

Effect of Atmosphere and Duration of Incubation on Primary Isolation of Group A Streptococci from Throat Cultures

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The optimal incubation conditions for isolation of group A streptococci from throat cultures are controversial. Therefore, we compared the effects of aerobic and anaerobic incubations after 24 and 48 h on the recovery of group A streptococci. Throat swabs submitted to the clinical laboratory were inoculated onto duplicate 5% sheep blood agar plates, incubated aerobically or anaerobically (GasPak jar) at 35°C, and examined semiquantitatively after 24 and 48 h. Group A streptococci were identified by the fluorescent-antibody technique. Of 1,040 specimens, 506 (48.6%) grew beta-hemolytic streptococci, including 200 (19.2%) group A streptococci. Group A streptococci were recovered significantly more often with anaerobic incubation than with aerobic incubation after 24 h (182 versus 138; $P < 0.001$) and after 48 h (193 versus 174; $P < 0.05$). Non-group A beta-hemolytic streptococci also were recovered significantly more often with anaerobic incubation after 24 and 48 h ($P < 0.001$). Colony counts were not affected by the incubation atmosphere. We conclude that incubation of throat cultures in an anaerobic atmosphere is superior to incubation in air for detection of group A streptococci. The greater sensitivity of anaerobic incubation, however, may not justify the extra laboratory effort and cost required to differentiate group A streptococci from the non-group A streptococci detected as a result of anaerobic incubation. Throat cultures should be examined after 24 and 48 h, especially if plates are incubated aerobically.

Opinions differ regarding the optimal incubation conditions for the isolation of group A streptococci from throat cultures. Some authors recommend an atmosphere of 5 to 10% CO₂, (9, 13); others recommend an anaerobic environment to enhance the beta-hemolysis produced by streptolysin O, an oxygen-labile hemolysin (1, 3, 6, 8, 10), and to suppress viridans streptococci that may inhibit the growth of group A streptococci (14). Still others recommend incubation in air (2, 7, 15). The optimal duration of incubation, 24 or 48 h, also is controversial. Most physicians in their office laboratories incubate throat cultures aerobically for 24 h and rely upon stabs in the agar to detect the beta-hemolysis of subsurface colonies growing under relative anaerobiosis.

The published data that support the various recommendations about incubation conditions are scanty and contradictory. Using sheep blood agar plates (BAPs) in a carefully controlled study, Murray et al. (12) found that aerobic, 3 to 5% CO₂, and anaerobic incubations are equally sensitive in recovery of group A streptococci. CO₂ and anaerobic conditions, however, have the serious disadvantage of also enhancing the

recovery of non-group A beta-hemolytic streptococci, which require extra laboratory effort to be distinguished from group A streptococci. The investigators concluded that aerobic incubation is preferred. Dykstra et al. (4), however, using similar methods, found that significantly fewer group A streptococci are detected with aerobic stabbed BAPs than by anaerobic incubation.

The choice of atmosphere and duration of incubation of throat cultures is important owing to the uncertainty that remains regarding the most sensitive technique and owing to the greater technical complexity and cost of anaerobic incubation. The purpose of this study was to compare the effects of aerobic and anaerobic incubations after 24 and 48 h on the isolation rate of group A streptococci from throat cultures.

MATERIALS AND METHODS

Source of specimens. We cultured 1,040 pharyngeal specimens submitted to the clinical microbiology laboratory at University Hospital over a 5-month winter-spring period. Throat swabs (Culturette; Marion Scientific, Div. Marion Laboratories, Inc., Kansas City, Mo.) were taken by house staff from children and adults, predominantly outpatients, with symptoms of upper respiratory tract infections.

TABLE 1. Effects of atmosphere and duration of incubation on recovery of beta-hemolytic streptococci from 1,040 throat cultures

Incubation atmosphere	Incubation time (h)	No. (%) of specimens yielding beta-hemolytic streptococci	
		Group A	Non-group A
Aerobic	24	138 (13.3)	107 (10.3)
Anaerobic	24	182 (17.5)	241 (23.2)
Aerobic or anaerobic	24	191 (18.4)	264 (25.4)
Aerobic	48	174 (16.7)	135 (13.0)
Anaerobic	48	193 (18.5)	276 (26.5)
Aerobic or anaerobic	48	200 (19.2)	306 (29.4)

Media, inoculation, and incubation. Duplicate plates of 5% sheep blood agar (Trypticase soy agar base; BBL Microbiology Systems, Cockeysville, Md.) were inoculated by rolling the swab over approximately one-sixth of the agar surface. The plates then were streaked with a loop in four quadrants, and the agar was stabbed in several areas. One BAP was incubated overnight at 35°C in room air, and the other was incubated overnight at 35°C in a GasPak jar (BBL). During half of the study, the aerobic BAP was inoculated first, and during the other half of the study, the anaerobic BAP was inoculated first.

Interpretation. The BAPs were examined by transmitted light after overnight (12- to 24-h) incubation and again after 48 h for the presence of colonies resembling beta-hemolytic streptococci. Suspicious colonies were tested by the direct fluorescent-antibody technique (5). The number of colonies of beta-hemolytic streptococci was estimated to be 1+ to 4+, depending upon whether colonies were found in the first, second, third, or fourth streak zone. Subculturing was done as necessary.

Statistical analysis. Differences in isolation rates were analyzed by using the McNemar test (11) for matched pairs.

RESULTS

The total number of throat swabs studied was 1,040 (Table 1). Beta-hemolytic streptococci were identified in 506 (48.6%) of the specimens; 200 (19.2%) of the specimens grew group A streptococci, and 306 (29.4%) grew non-group A streptococci. Recovery of group A streptococci was significantly more frequent with anaerobic

incubation than in air after 24 h (182 versus 138; anaerobic only, 53; aerobic only, 9; $P < 0.001$) and after 48 h (193 versus 174; anaerobic only, 26; aerobic only, 7; $P < 0.05$). Non-group A beta-hemolytic streptococci also were recovered significantly more often in an anaerobic atmosphere than in air after 24 h ($P < 0.001$) and after 48 h ($P < 0.001$).

Colony counts were not affected by the incubation atmosphere (Table 2). Colony counts of non-group A streptococci tended to be lower than counts of group A streptococci in both atmospheres. When non-group A streptococci were recovered, they were present in 1+ or 2+ amounts more than half the time. In contrast, group A streptococci generally were present in 3+ or 4+ amounts. Of the seven cultures positive for group A streptococci by aerobic incubation only after 48 h, five were in 1+ or 2+ amounts and two were in 3+ amounts. Of 26 cultures positive only by anaerobic incubation after 48 h, 15 were present in 1+ or 2+ amounts and 11 were present in 3+ or 4+ amounts.

Approximately 50% of the throat swabs were taken from children ≤ 14 years old. The rate of recovery of group A streptococci was greatest in the 5- to 14-year age group (34%) and lowest in the < 5 - and ≥ 20 -year age groups (14%). The order of inoculation of the two BAPs did not affect the rate of recovery of group A streptococci; 182 of the plates inoculated first were positive after 48 h compared with 184 of the plates inoculated second.

TABLE 2. Effects of atmosphere and duration of incubation on quantity of beta-hemolytic streptococci recovered from 1,040 throat cultures

Quantity	Incubation time (h)	No. (%) of specimens yielding beta-hemolytic streptococci			
		Anaerobic incubation		Aerobic incubation	
		Group A	Non-group A	Group A	Non-group A
1 or 2+	24	31 (3.0)	63 (6.1)	37 (3.6)	133 (12.8)
3 or 4+	24	107 (10.3)	44 (4.2)	145 (13.9)	108 (10.4)
1 or 2+	48	50 (4.8)	87 (8.4)	43 (4.1)	159 (15.3)
3 or 4+	48	124 (11.9)	48 (4.6)	150 (14.4)	117 (11.3)

DISCUSSION

The throat culture is one of the most common tests performed in clinical microbiology laboratories in this country. Yet there is no consensus regarding optimal laboratory methods. Because group A streptococci, the principal bacterial pathogens sought in throat cultures, often produce more intense beta-hemolysis under anaerobic conditions than under aerobic conditions, many microbiologists recommend that throat cultures be incubated routinely in an anaerobic atmosphere. Well-controlled studies documenting the superiority of anaerobic incubation are few, however, and two recent studies of the problem produced contradictory results (4, 12).

Our study confirmed the superiority of anaerobic incubation over aerobic incubation of throat cultures for detection of group A streptococci, as reported by Dykstra et al. (4). The difference in recovery rates was most marked after 24 h and was still statistically significant after 48 h of incubation. Like Murray et al. (12), we found that non-group A streptococci were recovered significantly more often with anaerobic incubation. In this study, the number of non-group A streptococci recovered was more than doubled by anaerobic incubation. Therefore, laboratory expense resulting from the extra step of differentiating more non-group A streptococci from group A streptococci may be considerable.

We conclude that anaerobic incubation of throat cultures increases the recovery rate of both group A and non-group A streptococci. Therefore, laboratory directors and physicians with office laboratories who seek maximum test sensitivity should choose anaerobic incubation. If aerobic incubation is selected, it is important to examine the plates after 24 and 48 h.

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