

## Selective Streptococcal Agar versus Blood Agar for Detection of Group A Beta-Hemolytic Streptococci in Patients with Acute Pharyngitis

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**In a study on acute pharyngitis in general practice, we compared a selective group A streptococcal agar (ssA) for the recovery of group A beta-hemolytic streptococci (GABHS) with sheep blood agar. All plates were incubated at 36°C in an atmosphere reinforced with 5% CO<sub>2</sub> for 48 h with a first reading after 24 h. A total of 197 GABHS isolates were obtained from 721 throat cultures on both media. The recovery of GABHS was significantly higher after 48 h of incubation for both media. With the ssA plate, we detected significantly more GABHS after 24 h as well as after 48 h of incubation. The ssA plate reduced normal flora qualitatively and quantitatively. In conclusion, ssA is more sensitive and specific for the detection of GABHS than sheep blood agar and moreover easier to read. We recommend incubation for 48 h.**

The diagnosis of pharyngitis due to group A beta-hemolytic streptococci (GABHS) is still widely based upon the isolation of the organism from throat cultures on blood agar. However, because of overgrowth of normal flora, detection of GABHS is often obscured, resulting in false-negative results or in delays in reporting due to time-consuming reisolation steps. Many efforts to improve recovery and identification, including the addition of antimicrobial agents that selectively inhibit normal flora, have been undertaken (2, 4, 6).

The purpose of this study was to evaluate a selective streptococcal agar (ssA) (TM Becton Dickinson, Erembodegem, Belgium), recently introduced in Belgium, for the improved isolation and direct primary-plate identification of GABHS.

In a 1-year study, 40 general practitioners collected 721 oropharyngeal swab specimens from patients, with ages ranging between 5 and 50 years, presenting with acute sore throats. All swabs were placed into an Amies transport medium (Sterilin, Stone, United Kingdom) and kept at 4°C until they were processed for culture (within 12 h).

Two different media were used for isolation: a sheep blood agar (SBA) plate (tryptic soy agar with 5% sheep blood) and an ssA plate (5% SBA containing crystal violet, colistin, and trimethoprim-sulfamethoxazole), both supplied by Becton Dickinson. Each swab, in random order, was inoculated on both media by rolling it over approximately one-sixth of the agar surface. The plates were streaked with a sterile wire loop. Hemolysis detection was enhanced by stabbing the initial inoculum area. A differential Bacitracin Low Disc (Bacitracin Low Diagnostic Tablets; Rosco, Taalstrup, Denmark) was placed on both plates at the junction of the initial inoculum zone and the second zone of streaking. All plates were incubated at 36°C in an atmosphere reinforced with 5% CO<sub>2</sub> for 48 h with a first reading after 24 h.

Detection of beta-hemolytic streptococci (BHS) was based on the colony morphology and beta-hemolysis on the surfaces and subsurfaces of the media. Presumptive identification of GABHS was based on bacitracin susceptibility.

BHS showing an inhibition zone around the bacitracin disc were isolated and retested for their susceptibilities to co-trimoxazole (Neo Sensitabs; Rosco) and bacitracin on an SBA plate (1). Colonies sensitive to bacitracin and resistant to co-trimoxazole were classified as GABHS. Co-trimoxazole-sensitive colonies were identified by an agglutination method (Streptococcal Grouping Kit; Oxoid, Basingstoke, United Kingdom). If growth of BHS was too sparse to accurately detect the presence of an inhibition zone or if overgrowth of normal flora obscured reading, the bacitracin susceptibility test was repeated by using the conventional pure-subculture technique on an SBA or an ssA plate, depending on the plate involved.

Growth was expressed semiquantitatively. We classified growth as 1+ if beta-hemolytic streptococci were present in the first inoculation zone, 2+ if they were seen in the first and second zones, and 3+ if they were detected in all streaked areas.

Statistical analysis was performed by using the McNemar test for comparison of paired samples with correction for continuity (8).

The effects of duration of incubation and type of medium on the recovery of GABHS are shown in Table 1. The total number of throat swabs studied was 721. GABHS were identified in 197 (27.3%) of the specimens. An incubation period of 48 h yielded significantly more GABHS on the ssA ( $P < 0.001$ ) and SBA ( $P < 0.001$ ) media than did a 24-h incubation period. None of the media allowed recovery of all 197 isolates, but ssA was significantly more sensitive than SBA after 24 h ( $P < 0.001$ ) as well as after 48 h ( $P < 0.001$ ) of incubation.

The recovery of BHS, other than group A, from the 721 throat swabs is shown in Table 2. The numbers of group B streptococci isolated on both plates were comparable. However, after 48 h of incubation, ssA yielded significantly fewer group C ( $P < 0.05$ ) and group G ( $P < 0.05$ ) streptococci than SBA.

The ssA plate reduced normal flora qualitatively and quantitatively (data not shown). Viridans group streptococci, in a small amount, were the sole normal-flora species encountered on ssA, whereas *Neisseria* spp., *Staphylococcus* spp., and viridans group streptococci were the predom-

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TABLE 1. Effects of duration of incubation and type of medium on the recovery of GABHS from 721 throat cultures

Medium	Duration of incubation (h)	No. of GABHS isolates recovered	% Detected
SBA	24	167	90.3
	48	181 <sup>a</sup>	91.9
ssA	24	183 <sup>b</sup>	98.9
	48	196 <sup>a,b</sup>	99.5
Both	24	185	
	48	197	

<sup>a</sup>  $P < 0.001$  for the difference in recovery of GABHS after 24 and 48 h of incubation.

<sup>b</sup>  $P < 0.001$  for the difference in recovery of GABHS on SBA and recovery on ssA after 24 and 48 h of incubation, respectively.

inant normal-flora species, in large amounts, present on SBA.

The recovery of GABHS from throat swab specimens on an SBA medium is still widely accepted for diagnosis of pharyngitis. However, GABHS present in a specimen may be missed for many reasons. The normal flora is known as the major responsible factor; it can overgrow the pathogen (3, 6, 7) and mask its expression of beta-hemolysis (3). We compared a selective medium, recently introduced in Belgium, with the standard SBA plate.

In this study, the ssA medium was superior to the SBA. It selectively reduced normal flora, facilitating both the recov-

TABLE 2. Effects of duration of incubation and type of medium on the recovery of BHS, other than group A, from 721 throat cultures

Medium	Duration of incubation (h)	No. of isolates in group:			
		B	C	G	F
SBA	24	3	6	9	1
	48	3	9 <sup>a</sup>	10 <sup>b</sup>	1
ssA	24	4	3	4	0
	48	5	3	4	0
Both	24	4	6	9	1
	48	5	9	10	1

<sup>a</sup>  $P < 0.05$  for the difference in recovery of group C streptococci on SBA and recovery on ssA after 48 h of incubation.

<sup>b</sup>  $P < 0.05$  for the difference in recovery of group G streptococci on SBA and recovery on ssA after 48 h of incubation.

ery and the identification of GABHS. The ssA medium yielded significantly more GABHS than SBA after both 24 and 48 h of incubation. These results correspond with results of a previous study (3) but disagree with results of the study by Huck et al. (5), who found SBA to be superior to ssA. As to the incubation period, in our experience 48 h was obligatory, since both ssA and SBA yielded significantly more GABHS after this length of time. For the SBA plate, this was in agreement with previous reports (7, 9). An additional aspect of cultures on the ssA medium was the significant reduction in the number of BHS of groups C and G after 48 h of incubation. This may be of interest in exclusive screening procedures for the presence of GABHS.

In conclusion, ssA is a very useful, selective culture medium. It is more sensitive and specific than SBA for the detection of GABHS and is easier to read because of the selective suppression of normal flora. Incubation for 48 h is recommended. However, if the screening of BHS other than group A has to be considered, an additional SBA plate should be inoculated.

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