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Standardized Procedure for Identification of Bacteria and Yeast Directly from Positive Blood Culture within 60 Minutes by Peptide Nucleic Acid Fluorescence In-Situ Hybridization

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Background

Hagerstown Medical Laboratory has implemented rapid molecular based Peptide Nucleic Acid Fluorescent In-Situ Hybridization tests (PNA FISH, AdvanDx Woburn, MA) that provide identification of organisms from positive blood cultures in 60 minutes. The protocol is designed to achieve the final identification of an organism in significantly less time than the standard PNA FISH procedure without comprising accuracy. The rapid results allow the laboratory to report critical results to physicians and pharmacists enabling timely, optimal therapy, thereby reducing patient length of stay and mortality rates.

The PNA FISH results are immediately reported to the unit along with appropriate antibiotic recommendations.

Since beginning this testing, we have performed a total of 466 PNA FISH analyses at a cost of approximately \$25,000. There were 166 positive results (35.6%). Estimating the savings at \$1,800 per positive result, the total savings is approximately \$298,800 (net savings \$273,800). On an annualized basis, the net savings is approximately \$657,000.

Materials and Methods

Three slides were prepared from positive blood culture; one gram stain and two PNA/FISH slides (prepared according to the manufacture package insert). Based on the gram stain morphologies PNA FISH slides were then hybridized with an appropriate probe. One slide was hybridized for 90 minutes, the second slide was prepared with a modified rapid protocol which reduced the hybridization step to 30 minutes and reduced stringent wash from 30 minutes to 15 minutes. For the *E. coli/ Ps. aeruginosa* Culture Identification Kit, the deionized water rinse step was eliminated to standardize all four procedures so that all PNA FISH assays can be prepared at the same time regardless of the morphology type.

Positive Blood culture bottle (BACTEC BD Sparks, MD)

- Plus+ Aerobic/F Medium
- Lytic/10 Anaerobic/F Medium
- Peds Plus+/F Medium

AdvanDx, Inc. PNA FISH (Woburn, MA)

- *Staphylococcus aureus*/Coagulase Negative Staphylococci Culture Identification Kit
- *Enterococcus faecalis*/Other enterococci Culture and Identification Kit
- *Escherichia coli/Pseudomonas aeruginosa* Culture and Identification kit
- *Candida albicans/Candida glabrata* Culture Identification Kit

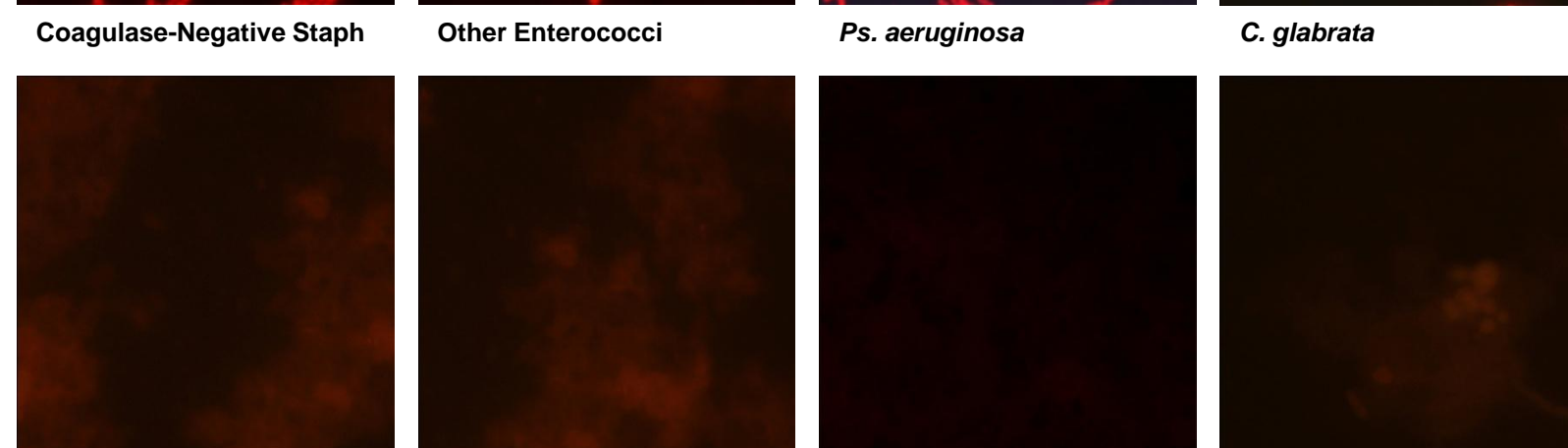
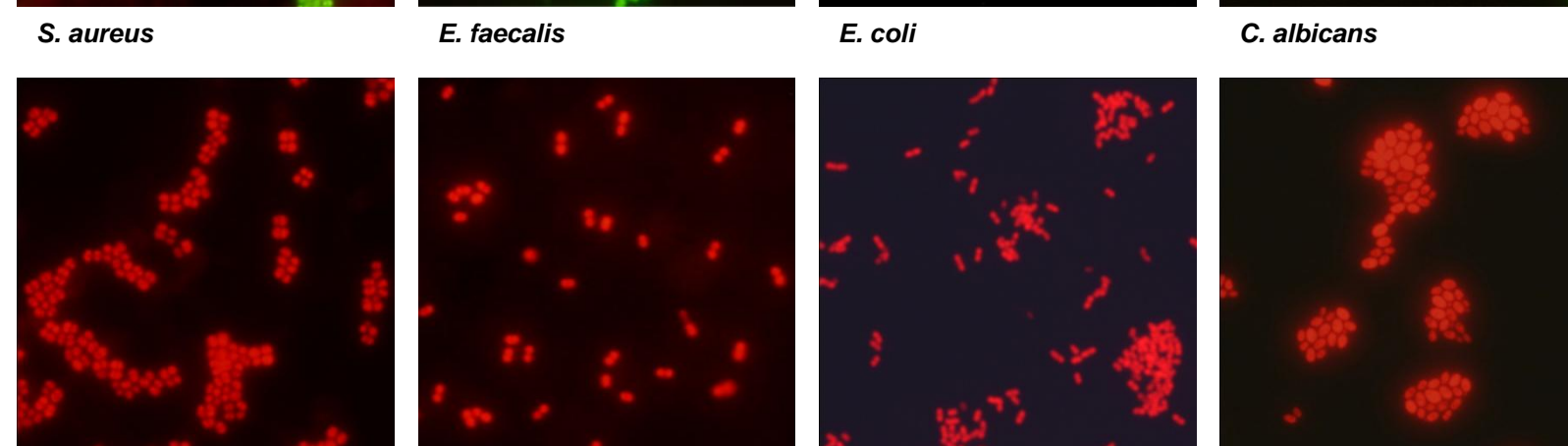
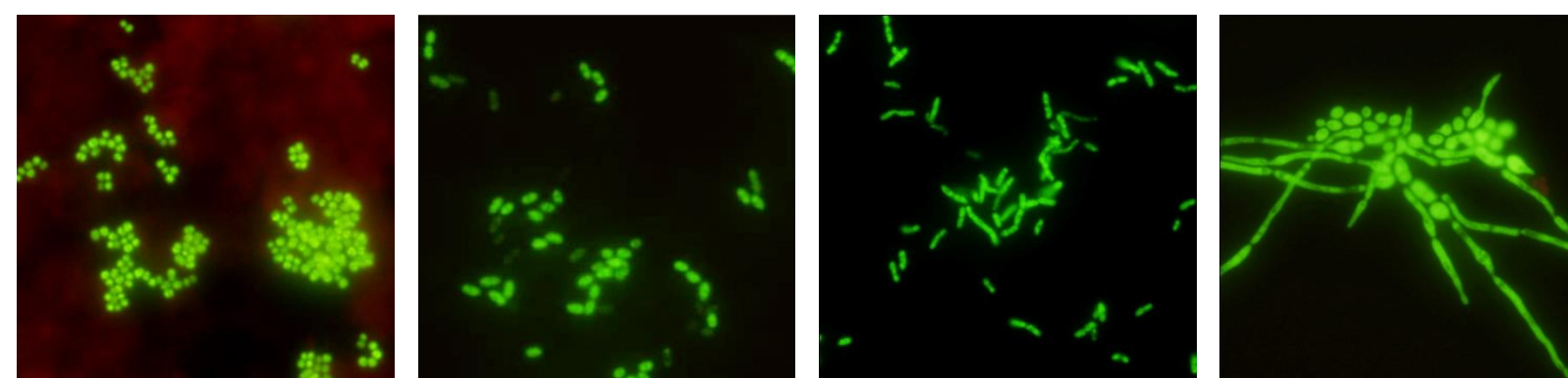
PNA FISH Procedure Steps	Standard Protocol (Time in minutes)	Rapid Protocol (Time in minutes)
Fix the Smear with 95% ETOH	20	10
Hybridization	30	30
**Water rinse	<1 min	-----
***Stringent Wash	30	15

***wash change with each new batch

**this step is only in *E. coli/ Ps. aeruginosa* Culture Identification Kit

*allow slide to air dry

As soon as the slide is dry cover slip with mounting medium and the slides are examine for fluorescent cells using a FITC/Texas Red Dual-band filter on a fluorescence microscope at 60x or 100x oil objectives.



Negative Negative Negative Negative

Results

446 Result of PNA FISH was compared to culture identification demonstrated 100% equivalence between 90 minutes

<i>S. aureus</i> /CNS PNA FISH Performance	PNA FISH Results		
Culture Results	<i>S. aureus</i>	CNS	Negative
Methicillin Sensitive <i>Staphylococcus aureus</i>	71		
Methicillin-Resistant <i>Staphylococcus aureus</i>	61		
<i>Staphylococcus epidermidis</i>		56	
<i>Staphylococcus sp.</i>		31	
<i>Micrococcus species</i>			4

<i>E. faecalis</i> /OE PNA FISH Performance	PNA FISH Results		
Culture Results	<i>E. faecalis</i>	OE	Negative
<i>Enterococcus faecalis</i>	27		
<i>Enterococcus faecium</i>		7	
<i>Streptococcus pneumoniae</i>			14
Beta- hemolytic <i>Streptococcus</i> (A,B,C and G)			10
Alpha-Hemolytic <i>Streptococcus</i>			53

<i>E. coli/ P. aeruginosa</i> PNA FISH Performance	PNA FISH Results		
Culture Results	<i>E. coli</i>	<i>P. aeruginosa</i>	Negative
<i>Escherichia coli</i>	41		
<i>Pseudomonas aeruginosa</i>		12	
Enterobacteriaceae			28
Anaerobes Gram negative rods			5

<i>C. albicans/ C. glabrata</i> PNA FISH Performance	PNA FISH Results		
Culture Results	<i>C. albicans</i>	<i>C. glabrata</i>	Negative
<i>Candida albicans</i>	9		
<i>Candida glabrata</i>		6	
<i>Candida tropicalis</i>			6
<i>Candida parapsilosis</i>			2
<i>Cryptococcus neoformans</i>			4

Performance Summary	Performance Parameters			
PNA FISH Tests	Sensitivity (Green)	Sensitivity (Red)	PPV	NPV
<i>S. aureus</i> /CNS PNA FISH	<i>S. aureus</i> 100%	CNS 100%	100%	100%
<i>E. faecalis</i> /OE PNA FISH	<i>E. faecalis</i> 100%	OE 100%	100%	100%
<i>E. coli/ P. aeruginosa</i> PNA FISH	<i>E. coli</i> 100%	<i>P. aeruginosa</i> 100%	100%	100%
<i>C. albicans/ C. glabrata</i> PNA FISH	<i>C. albicans</i> 100%	<i>C. glabrata</i> 100%	100%	100%

Antibiotic Recommendation according to PNA FISH result at Washington County Hospital (WCH).

- Positive for *E.coli*: ceftriaxone is 99% effective at WCH.
- Positive for *Ps. aeruginosa*: Cefepime is 85% effective at WCH
- Positive for Coagulase negative Staphylococcus: 98% contamination rate, evaluate the need for treatment.
- Positive for *E. faecalis*: penicillins are 86% effective at WCH.
- Positive for Other Enterococcus: linezolid is 94% effective at WCH.
- Negative for Enterococcus: penicillins are 89% effective at WCH.

Conclusion

- The rapid protocol decrease the staining time from 90 minutes to 60 minutes does not affect the intensity of the fluorescence stain.
- Rapid appropriate antimicrobial therapy.
- Reduce patient length of stay and cost saving for the hospital.

Bibliography

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 - I like to thank Tai Ton and Philip Onigman AdvanDx Inc. for providing materials, photograph and technical assistance.
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