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Comparison of Four Media for the Detection of Group A Streptococcus from Throat Specimens

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Abstract

Background: Culture is used commonly for the diagnosis of Group A beta-hemolytic streptococcus (GAS) infections. This study examined medium type and length of incubation for optimal and cost-effective culture of GAS.

Methods: The media included BBL Trypticase Soy with 5% Sheep Blood Agar incubated anaerobically (BAP), BBL Group A Selective Strep Agar with 5% Sheep Blood incubated in 5% CO_2 (BBL), Remel Strep A Isolation Agar incubated in 5% CO_2 (REM), and Hardy GBS Detect Agar incubated in ambient air (GBSD). A total of 699 throat swabs were placed into 200 µL of Tris EDTA buffer and vortexed for 1 min. Plates were inoculated with 25 µL of eluted specimen, streaked for isolation using a BD InocuLab, incubated at 35°C, and examined at 24 and 48 hours. Beta-hemolytic colonies were tested with Streptex® Latex Group A (Remel, Lenexa, KS) and occasionally with catalase reagent.

Results: A total of 63 GAS isolates were recovered with an overall positivity rate of 9%. The recovery rates for each medium at 24/48 h were: BAP 54/55 (86/87%), BBL & REM 51/57 (81/90%), and GBSD 57/58 (90/92%). Five (8%) isolates were missed by BAP due to low numbers, and 3 (5%) GAS were overgrown. Six (10%) isolates were missed by BBL due to a failure to grow. Four (6%) isolates were missed by REM due to low numbers, and 2 (3%) GAS failed to grow. Five (8%) isolates were missed by GBSD due to low numbers. The incidence of non-GAS beta-hemolytic colonies requiring latex testing for each medium at 24/48 h were: BAP 152/220 (22/31%), BBL 34/58 (5/8%), REM 29/35 (4/5%), and GBSD 72/105 (10/15%). The number of subcultures required for each medium at 24/48 h was: BAP 37/70 (5/10%), BBL 20/24 (3/3%), REM 12/13 (2/2%), and GBSD 19/28 (3/4%).

Conclusions: GBSD detected the most (90%) isolates after 24 hours. Incubation of GBSD and BAP beyond 24 hours did little to improve the sensitivity (2%), and had a negative impact on specificity. However, the sensitivity of BBL and REM increased by nearly 10% with the full 48 hours incubation. Although GBSD is the most expensive medium, it moderates the amount of latex testing required, reduces subcultures, eliminates the need for anaerobic or CO_2 incubation, and reduces labor and result turn-around-time by 50% by permitting culture completion at 24 instead of 48 hours. These findings prompted our lab to switch from BAP to GBSD for culture of GAS from throat swabs.

Introduction

Group A beta-hemolytic strep (GAS) is the most common bacterial agent associated with acute pharyngitis. GAS accounts for as much as 30% of pharyngitis cases in children, with lower rates of incidence in adult populations. Serious complications, including peritonsillar abscess, acute glomerulonephritis, and rheumatic fever are associated with GAS infection. Prompt diagnosis of GAS infection aids in the appropriate use of antibiotics and helps to alleviate symptoms and prevent sequelae. Although rapid antigen testing is frequently used to screen for GAS in patients with pharyngitis, collection of quality specimens from uncooperative children can be difficult. The rapid antigen test may not detect GAS from swabs containing low numbers of organism. For this reason, the American Academy of Pediatrics and the Infectious Diseases Society of America recommend that negative rapid antigen test results be confirmed by culture. The College of American Pathologists also states that, "If group A streptococcus direct antigen testing is performed, additional confirmatory testing is performed on negative samples."

A variety of culture methods have been used for the isolation of GAS from throat swabs. A non-selective blood agar plate (BAP) may be incubated anaerobically to enhance the growth and hemolysis of GAS. However, this method allows for the growth of a variety of organisms, resulting in the need for subcultures for further testing. Selective media for GAS are available from a variety of vendors. These media incorporate antimicrobial agents to suppress overgrowth by oropharyngeal flora. These media typically require incubation in 5 to 10% CO_2 or, if incubated aerobically, the agar must be stabbed to create areas with reduced oxygen to facilitate the formation of beta hemolysis. Although these selective media enhance the isolation of GAS, some isolates are inhibited, and the cultures must be incubated for two days before the culture can be finalized as negative.

This study examined four types of media and the length of incubation needed for the optimal and cost-effective culture of GAS. Three of the media are traditionally used for the isolation of GAS. The fourth medium, GBS Detect agar from Hardy Diagnostics, is a selective medium intended for the isolation of beta-hemolytic group B streptococcus (GBS) from vaginal/rectal specimens collected from pre-partum women. This medium contains hemolysis-enhancing ingredients that permit the detection of both hemolytic and non-hemolytic GBS when incubated under ambient atmospheric conditions. GAS will also grow on this medium and produce colonies with enhanced hemolysis when incubated under ambient atmospheric conditions.

Methods

The media included in this evaluation were, BBL Trypticase Soy with 5% Sheep Blood Agar incubated anaerobically (BAP), BBL Group A Selective Strep Agar with 5% Sheep Blood incubated in 5% CO_2 (BBL), Remel Strep A Isolation Agar incubated in 5% CO_2 (REM), and Hardy GBS Detect Agar incubated in ambient air (GBSD). A total of 699 throat swabs were cultured. Each specimen was placed into 200 µL of Tris EDTA buffer and vortexed for 1 minute. Plates were inoculated with 25 µL of eluted specimen, streaked for isolation using a BD InocuLab, incubated at 35 ± 2°C, and examined at 24 and 48 hours. Beta-hemolytic colonies were tested with Streptex® Latex Group A (Remel, Lenexa, KS) and occasionally with catalase reagent.

Figures 1a-d: The appearance of GAS on each medium. All media were inoculated with the same specimen and incubated for 24 hours.

Figure 1a: BAP (anaerobic)

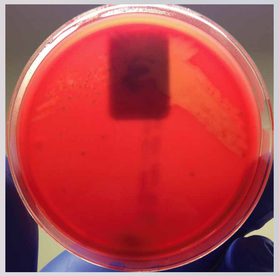

Figure 1b: BBL Group A Selective Strep Agar (5% CO_2)

Figure 1c: Remel Strep A Isolation Agar (5% CO_2)

Figure 1d: GBS Detect Agar (aerobic)

Results

A total of 63 GAS isolates were recovered with an overall positivity rate of 9%. The recovery rates for each medium at 24/48 hours were: BAP 54/55 (86/87%), BBL & REM 51/57 (81/90%), and GBSD 57/58 (90/92%). Five (8%) isolates were missed by BAP due to low numbers. This was determined by the fact that only 1 to 3 colonies grew on one or more of the companion media, suggesting that the number of GAS was near the limit of detection. Three (5%) GAS were undetectable due to overgrowth by other flora. Six (10%) isolates were not detected by BBL due to a failure to grow. The companion media for these six cultures grew GAS in the 2nd, 3rd, or 4th quadrants, indicating that there were sufficient numbers of GAS in the inoculum to be detected. Four (6%) isolates were missed by REM due to low numbers, and 2 (3%) GAS failed to grow. Five (8%) isolates were missed by GBSD due to low numbers. The incidence of non-GAS beta-hemolytic colonies requiring latex testing for each medium at 24/48 hours were: BAP 152/220 (22/31%), BBL 34/58 (5/8%), REM 29/35 (4/5%), and GBSD 72/105 (10/15%). The number of subcultures required for each medium at 24/48 hours was: BAP 37/70 (5/10%), BBL 20/24 (3/3%), REM 12/13 (2/2%), and GBSD 19/28 (3/4%).

Colonies of GAS isolates growing on GBS Detect agar were consistently larger than colonies on the other media. Although impractical to assign a quantitative value to the latex agglutination test, inoculum selected from GBS Detect agar agglutinated with the latex reagent quickly and was typically visible earlier than when the test was performed with inoculum from the other media. This is probably due to the larger colonies available for testing on the GBS Detect agar.

Performance of Each Medium for Detection of GAS

	GAS Detected No. (%)		Reason for Failure to Detect GAS No. (%)		
Medium (atmosphere)	24 h	48 h	Low Numbers	Overgrown	Failed to Grow
BAP (anaerobic)	54 (86)	55 (87)	5 (8)	3 (5)	0
BBL (5% CO_2)	51 (81)	57 (90)	0	0	6 (10)
REM (5% CO_2)	51 (81)	57 (90)	4 (6)	0	2 (3)
GBSD (aerobic)	57 (90)	58 (92)	5 (8)	0	0

Additional Labor Associated with Each Medium

	Subculture No. (%)		Latex Testing Needed for non-GAS Beta Strep No. (%)	
Medium (atmosphere)	24 h	48 h	24 h	48 h
BAP (anaerobic)	37 (5)	70 (10)	152 (22)	220 (31)
BBL (5% CO_2)	20 (3)	24 (3)	34 (5)	58 (8)
REM (5% CO_2)	12 (2)	13 (2)	29 (4)	35 (5)
GBSD (aerobic)	19 (3)	28 (4)	72 (10)	105 (15)

Cost Analysis for GAS Culture

Based on annual test volume = 15,600

Method	Annual Supply & Labor Cost
Blood Agar Plate (anaerobic*) • 2-day incubation and review • 10% of tests required subculture • 31% required latex testing for non-GAS	$29,530
BBL Group A Selective agar (5% CO_2) • 2-day incubation and review • 3% of tests required subculture • 8% required latex testing for non-GAS	$22,317
Remel Strep A Isolation agar (5% CO_2) • 2-day incubation and review • 2% of tests required subculture • 5% required latex testing for non-GAS	$21,174
Hardy GBS Detect agar (ambient) • 1-day incubation and review • 3% of tests required subculture • 10% required latex testing for non-GAS	$15,768

*The cost of using anaerobe jars was not factored into this cost analysis. Our lab uses the Anoxomat system, with a minimal cost per jar. For labs with gas-pack systems, the cost could be readily calculated.

Conclusions

Hardy's GBS Detect agar detected the most (90%) isolates after 24 hours. Incubation of GBS Detect and BAP beyond 24 hours yielded marginal improvement in the sensitivity (2%) and had a negative impact on specificity. In contrast, the sensitivity of BBL Group A Selective agar and Remel Strep A Isolation agar increased by nearly 10% with a full 48 h incubation. In this study, as many as 10% of the GAS isolates failed to grow on a GAS-selective medium (BBL), suggesting that the use of a second, non-selective plate, may be necessary to increase sensitivity. No isolates failed to grow on GBS Detect agar or BAP.

Hardy's GBS Detect agar is a unique medium, providing both selective qualities and hemolysis-enhancement. Although GBS Detect is the most expensive medium, it moderates the amount of latex testing required, reduces the number of subcultures, eliminates the need for anaerobic or CO_2 incubation, and reduces labor and result turn-around-time by 50% by permitting culture completion at 24 instead of 48 hours. These findings prompted our lab to switch from anaerobic BAP to GBS Detect for culture of GAS from throat swabs. Our estimated annual cost saving is nearly $13,800, representing a cost reduction of almost 50%. The conversion to GBS Detect agar was well-received by technical staff. The reduction in latex testing and the elimination of second-day culture review significantly reduced labor. The streamlined testing has been beneficial during periods of reduced staffing.