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NOTES

Identification of *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* in Blood Cultures: a Multicenter Performance Evaluation of a Three-Color Peptide Nucleic Acid Fluorescence *In Situ* Hybridization Assay▽

Phyllis Della-Latta,[1] Hossein Salimnia,[2] Theresa Painter,[3] Fann Wu,[1] Gary W. Procop,[4] Deborah A. Wilson,[4] Wendy Gillespie,[5] Alayna Mender,[6] and Benjamin S. Crystal[6]*

Columbia University Medical Center, NewYork-Presbyterian Hospital, New York, New York[1]; Detroit Medical Center and Wayne State University School of Medicine, Detroit, Michigan[2]; Detroit Medical Center, Detroit, Michigan[3]; Cleveland Clinic, Cleveland, Ohio[4]; Lahey Clinic, Burlington, Massachusetts[5]; and AdvanDx Inc., Woburn, Massachusetts[6]

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A multicenter evaluation was undertaken to evaluate the performance of a new three-color peptide nucleic acid fluorescence *in situ* hybridization assay that identifies isolates directly from blood cultures positive for Gram-negative bacilli (GNB). The assay correctly identified 100% (186/186) of the *Escherichia coli* isolates, 99.1% (109/110) of the *Klebsiella pneumoniae* isolates, and 95.8% (46/48) of the *Pseudomonas aeruginosa* isolates in this study. Negative assay results were correctly obtained for 162 of 165 other GNB (specificity, 98.2%).

Gram-negative bacilli (GNB) are associated with bloodstream infections (BSI) resulting in significant mortality, particularly in patients in intensive care units (ICUs). Among the most prevalent GNB pathogens are *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* (4, 8). The selection of empirical therapy for GNB infections is challenging due to inherent antimicrobial resistance among many *P. aeruginosa* strains and emerging resistance to carbapenem antimicrobial agents among *K. pneumoniae* isolates. Rapid same-day pathogen identification (ID) directly from newly positive blood culture bottles can impact the appropriate selection of empirical therapy, an important factor in improving patient outcomes (7). Peptide nucleic acid fluorescence *in situ* hybridization (PNA FISH) assays (AdvanDx Inc.) have been shown to compare favorably to automated phenotypic ID methods and can produce results in approximately 90 min (10). A limitation of the current FDA-cleared GNB PNA FISH assays (*E. coli*/*P. aeruginosa* PNA FISH and *E. coli*, *K. pneumoniae* [EK]/*P. aeruginosa* PNA FISH) is the inability to differentiate *E. coli* from *K. pneumoniae*. This multicenter study evaluated the performance of GNR (Gram-negative rod) Traffic Light PNA FISH (not FDA cleared at the time of this study), the first rapid PNA FISH assay to identify 3 major GNB (*E. coli*, *K. pneumoniae*, and *P. aeruginosa*) directly from newly positive blood cultures.

Four U.S. study sites collected GNB-positive clinical blood cultures. The phenotypic ID method for each laboratory was performed using either MicroScan (Siemens Healthcare Diagnostics, Tarrytown, NY) or VITEK2 (bioMérieux, Inc., Durham, NC). Data collected for this study had no patient identifiers, and only blood cultures drawn for clinical purposes were used. Institutional Review Board (IRB) approval or exemption was obtained for the study protocol from each institution. Each site used one of the following three automated, continuously monitoring blood culture systems: BacT/Alert (bioMérieux, Inc., Durham, NC), BACTEC (BD Diagnostics, Spark, MD), or VersaTrek (Trek Diagnostic Systems, Cleveland, OH). For each sample, GNR Traffic Light PNA FISH was performed, according to the manufacturer's instructions. Slides were examined with fluorescence microscopy (60× or 100× oil objective, dual-bandpass fluorescein isothiocyanate/Texas Red filter). Results were interpreted as positive when fluorescent cells were seen in multiple fields of view, as follows: green for *E. coli*, red for *P. aeruginosa*, and yellow for *K. pneumoniae*. Additionally, EK/*P. aeruginosa* PNA FISH was performed on each sample (data not shown). GNR Traffic Light PNA FISH was performed concurrently with the routine laboratory ID on excess blood culture material. The operator of the PNA FISH testing at each study site was blinded to the results of the routine laboratory ID until all testing was complete.

A total of 490 clinical blood cultures positive for GNB by Gram staining, and 4 blood culture bottles spiked with clinical *P. aeruginosa* isolates were included in the study. Automated phenotypic ID methods identified 537 isolates, including 43 in mixed growth cultures of 2 or more organisms. Of the organisms identified, 186 (34.6%) were *E. coli*, 110 (20.5%) were *K. pneumoniae*, and 48 (8.9%) were *P. aeruginosa*. Of the 43 mixed cultures, 15 were identified as having 2 of the GNR Traffic Light PNA FISH target organisms

* Corresponding author. Mailing address: AdvanDx Inc., 400 TradeCenter, Woburn, MA 01801. Phone: (781) 376-0009. Fax: (781) 376-0111. E-mail: bsc@advandx.com.

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TABLE 1. Performance characteristics of the GNR Traffic Light PNA FISH assay[g]

Study site	GNR Traffic Light PNA FISH assay result (no. of isolates correctly identified/total no. of isolates [%])				Blood culture system[e]
	Sensitivity			Specificity of other GNB	
	E. coli	*K. pneumoniae*	*P. aeruginosa*		
A	35/35	17/17	7/9[b]	35/37[c]	BACTEC
B	31/31	30/30	11/11	36/36	BacT/Alert
C	87/87	54/54	16/16	62/63[d]	BACTEC
D	33/33	8/9[a]	12/12[f]	29/29	VersaTrek
Total	186/186 (100)	109/110 (99.1)	46/48 (95.8)	162/165 (98.2)	

[a] One false negative for *K. pneumoniae* was in a mixed culture of *E. coli* and *K. pneumoniae.*
[b] Both false negatives were in mixed cultures of *P. aeruginosa* and *K. pneumoniae.*
[c] One *E. cloacae* sample produced a green false positive (negative upon retest), and one *A. baumannii* sample produced a red false positive.
[d] One false-positive *A. radioresistens* sample in a mixed culture with *Enterococcus faecalis.*
[e] A total of 509 blood cultures were tested, including 15 mixed cultures with dual-color PNA FISH results.
[f] Includes 4 spiked samples.
[g] The 95% confidence intervals for *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and other GNB were 98.4 to 100, 95.0 to 99.9, 85.8 to 99.5, and 94.8 to 99.6, respectively.

present and were therefore expected to display 2 fluorescence results. GNR Traffic Light PNA FISH correctly identified 100% (186/186) of the *E. coli* isolates, 99.1% (109/110) of the *K. pneumoniae* isolates, and 95.8% (46/48) of the *P. aeruginosa* isolates (Table 1). One false negative for *K. pneumoniae* and two false negatives for *P. aeruginosa*, all in mixed cultures, were recorded (isolates were not available for retesting). According to the manufacturer's labeling of the other PNA FISH assays, such as *E. coli*/*P. aeruginosa* PNA FISH, the limit of detection (LOD) is 10^5 CFU. In mixed cultures, it is possible that one organism may out grow the other and that the bottle may signal positive on the automated blood culture device before both organisms reach this LOD for the PNA FISH assay. The specificity of the test was 98.2% (162/165). Mixed cultures which contained more than one isolate other than *E. coli*, *P. aeruginosa*, or *K. pneumoniae* (28 in all) were counted once in the specificity calculations, since only one negative result could be expected to be observed at a time. One *Enterobacter cloacae* sample gave a false-positive *E. coli* (green) result (negative upon repeat testing), and one *Acinetobacter baumannii* sample gave a false-positive *P. aeruginosa* (red) result (isolate/sample unavailable for additional testing). Another false-positive *P. aeruginosa* (red) result occurred with a sample containing *Acinetobacter radioresistens*, a rare clinical isolate. Analysis of publically available sequences (http://www.ncbi.nlm.nih.gov) revealed a partial complementary sequence similarity for *A. radioresistens* with the *P. aeruginosa* PNA probe.

Inappropriate initial antibiotic selection and delays in starting effective antimicrobial therapy have been shown to adversely impact mortality rates in patients with GNB BSI (6, 7, 9) and, for *Pseudomonas* BSI, have been associated with a longer length of hospital stay (9). Rapid identification of *E. coli*, *P. aeruginosa*, or *K. pneumoniae* from blood culture bottles has the potential to impact clinical decisions by allowing an early choice of effective targeted antimicrobial therapy and, hence, better patient outcomes and reduced lengths of hospital stays. Once the species has been identified, a therapeutic regimen with antipseudomonal activity such as tobramycin or piperacillin-tazobactam may be chosen for *P. aeruginosa* infection and ceftriaxone or other suitable drugs for *E. coli* bacteremia. Early identification of *K. pneumoniae* in the blood culture would enable rapid initiation of appropriate therapy based on the resistance pattern of this organism in a given institution, such as the rate of extended-spectrum β-lactamase (ESBL) carriage or prevalence of carbapenem resistance. Rapid PNA FISH results negative for *E. coli*, *P. aeruginosa*, or *K. pneumoniae* have been shown to indicate an increase in the likelihood of an isolate being resistant to a cephalosporin, further supporting the concept of rapid pathogen ID impacting therapeutic decisions (5).

This study demonstrated that GNR Traffic Light PNA FISH is a highly sensitive and specific assay for identifying the most common GNB recovered from newly positive blood culture bottles. The time to results for GNR Traffic Light PNA FISH from a positive blood culture is approximately 90 min (hands-on technologist time is approximately 10 min per test), compared to 1 to 3 days for conventional phenotypic methods. While assessing the actual cost of GNR Traffic Light PNA FISH implementation was beyond the scope of this study, the approximate cost per test based on the manufacturer's list price is $39 (not including controls), with a Medicare reimbursement of $84.66. As suggested by studies of other PNA FISH assays, lab expenses for implementation of GNR Traffic Light PNA FISH would be justifiable when measured against improved patient outcomes, reduced lengths of hospital stays, and judicious use of antimicrobial therapy (1, 2, 3). The direct effect of GNR Traffic Light PNA FISH on the selection of appropriate empirical therapy, subsequent patient outcomes, and cost benefits would be areas of interest for future studies.

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