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Direct Identification of *Streptococcus pyogenes* by a Rapid Intrinsic Fluorescence Method

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Abstract

Background: A study was performed to determine the ability of an investigational, rapid, optical analysis system to detect and identify *Streptococcus pyogenes* (group A streptococci, GAS) from pharyngeal specimens when compared to culture and organism identification methods. POCARED™ P-1000™ system (POCARED Diagnostics Ltd.) is an automated rapid system that employs intrinsic fluorescence, optical data analysis and artificial intelligence methods to analyze multi-dimensional optical characteristics of microorganisms. The P-1000™ captures the emitted light from the interaction between photons and molecules to detect the pathogens' unique optical properties and subsequently an algorithm determines results.

Methods: Clinical ESwab™ (Copan, Italy) specimens were first plated onto *Streptococcus* selective agar plates (Hy Labs, Israel). Bacitracin discs were placed on the densest area on each inoculated agar plate. An agglutination test for GAS (BD Streptocard™ Enzyme Latex Test) was performed on each hemolytic isolate in order to confirm the presence of GAS. Following inoculation, the remaining volume of each clinical ESwab™ solution was diluted 1:20 in phosphate buffered saline (PBS) followed by 5µm filtration and then processed with POCARED™'s Samples Processor (SP). Subsequently, each processed clinical ESwab™ sample was analyzed by the P-1000™ system.

Results: There were 627 pharyngeal ESwabs™ tested. The standard plate culture and identification methods yielded 143 positives and 484 negatives for GAS. POCARED™'s P-1000™ system correctly identified 135/143 positive specimens and 460/484 negative specimens. The sensitivity and specificity for GAS were 94% and 95%, respectively.

Conclusion: In summary, POCARED™'s P-1000™ system is a fully automated platform that can rapidly and accurately detect and identify GAS in 5 min. directly from the clinical specimen eliminating the need for media, reagents organism isolation, and further testing.

Introduction:

Streptococcus pyogenes (group A streptococci, GAS) is responsible for a wide range of symptomatology ranging from mild pharyngitis and impetigo to severe invasive soft tissue infections, which may result in death (1). A recent increase in variety, severity and sequelae of *Streptococcus pyogenes* infections, and a resurgence of severe invasive infections was reported (2). GAS is the most common bacterial cause of pharyngitis, accounting for 10% to 30% of all cases (3). The current gold standard for laboratory diagnosis of GAS pharyngitis is a culture of pharyngeal swab specimen onto *Streptococcus* selective agar. These cultures are screened for the presence of beta-hemolytic colonies, which are positively identified as GAS using standard biochemical tests e.g., catalase, pyrrolidonyl arylamidase, and latex agglutination for type-specific antigen tests. While sensitive, culture requires up to 48 h of incubation for detection of GAS eliminating the ability to definitely diagnose the infection during the patient visit.(3). Rapid accurate detection of GAS in pharyngeal specimens from individuals suffering from pharyngitis aids in optimizing the management of antibiotic therapy for these patients.

POCARED™ P-1000™ Platform (Figure 1) is an automated, rapid, reagentless platform that employs intrinsic fluorescence, optical data analysis and artificial intelligence methods to analyze multi-dimensional optical characteristics of microorganisms. An advance fluorometer utilizes a UV light source to excite the microorganisms in the sample to create fluorescent energy (autofluorescence). Neither reagents nor tagging is required to generate the autofluorescence signal. The intensity and spectral content of the autofluorescence signal is captured by a photodetector. The captured signal is converted into a mathematical model that is used to provide the results.

In this study we've determined the ability of POCARED™ P-1000™ Platform to detect and identify GAS from clinical ESwabs™ of pharyngeal specimens.

Methods:

There were 627 pharyngeal ESwabs™ (Copan, Italy) tested and analyzed in this study. These ESwabs™ were first cultured onto Streptococcus selective agar (Hy labs, Israel), and then the remaining samples' volumes were processed by POCARED™'s Sample Processor (SP) and analyzed by the P-1000™ Platform as follows:

1. <u>Culture of clinical pharyngeal ESwabs™:</u>

1.1 Each ESwab™ sample was plated by the Walk-Away Specimen Processing (WASP®) automated instrument (Copan, Italy), using the 30µl loop, onto *Streptococcus* selective agar.

1.2 A 0.04U Bacitracin disc (BD, USA) was placed on the densest area of each inoculated agar plate.

1.3 All plates were incubated at 36°C with 5% CO_2 (enhance the β-hemolysis) for up to 24

hours (Figure 2). Colonies demonstrating no β-hemolysis after the first 24 hours were reincubated for additional 24 hours.

2. SP and P-1000™ process and analyzing of clinical pharyngeal ESwabs™:

2.1 Following specimen inoculation, 19 ml of PBS were added to each of the samples in order to reach a final volume of 20ml.

2.2 Each diluted clinical ESwab™ sample was filtered through a 5µm Supor® membrane.

2.3 The filtered samples were processed then with POCARED™'s SP in order to clean and concentrate the sample into a volume of 1.4 ml.

2.4 At the completion of sample processing, each sample was analyzed using the P-1000™ Platform.

2.5 Overall processing and analyzing time with POCARED™'s SP and P-1000™ Platform was approximately 5 minutes per specimen.

Results:

1 All bacitracin inhibition zones that were ≥12 mm were reported as "GAS positive" (Figure 2) (4).

2 All bacitracin inhibition zones that were <12 mm were further analyzed using a serological test (BD Streptocard™ Enzyme Latex Test). All samples showing agglutination were reported as "GAS positive", whereas samples showing no agglutination were reports as "GAS negative". Note: Preliminary tests and previous studies showed that groups B, C and G streptococci may produce zones of inhibition (false-positive result) that are ≤10 mm (4).

3 The standard plate culture and identification methods yielded 143 positives and 484 negatives for GAS.

4 Results of 627 pharyngeal ESwabs™ analyzed by POCARED™ P-1000™ Platform are shown in Figure 3. The graph depicts the values assigned by the mathematical algorithm to each autofluorescence signal.

5 The values fell into two distinct groups, which are corresponding with the culture results, e.g. "positive" and "negative" for GAS with a cutoff value of 0.4 that was selected by the algorithm to separate the two groups.

6 The samples in the group below the threshold value are negative for GAS, whereas the samples in the group above the threshold value are positive for GAS.

7 The P-1000™ Platform correctly identified 135/143 positive specimens and 460/484 negative specimens.

8 The sensitivity and specificity values of the P-1000™ Platform were calculated as follows:

		Culture results	
		Positive	Negative
P-1000™ results	Positive	True positive	False positive
	Negative	False negative	True negative

$$Sensitivity = \frac{True\ positive}{True\ positive + False\ negative}$$

$$Specificity = \frac{True\ negative}{True\ negative + False\ positive}$$

9 The test performance showed that the sensitivity and specificity calculated for GAS were 94% and 95%, respectively.

Conclusions:

1 POCARED™'s P-1000™ Platform has demonstrated its ability to provide comparable results with the gold standard performance for GAS detection and identification.

2 The sensitivity and specificity of POCARED™'s P-1000™ Platform compared with the Gold Standard were 94% and 95%, respectively.

3 POCARED™'s P-1000™ Platform is a fully automated platform that provides accurate detection and identification of GAS within 5 min. directly from the clinical specimen eliminating the need for media, reagents, organism isolation, and further testing.

References:

1 Reglinski M, Sriskandan S. 2014. The contribution of group A streptococcal virulence determinants to the pathogenesis of sepsis. Virulence. Jan 1;5(1):127-36.

2 Todar, Ken. 2002. "*Streptococcus pyogenes*" http://textbookofbacteriology.net/streptococcus.html.

3 Anderson NW1, Buchan BW, Mayne D, Mortensen JE, Mackey TL, Ledeboer NA. 2013. Multicenter clinical evaluation of the illumigene group A Streptococcus DNA amplification assay for detection of group A Streptococcus from pharyngeal swabs. J Clin Microbiol. 51(5): 1474-7.

4 Stoner RA. 1978. Bacitracin and co-agglutination for grouping of beta-hemolytic streptococci. J Clin Microbiol. 7(5): 463-6.

Figure 1: POCARED™ P-1000™ Platform

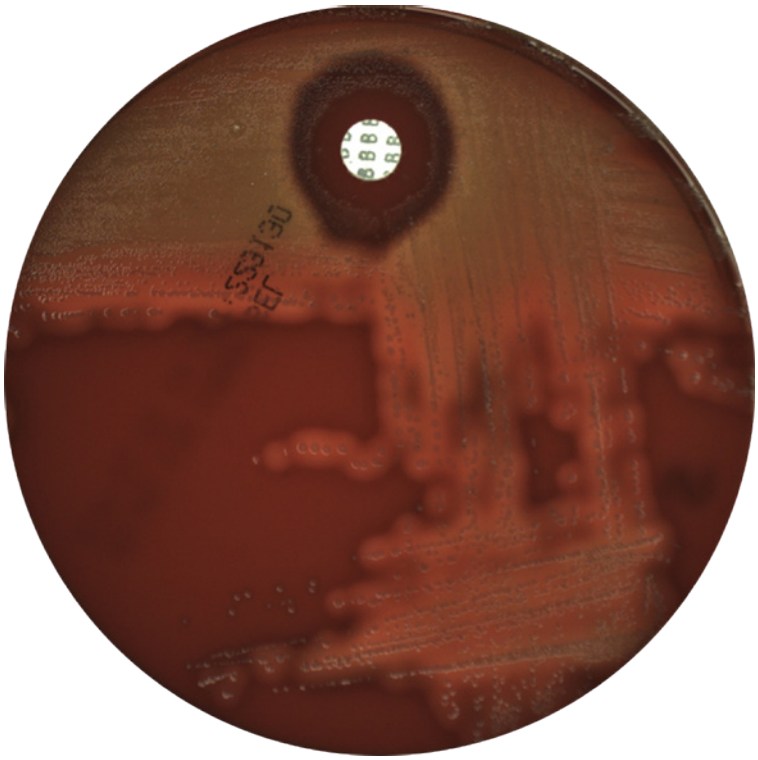

Figure 2: Pharyngeal clinical eSwab cultured on streptococcal selective agar with bacitracin disc showing β-hemolysis with inhibition zone.

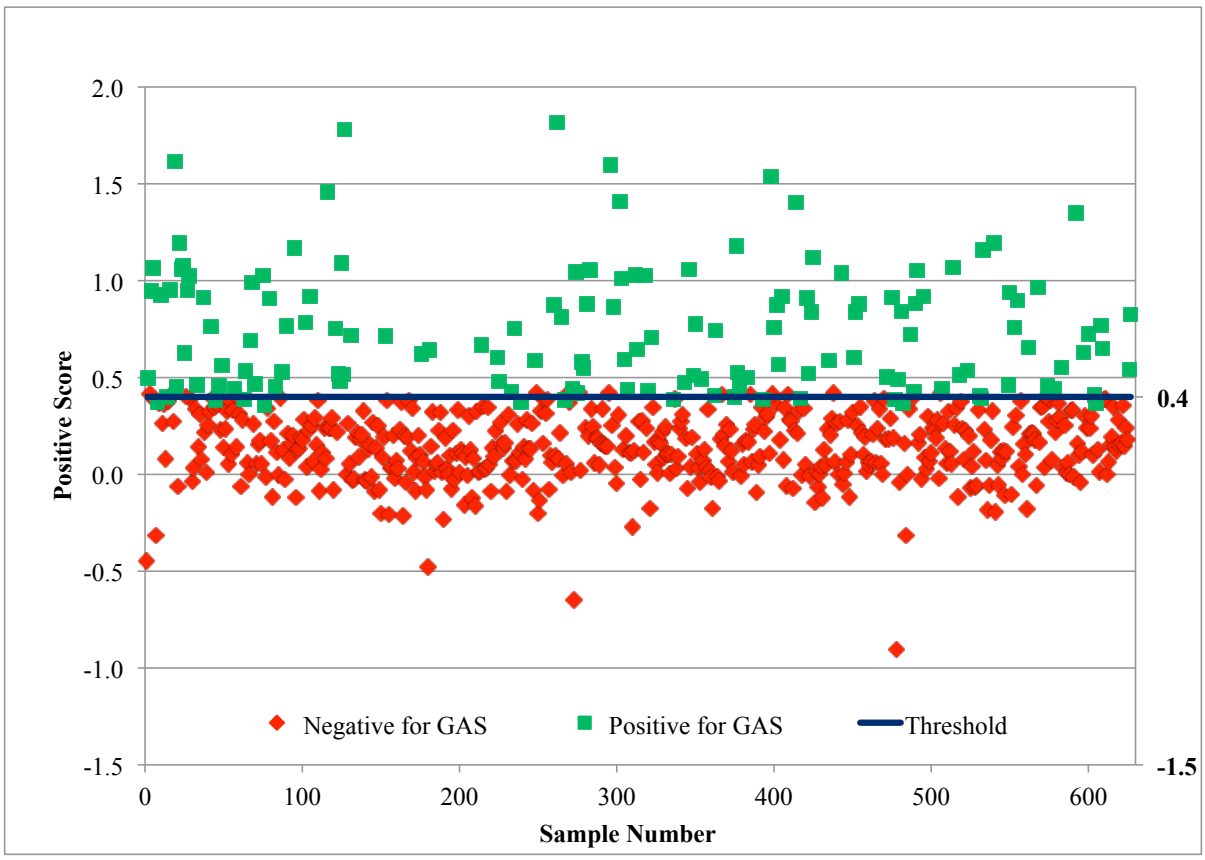

Figure 3: Algorithm- assigned values based on autofluorescence signal of 627 pharyngeal clinical eSwabs.