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An Equivalency Study Using The New Spectra Group A Strep Agar: A New Chromogenic Medium For The Detection Of Group A Streptococci From Clinical Throat Samples

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Abstract

Background: The Thermo Scientific™ Spectra™ Strep A Agar (R01812, Thermo Fisher Scientific Lenexa, Kansas) is a selective and differential chromogenic medium intended for use in the qualitative detection and identification of *Streptococcus pyogenes* (Group A *Streptococcus* (GAS)) taken directly from clinical throat specimens. An equivalency study was conducted to compare the performance of the new Spectra Strep A Agar to Thermo Scientific™ Remel™ Blood Agar (Tryptic Soy Agar with 5% Sheep Blood (BAP)) (R01202) with a Thermo Fisher™ Oxoid™ Bacitracin Antimicrobial Susceptibility disc (CT0005B).

Methods: A total of 366 non-consecutive clinical throat swab samples from patients with suspected infection from *Streptococcus pyogenes* were included in this study. These samples were inoculated within 24 hours of collection onto the test media; Spectra Strep A agar and the reference non-selective BAP with the Bacitracin Antimicrobial Susceptibility disc. Additional GAS testing was conducted with 29 negative clinical throat swab samples which were spiked with at least 1 x 10^5 CFU/ml of different GAS clinical isolates originally obtained from throat swabs. Presumptive positive GAS colonies from both the test and reference media, were confirmed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF).

Results: Of 366 samples and 29 spiked samples, 45 were considered true-positive, with GAS having been isolated and confirmed on at least 1 of the 2 culture media tested. Sensitivity of the Spectra Strep A Agar plate and the reference BAP with Bacitracin disc was 97.8% and 83.3% respectively. The specificity of the Spectra Strep A Agar plate and the reference BAP with Bacitracin disc was 99.7% and 93.8% respectively.

Conclusions: The Spectra Strep A Agar displayed greater sensitivity and specificity than the BAP with bacitracin disc, for the detection and isolation of GAS from throat samples. Overall, the data from this study supports the safe and effective use of the Spectra Strep A Agar, when used according to the intended purpose.

*Abstract has been amended from the original version.

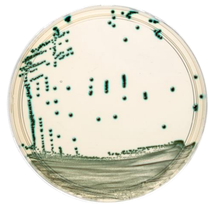
Introduction

Streptococcus pyogenes can cause a wide variety of diseases in humans including throat infections, skin and soft-tissue infections, severe systemic disease and long-term complications such as rheumatic fever.[1] GAS is the most common cause of acute pharyngitis, causing 20% to 30% of pharyngitis in children and 5% to 15% in adults.[2] Rapid, accurate diagnosis of streptococcal pharyngitis followed by appropriate antimicrobic therapy is important to prevent severe systemic disease and long-term complications in patients and to reduce the spread of the disease.[3] Inappropriate antimicrobial use contributes to the development of antimicrobial resistance. Differential diagnosis between GAS and viral pharyngitis based on clinical examination is unreliable, unless the patient presents with clear viral symptoms[2]. The American Academy of Pediatrics recommends that in children and adolescents, negative rapid antigen test results should be confirmed by throat culture.[3] Throat culture remains the gold standard diagnostic test with high sensitivity, compared to some rapid antigen tests, and a lower cost than molecular methods.

Spectra Strep A Agar is a selective and differential chromogenic medium intended for use in the qualitative detection and identification of Group A Streptococci directly from clinical specimens. GAS will grow as orange colonies, 0.5 - 1.5 mm, on the medium (see Figure 1). Non-target organisms will be inhibited or grow as green, blue, cream or white colonies, pinpoint – 4 mm, on the Spectra Strep A Agar (See Figure 2). Alternative methods for GAS isolation and identification include selective and non-selective blood agars to observe beta-hemolysis and Bacitracin discs to observe zones of inhibition.

Figure 1. Growth of target organism *Streptococcus pyogenes* on Spectra Strep A Agar.

Figure 2. Growth of non-target organisms on Spectra Strep A Agar.



E. faecalis ATCC® 29212

S. dysagalactiae ATCC® 12388

Materials and methods

Sample Preparation

A total of 366 non-consecutive clinical throat swab samples, sent to the Medical College of Wisconsin for GAS screening from August 2021 to January 2022, were included in this study. One specimen per patient was used for this study. Samples were collected using either CultureSwab™ Collection and Transport System (BBL, Becton Dickinson, Sparks, MD) or eSwab™ Transport System (Copan Diagnostics, Murrieta, CA), which were used in accordance with the manufacturer's instructions. Additional GAS testing was conducted using 29 confirmed negative throat swab samples, inoculated with clinical GAS isolates at inoculums of at least 1 x 10^5 CFU/ml. The 29 clinical GAS isolates used for spike testing were originally isolated from throat swab samples.

Test Methods

Throat swab samples were each inoculated and streaked within 24 hours of collection, directly onto a Spectra Strep A agar plate and a reference BAP, with a Bacitracin disc placed over the heavily inoculated area of the BAP. The order in which the test and reference method plates were inoculated was alternated to avoid bias and the incubation conditions were according to manufacturer recommendations. The Spectra Strep A agar plates were examined for the presence of orange colonies after a 24 hour incubation. Orange colonies observed on the Spectra Strep A Agar were also confirmed by MALDI-TOF MS identification. The BAP plates were examined for the presence of a zone of inhibition around the Bacitracin disc, and beta-hemolytic colonies after a 24 or 48 hour incubation. A Gram stain and catalase test was conducted on beta-hemolytic colonies from the BAP plate. Presumptive GAS colonies (beta-hemolytic, catalase negative, Gram positive cocci) on the BAP were confirmed by MALDI-TOF MS identification.

Data Analysis

The sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) were calculated, for both the Spectra Strep A Agar test method and the BAP with a Bacitracin disc reference method.

Results

Table 1 shows the comparative performance of Spectra Strep A Agar and non-selective BAP with Bacitracin disc. Samples were considered as true positive for GAS if at least 1 out of 2 culture media yielded GAS, as confirmed by MALDI-TOF MS identification. Of the 395 samples included in the study, 45 were considered true-positive specimens.

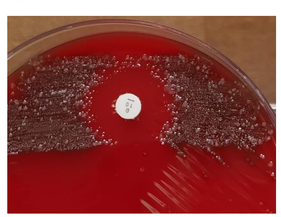
Table 1. Comparative performance of Spectra Strep A Agar and non-selective BAP with Bacitracin disc

	Spectra Strep A Agar	BAP with Bacitracin disc
True Positives	44	35
False Positives	1	22
True Negatives	349	331
False Negatives	1	7
Sensitivity	97.8% (95% CI = 96.3 - 99.2)	83.3% (95% CI = 79.7 - 87.0)
Specificity	99.7% (95% CI = 99.2 - 100)	93.8% (95% CI = 91.4 - 96.2)
PPV	97.8% (95% CI = 96.3 - 99.2)	61.4% (95% CI = 56.6 - 66.2)
NPV	99.7% (95% CI = 99.2 - 100)	97.9% (95% CI = 96.5 - 99.3)

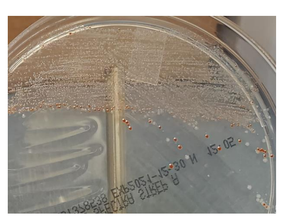
The sensitivity of the non-selective BAP with Bacitracin disc was lower than expected. This is likely due to overgrowth of non-target microbial flora making it difficult to detect and isolate beta-hemolytic colonies. Also 3 of the samples recorded as true positives on the Spectra Strep A agar were recorded as false positives on the BAP with Bacitracin disc. In these cases, the technician had been unable to pick the right colony on the BAP for MALDI-TOF identification.

As expected, the specificity of the Spectra Strep A Agar was greater than that of the non-selective BAP with Bacitracin disc. Figure 3 shows the BAP with Bacitracin disc and Spectra GAS test plate for a positive clinical throat swab sample. As can be seen in Figure 3, with reduced growth of non-target organisms, orange GAS colonies were easier to identify and select for confirmatory testing on the Spectra GAS agar in comparison to the BAP with Bacitracin disc.

Figure 3. Growth from a clinical throat swab sample on a BAP and Spectra Strep A Agar.



BAP with a Bacitracin disc



Spectra Strep A Agar

Conclusions

In this study, the Spectra Strep A Agar displayed greater sensitivity, specificity, PPV and NPV than the BAP with Bacitracin disc, for the detection and isolation of GAS from throat samples. The selectivity and chromogenic differentiation of colonies using Spectra Strep A Agar offers the user clear identification of GAS. Overall, the data from this study supports the safe and effective use of the Spectra Strep A Agar, when used according to the intended purpose.

Additional studies to evaluate the performance of Spectra Strep A Agar for the detection and isolation of GAS, using digital imaging software such as WASPLab™ Chromogenic Detection Module (Copan Diagnostics, Murrieta, CA) to analyze the plates compared to technologist plate reading, would be beneficial. A study by Van TT et al.[4] showed improved sensitivity with CDM software in comparison to manual plate image reading for the detection of GAS on chromogenic media. The use of digital imaging has the potential to reduce the labor cost and improve the speed and accuracy of results and ultimately improve patient care.

Acknowledgements

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