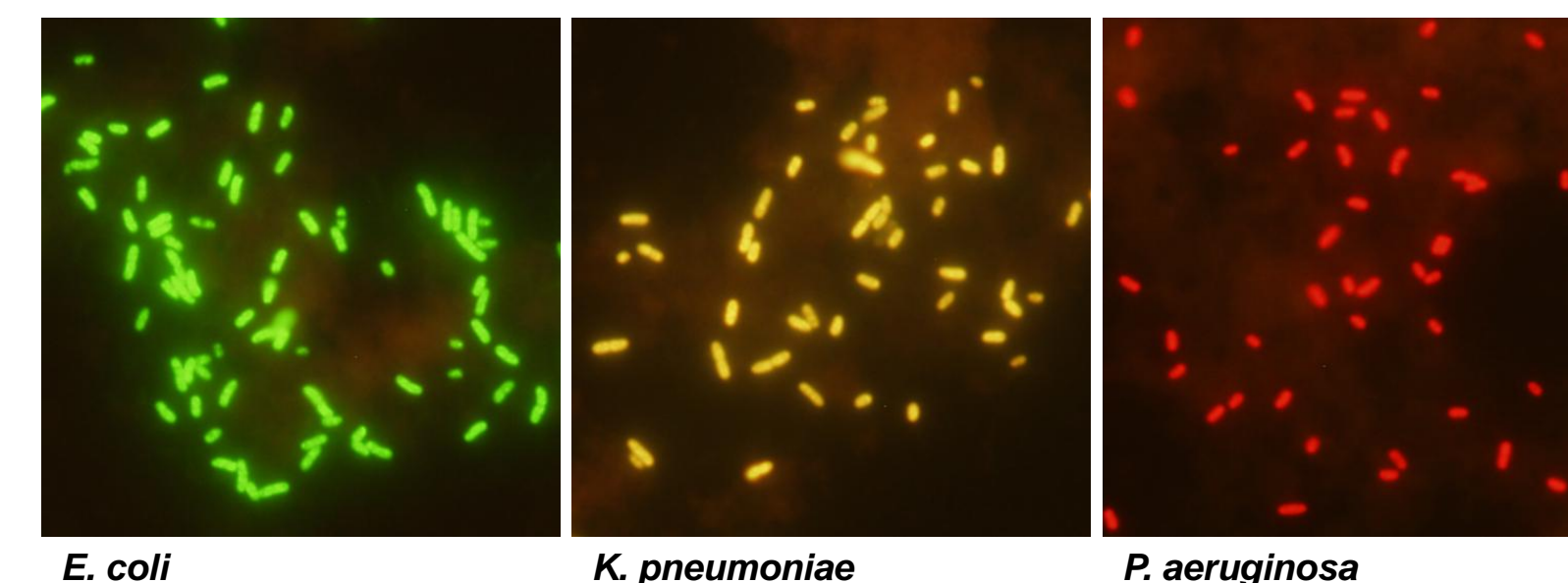
### Wash

- Immerse slide in dH<sub>2</sub>O and remove coverslip
- Transfer slide to Wash Solution
- Wash for 30 min. at 55°C

### View Results

- Examine using fluorescence microscope equipped with a FITC/Texas Red filter and 60x or 100x oil objective

**Figure 2:** GNR Traffic Light PNA FISH Results



## Results

**Table 1:** GNR Traffic Light Results for Unknown Blood Cultures

| Conventional ID  | GNR Traffic Light™ Result |        |     |          |
|--|---------------------------|--------|-----|----------|
|  | GREEN                     | YELLOW | RED | NEGATIVE |
| <i>Escherichia coli</i>                                | 30                        |        |     |          |
| <i>Escherichia coli</i> & CNS                          | 2                         |        |     |          |
| <i>Escherichia coli</i> & <i>Klebsiella pneumoniae</i> | 2                         | 1*     |     |          |
| <i>Pseudomonas aeruginosa</i>                          |                           |        | 8   |          |
| <i>Klebsiella pneumoniae</i>                           |                           | 9      |     |          |
| <i>Klebsiella oxytoca</i>                              |                           |        |     | 4        |
| <i>Clostridium spp.</i>                                |                           |        |     | 4        |
| <i>Clostridium tertium</i>                             |                           |        |     | 1        |
| <i>Bacteroides vulgatus</i>                            |                           |        |     | 1        |
| <i>Citrobacter freundii</i>                            |                           |        |     | 1        |
| <i>Sphingomonas paucimobilis</i>                       |                           |        |     | 1        |
| <i>Moraxella spp.</i>                                  |                           |        |     | 1        |
| <i>Bacteroides thetaiotamicron</i>                     |                           |        |     | 1        |
| <i>Bacteroides fragilis</i> group                      |                           |        |     | 1        |
| <i>Enterobacter cloacae</i>                            |                           |        |     | 4        |
| Coagulase-negative <i>Staphylococcus</i>               |                           |        |     | 1        |
| <i>Proteus mirabilis</i>                               |                           |        |     | 1        |
| <i>Enterobacter aerogenes</i>                          |                           |        |     | 2        |
| <i>Pseudomonas luteola</i>                             |                           |        |     | 1        |

\*GNR Traffic Light PNA FISH positive for *E. coli*, *K. pneumoniae* was not detected during initial testing. Retesting of isolates with GNR Traffic Light PNA FISH correctly identified both *E. coli* and *K. pneumoniae*.

All *E. coli* (34/34) produced a green positive result, the majority of *K. pneumoniae* (10/11) produced a yellow positive result and all *P. aeruginosa* (8/8) produced a red positive result. GNR Traffic Light PNA FISH provided an accuracy of 98.6% (74/75) with 75 positive blood cultures as compared to standard methods (Table 1).

The 1 false negative result (*K. pneumoniae*) was found in a mixed culture of *E. coli* and *K. pneumoniae*. Retesting of the isolate produced a positive yellow result. It is likely the false result was due to a CFU level of *K. pneumoniae* below the sensitivity (10<sup>5</sup> CFU/mL) of the assay caused by the presence of another organism.

## Conclusion

GNR Traffic Light PNA FISH provides a highly accurate identification of three major pathogens in under 90 minutes. This assay improves upon currently available PNA FISH assays by providing specific identification of *K. pneumoniae* from positive blood culture bottles.

The simultaneous identification of three significant pathogens in less than 90 minutes permits for a substantial reduction in time to species identification compared to standard methods. Same day species identification of blood cultures containing Gram-negative rods will allow physicians to make optimal treatment decisions in a time not currently possible.

## Bibliography

1. United States. Centers for Disease Control and Prevention. Department of Health and Human Services. *Klebsiella pneumoniae* (*K. pneumoniae*). 17 Mar. 2009. 10 Apr. 2010 <[http://www.cdc.gov/ncidod/dhqp/ar\\_kp.html](http://www.cdc.gov/ncidod/dhqp/ar_kp.html)>.