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Diagnosis of Group A Streptococcal Infections Directly from Throat Secretions

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The diagnosis of group A streptococcal disease still relies on isolation of group A streptococcal strains on sheep blood agar followed by presumptive identification based on bacitracin sensitivity or the results of the more precise serogrouping methods such as the Lancefield precipitin test. A technique that would permit rapid identification of streptococcal infections directly from throat secretions would allow immediate appropriate antimicrobial therapy for the management of streptococcal infections to be started. We have been able to identify soluble group A antigen directly from throat secretions by using a latex agglutination test. In a clinical trial in which latex (Streptex group A) and conventional culturing techniques were used, 53 throat secretion cultures were tested: 26 were positive by both procedures, 5 were positive by culture only, 3 were positive by the latex agglutination test only, and 19 were negative by both tests.

The early identification of group A streptococci is essential for selecting appropriate antimicrobial therapy to prevent suppurative complications of streptococcal disease. Because clinical diagnosis of streptococcal pharyngitis is unreliable, (1, 5) throat cultures are necessary for the management of patients. Although beta-hemolytic streptococci can be recovered within 18 to 24 h from a throat culture, the widely used bacitracin disk susceptibility test for differentiating group A streptococci from other beta-hemolytic streptococci requires another 18 to 24 h. The tests described more recently in which sensitized staphylococci (3) (COAG reagent [Phadebact]; Pharmacia Diagnostics) or the latex agglutination test (Streptex; Wellcome Research Laboratories) have made precise identification easier but have not reduced the time required for identification. Because of the delay in getting culture results, many physicians either do not take cultures or may, while the patient is in the office, administer antimicrobial therapy to play it safe in case the culture later yields beta-hemolytic group A streptococci.

Other previously described rapid methods of streptococcal detection are the fluorescent-antibody technique (4) and counterimmunoelectrophoresis (2). However, neither of these techniques have come into widespread use.

A simple technique that would permit identification of streptococci directly from throat secretions would allow immediate application of appropriate antimicrobial therapy in the management of streptococcal infections. It would also be valuable as an aid in the detection of group A streptococci in health surveys. Such a method appears to be the latex agglutination test. This application of latex particles sensitized with specific antibody (Streptex group A; Wellcome) to detect streptococcal antigen directly from throat secretions is described here.

MATERIALS AND METHODS

Clinical samples. Individuals reporting to the Marine Corps dispensary, San Diego, Calif., with sore throat complaints gargled with approximately 10 ml of 0.01 M phosphate-buffered saline, pH 7.2, or nutrient broth (Difco Laboratories, Detroit). Throat swabs were taken at the same visit just before the patients gargled. The swabs were streaked onto sheep blood agar plates for streptococcal isolation and identification. Material obtained from the gargling was cultured on sheep blood agar for group A streptococcus identification and tested for streptococcal group A antigen by latex agglutination as described below.

Latex test. Latex (Streptex A; Wellcome, lots K0271 and K5674) was used as follows. Gargle material was centrifuged at 600 × *g* for 10 min. The clear supernatant was removed and heated in a boiling water bath for 3 min. After the supernatant had cooled, 5 drops (0.025 ml each) were transferred to a test tube (10 by 75 mm), and 1 drop (0.025 ml) of a freshly prepared trypsin solution was added (0.01% suspension of 1:250 trypsin [Difco, lot A145356]). The mixture was heated at 37°C for 30 min. One drop of the trypsinized gargle was mixed with 1 drop of Streptex group A on a microscopic slide. The slide was tilted to and fro for 3 to 5 min. Agglutination readings were made with a stereomicroscope. A control for nonspecific agglutination of the latex particles was made by using Streptex group B (Wellcome; lot K7971).

Sensitivities of latex agglutination, staphylococcal coagglutination, and counterimmunoelectrophoresis in detecting antigen. Tenfold dilutions of an antigen in phosphate-buffered saline were prepared from a stock

TABLE 1. Comparison of latex agglutinations, staphylococcal coagglutinations, and counterimmunoelectrophoresis in terms of detection of group A streptococcal antigen[a]

Group A streptococcal antigen dilution	Test result		
	CIE	Latex	COAG
10^{-1}	+	+	+
10^{-2}	−	+	+
10^{-3}	−	+	+
10^{-4}	−	+	−
10^{-5}	−	+	−
10^{-6}	−	+	−
10^{-7}	−	+	−
10^{-8}	−	+	−
10^{-9}	−	±	−
10^{-10}	−	±	−

[a] +, Positive reaction; −, negative reaction; ±, weakly positive. COAG is the Phadebact streptococcus group A reagent (lot 0851). Latex is the Wellcome Streptex A reagent (lot K0271). CIE, Counterimmunoelectrophoresis.

strain of group A streptococci. The antigen was extracted by autoclaving (6). Testing for antigen by the latex agglutination, staphylococcal coagglutination, and counterimmunoelectrophoresis methods was in accordance with the manufacturer's directions or with previously described methods (2).

RESULTS

The sensitivities of the counterimmunoelectrophoresis, staphylococcal coagglutination, and latex agglutination tests in the detection of streptococcal group A antigen from culture extracts are shown in Table 1. The latex agglutination test was consistently the most sensitive of the three tests. In testing gargle material for the presence of streptococcal antigen, we found that the majority of specimens caused spontaneous agglutination of the latex agglutination test reagent. Our preliminary studies indicate that trypsin "destroys" the factor(s) responsible for the nonspecific agglutination but does not interfere with latex-antigen complexing when antigen is present. In a clinical trial in which conventional culturing and latex agglutination were compared in terms of ability to identify group A streptococcal infections, a total of 53 throat specimens were collected: 26 were positive by both test procedures, 5 were positive by culturing but negative by the latex agglutination test, 3 were positive by the latex agglutination test but negative by culturing, and 19 were negative by both tests. An example of a positive test result is shown in Fig. 1.

DISCUSSION

Testing complex biological secretions for antigen has been troublesome because of nonspecific factors which can interfere with test interpretation. With the use of latex in highly contaminated fluids such as the gargle material described here, "nonspecific" agglutination of the latex was a major problem. The substance in gargle material causing agglutination of the latex particles is, at this time, unknown. The material was resistant to heat treatment in a boiling water bath for at least 5 min and was not dialyzable. Attempts to destroy the agglutinating property of the substance with various concentrations of trypsin or pronase B were successful over a broad range of enzyme concentrations: trypsin at 0.002 to 0.1% and pronase B at 0.01 to 2 mg/ml (pronase B at final concentrations of 12 mg/ml or greater spontaneously agglutinated the latex). This suggests that the agglutinating substance has a protein nature. Our experience with both of these enzymes indicates that their use may be a relatively simple solution to the nonspecific agglutination of latex by biological secretions.

We have also applied enzyme-linked immunosorbent assay procedures to identify streptococcal antigen from throat secretions. Although antigen could be detected in the secretions by these procedures, nonspecific reactions were so frequent that test results were difficult to interpret.

We are unable to explain the discrepancies between the culture results and the latex agglutination test results. Although no quantitative studies were made in these preliminary experiments, our casual observations did not suggest that the latex-negative, culture-positive results were due to low colony counts (<10 colonies). One explanation could be that the clinical stage of the disease was one at which very little, if any, soluble antigen was being expressed. This will require further study.

In this preliminary study, the latex agglutination test compared favorably ($P < 0.01$) with the conventional culture method in the identification

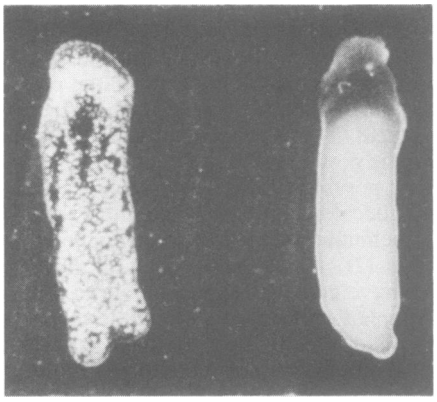

FIG. 1. A typical latex agglutination test with Streptex A reagent (left). Streptex B reagent was used as a negative control (right).

of group A streptococcal infections. If future studies confirm the results of these observations, diagnosis of streptococcal infections could readily be made a relatively simple routine office procedure. Further studies will have to be made on possible cross-reactions of Streptex C and D reagents. The optimal method of acquiring throat samples from young children has yet to be determined.

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