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Multicenter Evaluation of a New Shortened Peptide Nucleic Acid Fluorescence *In Situ* Hybridization Procedure for Species Identification of Select Gram-Negative Bacilli from Blood Cultures[▽]

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A shortened protocol for two peptide nucleic acid fluorescence *in situ* hybridization (PNA FISH) assays for the detection of Gram-negative bacilli from positive blood cultures was evaluated in a multicenter trial. There was 100% concordance between the two protocols for each assay (368 of 368 and 370 of 370 results) and 99.7% (367 of 368 and 369 of 370 results) agreement with routine laboratory techniques.

Bloodstream infections (BSI) caused by Gram-negative bacilli (GNB) are associated with significant mortality representing roughly 22% of all BSI (1, 4). In recent years, increased multidrug resistance in these pathogens has highlighted the importance of rapid identification and appropriate therapy (2, 3, 6). Conventional phenotypic and biochemical methods for identification of GNB bacteremia routinely involve Gram staining and subculturing from positive blood cultures followed by testing with biochemical and/or automated devices and may require 1 to 3 days to complete. The peptide nucleic acid fluorescence *in situ* hybridization (PNA FISH) platform provides identification of select GNB within hours. Three FDA-cleared GNB PNA FISH assays (AdvanDx, Woburn, MA) are currently available: *Escherichia coli* PNA FISH (single-color assay), *E. coli*/*Pseudomonas aeruginosa* PNA FISH (dual-color assay distinguishing *E. coli* and *P. aeruginosa*), and EK/*P. aeruginosa* PNA FISH (dual-color assay distinguishing *P. aeruginosa* from *E. coli* and/or *Klebsiella pneumoniae*).

The purpose of this study was to compare the performances of shortened procedures for *E. coli*/*P. aeruginosa* PNA FISH and EK/*P. aeruginosa* PNA FISH to the original procedures and to conventional laboratory identification techniques.

A total of 368 GNB-positive blood cultures bottles from four U.S. sites were evaluated. All samples were deidentified remnant clinical specimens; routine identification results were left blinded until PNA FISH testing was completed. Two automated microbial blood culture detection systems were used: BacT/Alert 3D (bioMérieux, Durham, NC) and Bactec 9240 (BD, Cockeysville, MD). The GNB-positive BacT/Alert bottles included 49 standard aerobic (SA) and 51 standard anaerobic (SN). GNB-positive Bactec bottles included 61 standard/10 aerobic, 56 standard anaerobic, 62 plus aerobic, 35 plus anaerobic, 41 lytic/10 anaerobic, and 13 Peds Plus. The study protocol was approved by the institutional review board at each institution.

Samples were analyzed with both *E. coli*/*P. aeruginosa* and

TABLE 1. Performance of shortened *E. coli*/*P. aeruginosa* PNA FISH versus routine laboratory identification methods and standard PNA FISH

Study site	Sensitivity for *E. coli*		Sensitivity for *P. aeruginosa*		Specificity		Blood culture system used
	Routine	Stnd[a]	Routine	Stnd	Routine	Stnd	
A	51/51	51/51	12/12	12/12	54/54	54/54	Bactec
B	51/51	51/51	9/9	9/9	40/40	40/40	BacT/Alert
C	17/17	17/17	7/7	7/7	51/51	51/51	Bactec
D	32/32	32/32	7/8[b]	7/7	36/36	37/37	Bactec
Total (n = 368)	100% (151/151)	100% (151/151)	97.2% (35/36)	100% (35/35)	100% (181/181)	100% (182/182)	
95% CI	98.0–100	98.0–100	85.5–99.9	91.8–100	98.4–100	98.4–100	

[a] Stnd, standard PNA FISH procedure.
[b] One sample was identified as a mixed *P. aeruginosa*/VRE culture by routine methods and was negative for both the rapid and standard procedures.

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TABLE 2. Performance of shortened EK/*P. aeruginosa* PNA FISH versus routine laboratory identification methods and standard PNA FISH

Study site	Sensitivity for *E. coli* and/or *K. pneumoniae*		Sensitivity for *P. aeruginosa*		Specificity		Blood culture system used
	Routine	Stnd[a]	Routine	Stnd	Routine	Stnd	
A	65/65[b]	65/65[b]	12/12	12/12	42/42	42/42	Bactec
B	69/69	69/69	9/9	9/9	22/22	22/22	BacT/Alert
C	34/34	34/34	7/7	7/7	34/34	34/34	Bactec
D	47/47	47/47	7/8[c]	7/7[c]	21/21	22/22	Bactec
Total (n = 370)	100% (215/215)	100% (215/215)	97.2% (35/36)	100% (35/35)	100% (119/119)	100% (120/120)	
95% CI	98.6–100	98.6–100	85.5–99.9	91.8–100	97.5–100	97.5–100	

[a] Stnd, standard PNA FISH procedure.
[b] Two mixed *P. aeruginosa* and *K. pneumoniae* samples.
[c] One sample was identified as a mixed *P. aeruginosa*/VRE culture by routine methods and was negative for both the rapid and standard procedures.

EK/*P. aeruginosa* PNA FISH assays using both the standard and shortened procedures (four tests per sample). The two assays share the same procedure and components, with the exception of the specific PNA FISH probe solution. Results of the PNA FISH assays were read independently by test operators blinded to results obtained by standard methods: Vitek (bioMérieux), MicroScan (Siemens), and/or API (bioMérieux). The shortened procedure eliminated the 10-min ethanol immersion step and shortened the hybridization step from 90 to 30 min. Slides were examined under a fluorescence microscope (60× or 100× oil objective) equipped with a dual-bandpass fluorescein isothiocyanate/Texas Red (FITC/TXR) filter. For both assays, positive samples were determined as multiple bright fluorescent rods in multiple fields of view: green for *E. coli* and red for *P. aeruginosa*. In the EK/*P. aeruginosa* PNA FISH assay, *K. pneumoniae* and *E. coli* were identical in morphology and degree of green fluorescence.

Sensitivity, specificity, and total agreement were calculated. Exact 95% confidence intervals (CIs) were determined using the online calculator provided at http://www.measuringusability.com/.

A total of 383 GNB (from 368 blood cultures; 15 mixed cultures), including 151 *E. coli*, 36 *P. aeruginosa*, and 65 *K. pneumoniae* isolates and 131 organisms from 35 other species, were identified by the routine techniques of the four laboratories. There was 100% agreement between the new and standard procedures for both *E. coli*/*P. aeruginosa* PNA FISH and EK/*P. aeruginosa* PNA FISH (368 of 368 and 370 of 370 results, respectively). In two mixed cultures of *P. aeruginosa* and *K. pneumoniae*, EK/*P. aeruginosa* PNA FISH produced both red and green results, which accounts for the greater number of positive results obtained by EK/*P. aeruginosa* PNA FISH (370) than the number of culture bottles sampled (368).

Compared to routine identification methods, all 151 (100% sensitivity) *E. coli* isolates were correctly identified by both the standard and shortened procedures of *E. coli*/*P. aeruginosa* PNA FISH. Likewise, all 215 (100% sensitivity) *E. coli* and/or *K. pneumoniae* isolates were correctly identified by both of the EK/*P. aeruginosa* PNA FISH procedures. The sensitivity for *P. aeruginosa* for both assays with both procedures was 97.2% (35 of 36 results). One sample which contained a mixed culture of *P. aeruginosa* and vancomycin-resistant *Enterococcus* (VRE) was negative for *P. aeruginosa* by both PNA FISH methods and assays. The specificity of *E. coli*/*P. aeruginosa* PNA FISH for both procedures was 100% (181 of 181 results) and 100% (119 of 119 results) for EK/*P. aeruginosa* PNA FISH compared to routine methods (Tables 1 and 2).

This multicenter evaluation found both *E. coli*/*P. aeruginosa* and EK/*P. aeruginosa* PNA FISH to have excellent agreement between the shortened and standard procedures as well as with standard laboratory techniques. Both PNA FISH assays failed to detect *P. aeruginosa* in one mixed culture with VRE. Isolates from the sample were not available for further analysis.

A total of 65 samples (including 7 mixed cultures) were identified as having *K. pneumoniae* by conventional techniques. While the EK/*P. aeruginosa* PNA FISH is not designed to distinguish between *E. coli* and *K. pneumoniae*, PNA FISH informs the provider that a member of the *Enterobacteriaceae* (versus a *Pseudomonas* species) is causing the BSI, providing potentially important information for guiding more appropriate empirical therapy earlier in the septic course, an improvement over Gram stain morphology alone (5). As *P. aeruginosa* is resistant to many antibiotics used for *E. coli* and *K. pneumoniae* and the incidence of resistance to extended-spectrum beta-lactamases and carbapenemases in *K. pneumoniae* is expected to increase, the potential clinical utility of a three-color assay for specific identification of each organism is evident and should be a topic of future investigation. Of further interest in the study were two mixed cultures of *K. pneumoniae* and *P. aeruginosa*, both of which were correctly identified by EK/*P. aeruginosa* PNA FISH in less time than would have been possible by culture-based methods.

Given the increasing resistance to antimicrobial agents, particularly among the GNB, the advent of assays that can identify pathogens within hours represents a potential advantage over traditional methods which may take 1 to 3 days. A limitation of the current studies is that no measurements of workflow time were recorded; therefore, the impact of the shortened protocol on workflow cannot be assessed. Future studies which measure actual assay hands-on time and time to reporting would be helpful in assessing the shortened procedure's impact on workflow and/or time to directed therapy.

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