

Amended Abstract

Background: *Acinetobacter* is a Gram-negative rod most commonly found in soil and water. However, this organism can also be found on the skin surface of healthy individuals. Although there are many species that can cause human disease, *Acinetobacter baumannii* accounts for 80% of infections reported. Infections most commonly occur in healthcare environments and tend to affect seriously ill or immunocompromised patients.

Acinetobacter PNA FISH® is a rapid fluorescence in situ hybridization (FISH) test using fluorescently labeled peptide nucleic acid (PNA) probes targeting all *Acinetobacter* species. This assay generates a green fluorescent signal for *Acinetobacter* as observed through a fluorescence microscope.

Method: This study included 111 known and 36 unknown samples to determine the performance of the assay. The 36 unknown were routine blood cultures initially identified as Gram-negative rods (GNR) from a clinical microbiology laboratory. The 111 known were ATCC/NRRL strains of various organisms grown in blood cultures overnight. The known strains included 93 GNRs, 9 GPCs, 6 yeasts and 3 GPRs. Smears of blood cultures were heat fixed onto microscope slides, a drop of *Acinetobacter* 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. Positive results were determined by the presence of green fluorescent cells in multiple fields while negative results were determined by the absence of green fluorescence.

Results: *Acinetobacter* PNA FISH® correctly identified 100% (147/147) of the samples as compared to culture. The collective assay sensitivity was 100% (19/19). The specificity was 100% (128/128).

Conclusion: *Acinetobacter* PNA FISH® is a rapid and accurate method for the identification of *Acinetobacter* species directly from blood cultures containing Gram-negative rods. This assay provides precise identification of *Acinetobacter* from positive blood cultures in less than 90 minutes.

Introduction

Acinetobacter is a Gram-negative rod most commonly found in soil and water. However, this organism can also be found on the skin surface of healthy individuals. Although there are many species that can cause human disease, *Acinetobacter baumannii* accounts for 80% of infections reported. Infections most commonly occur in healthcare environments and tend to affect seriously ill or immunocompromised patients¹.

Acinetobacter baumannii has developed significant resistance to multiple antimicrobial drugs, which makes it even more difficult to treat². Known for being associated with nosocomial pneumonia and bacteremia, *A. baumannii* is also becoming associated with infections from trauma patients³. These trauma patients include soldiers who served and were injured in Iraq and Afghanistan; but received treatment in the United States. The *A. baumannii* colonized in the soldiers' wounds then become hospital-acquired infections in immunocompromised patients; which can lead to death due to its multi-drug resistance. Of the numerous infections *Acinetobacter* can cause, blood stream infections are perhaps one of the most severe. Therefore, identifying *Acinetobacter* in a timely matter is crucial for the survival of the patient.

Acinetobacter PNA FISH® is a rapid fluorescence *in situ* hybridization (FISH) test using fluorescently labeled peptide nucleic acid (PNA) probes targeting rRNA targets of all *Acinetobacter* species. This assay generates a green fluorescent signal for *Acinetobacter* as observed through a fluorescence microscope.

Materials and Methods

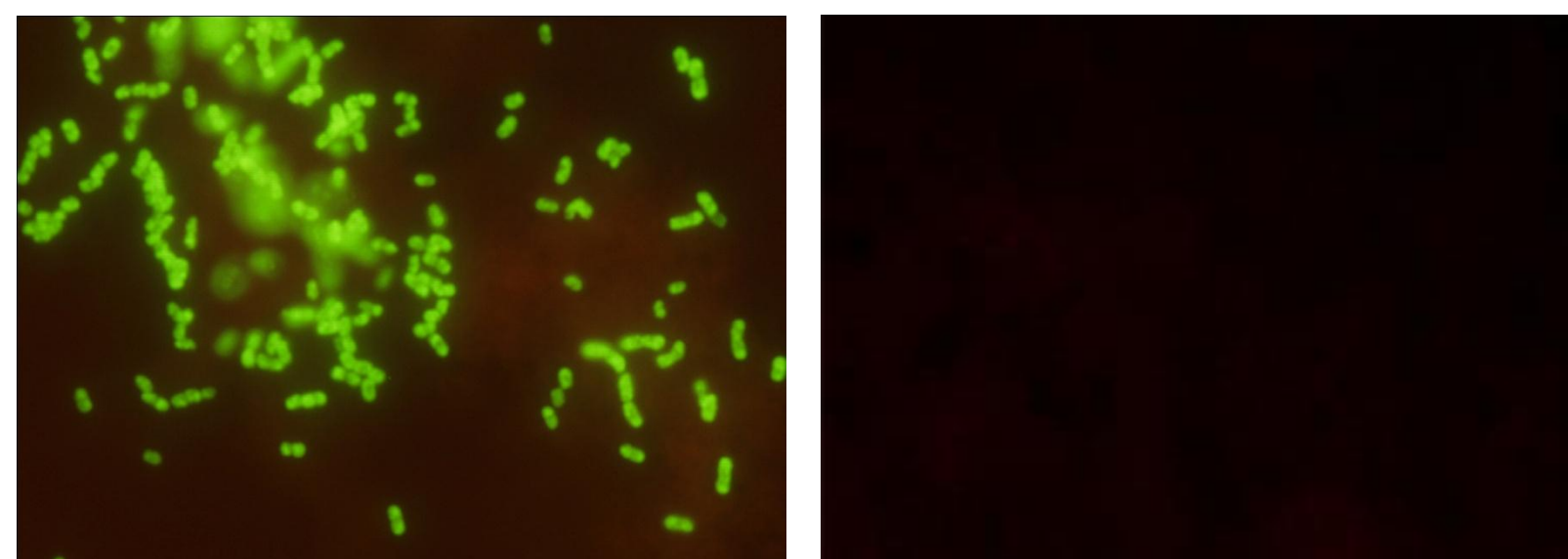
This study included a total of 147 samples to determine the performance of the assay. The study contained 36 unknown and 111 known samples. The 36 unknown were routine blood cultures initially identified as Gram-negative rods (GNR) from the clinical microbiology laboratory at Lahey Clinic (Burlington, MA). The 111 known samples were ATCC/NRRL strains of various organisms grown in blood culture media overnight. The known strains included 93 GNRs, 9 GPCs, 6 yeasts and 3 GPRs. See Figure 1 for PNA FISH procedure.

Figure 1: *Acinetobacter* PNA FISH Procedure



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|--|---|---|--|
| <p>Prepare Smear</p> <ul style="list-style-type: none"> • Add drop of GN Fixation Solution • Add drop from BC+ • Heat Fix GNR onto slide for 20 min. at 55°C | <p>Hybridize</p> <ul style="list-style-type: none"> • Add PNA probe • Add coverslip • Hybridize for 30 min. at 55°C | <p>Wash</p> <ul style="list-style-type: none"> • Immerse slide in dH₂O and remove coverslip • Transfer slide to Wash Solution • Wash for 30 min. at 55°C | <p>View Results</p> <ul style="list-style-type: none"> • Examine using fluorescence microscope equipped with a FITC/Texas Red filter and 60x or 100x oil objective |
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Figure 2: Example Images of *Acinetobacter* PNA FISH



Positive (Left) and Negative (Right)

Results

Table 1: *Acinetobacter* PNA FISH Results for Known Isolates

Organisms	PNA FISH Result
<i>Acinetobacter baumannii</i>	Positive (3/3)
<i>Acinetobacter baylyi</i>	Positive (1/1)
<i>Acinetobacter calcoaceticus</i>	Positive (1/1)
<i>Acinetobacter haemolyticus</i>	Positive (2/2)
<i>Acinetobacter johnsonii</i>	Positive (1/1)
<i>Acinetobacter junii</i>	Positive (1/1)
<i>Acinetobacter lwoffii</i>	Positive (2/2)
<i>Acinetobacter radioresistens</i>	Positive (1/1)
<i>Acinetobacter schindleri</i>	Positive (1/1)
<i>Acinetobacter species</i>	Positive (5/5)
<i>Acinetobacter ursingii</i>	Positive (1/1)
<i>Bacillus species</i>	Negative (2/2)
<i>Bacteroides species</i>	Negative (3/3)
<i>Brevundimonas species</i>	Negative (2/2)
<i>Burkholderia cepacia</i>	Negative (1/1)
<i>Candida species</i>	Negative (4/4)
<i>Citrobacter freundii</i>	Negative (1/1)
<i>Comamonas testosteroni</i>	Negative (1/1)
<i>Corynebacterium jeikeium</i>	Negative (1/1)
<i>Cryptococcus neoformans</i>	Negative (1/1)
<i>Delftia acidovorans</i>	Negative (1/1)
<i>Enterobacter species</i>	Negative (2/2)
<i>Enterococcus species</i>	Negative (2/2)
<i>Escherichia coli</i>	Negative (12/12)
<i>Escherichia hermanii</i>	Negative (1/1)
<i>Escherichia vulneris</i>	Negative (1/1)
<i>Fusobacterium necrophorum</i>	Negative (1/1)
<i>Herbaspirillum huttiense</i>	Negative (1/1)
<i>Klebsiella oxytoca</i>	Negative (1/1)
<i>Klebsiella pneumoniae</i>	Negative (11/11)
<i>Klebsiella pneumoniae subsp. Ozaenae</i>	Negative (1/1)
<i>Klebsiella pneumoniae subsp. Pneumoniae</i>	Negative (1/1)
<i>Klebsiella pneumoniae subsp. Rhinoscleromatis</i>	Negative (1/1)
<i>Pasteurella multocida</i>	Negative (1/1)
<i>Propionibacterium acnes</i>	Negative (1/1)
<i>Proteus species</i>	Negative (3/3)
<i>Pseudomonas perticinigena</i>	Negative (1/1)
<i>Pseudomonas aeruginosa</i>	Negative (7/7)
<i>Pseudomonas alcaligenes</i>	Negative (1/1)
<i>Pseudomonas chlororaphis</i>	Negative (1/1)
<i>Pseudomonas fluorescens</i>	Negative (2/2)
<i>Pseudomonas fulva</i>	Negative (1/1)
<i>Pseudomonas luteola</i>	Negative (1/1)
<i>Pseudomonas mendocina</i>	Negative (1/1)
<i>Pseudomonas mucidolens</i>	Negative (1/1)
<i>Pseudomonas nitroreducens</i>	Negative (1/1)
<i>Pseudomonas pseudoalcaligenes</i>	Negative (1/1)
<i>Pseudomonas putida</i>	Negative (2/2)
<i>Pseudomonas stutzeri</i>	Negative (1/1)
<i>Pseudomonas veronii</i>	Negative (1/1)
<i>Saccharomyces cerevisiae</i>	Negative (1/1)
<i>Salmonella enterica</i>	Negative (1/1)
<i>Serratia marcescens</i>	Negative (1/1)
<i>Shigella species</i>	Negative (3/3)
<i>Staphylococcus species</i>	Negative (4/4)
<i>Stenotrophomonas maltophilia</i>	Negative (1/1)
<i>Streptococcus species</i>	Negative (2/2)

Table 2: *Acinetobacter* PNA FISH Results for Unknown Blood Cultures

Organisms	PNA FISH Result
<i>Escherichia coli</i>	Negative (18/18)
<i>Klebsiella pneumoniae</i>	Negative (3/3)
<i>Pseudomonas aeruginosa</i>	Negative (4/4)
<i>Escherichia coli</i> & Coagulase-negative <i>Staphylococcus</i>	Negative (2/2)
<i>Escherichia coli</i> & <i>Klebsiella pneumoniae</i>	Negative (2/2)
Coagulase-negative <i>Staphylococcus</i>	Negative (1/1)
<i>Proteus mirabilis</i>	Negative (1/1)
<i>Enterobacter cloacae</i>	Negative (2/2)
<i>Enterobacter aerogenes</i>	Negative (2/2)
<i>Bacteroides thetaiotamicron</i>	Negative (1/1)

Acinetobacter PNA FISH® correctly identified 100% (147/147) of the samples. The collective assay sensitivity was 100% (19/19). The specificity was 100% (128/128).

Conclusion

Acinetobacter PNA FISH® is a rapid and accurate method for the identification of *Acinetobacter* species directly from cultures containing Gram-negative rods. This assay provides precise identification of *Acinetobacter* from positive blood cultures in less than 90 minutes.

The rapid identification of this organism will allow the appropriate intervention in respect to treatment considerations and the prevention of hospital acquired infections.

Bibliography

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3. Sebeny PJ, Riddle MS, Petersen K. *Acinetobacter baumannii* skin and soft-tissue infection associated with war trauma. Clin Infect Dis. 2008 Aug 15;47(4):444-9.