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Rapid Diagnosis of Group A Streptococcal Antigen Extracted Directly from Swabs by an Enzymatic Procedure and Used to Detect Pharyngitis

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Coagglutination after enzymatic digestion is a widely used, rapid procedure for serogrouping isolated colonies of beta-hemolytic streptococci. We tried to determine whether the same procedure could be used for the detection of group A streptococcal antigen directly from swabs used to take throat samples. This was achieved by incubating the swabs immersed in a small quantity of lytic extract obtained from cultures of the Maxted strain of *Streptomyces griseus* and testing the supernatant fluid by coagglutination. Of 538 throat swabs tested, blindly comparing the results of conventional cultures and rapid antigen detection, both tests were negative in 480 and both were positive in 49 swabs. In six cases, culture was positive and the rapid test was negative, but only one swab was from a patient with acute pharyngitis. In three cases, cultures were negative but the rapid test showed a strongly positive reaction. No special instructions were given to the physicians taking the samples. We conclude that this rapid antigen detection test, giving results in approximately 1 h, is an economic and reliable procedure for the detection of group A streptococcal antigen directly from throat samples.

Diagnosis of group A streptococcal pharyngitis relies mainly on the isolation by culture and further characterization of beta-hemolytic colonies. This procedure requires no less than 24 to 48 h.

Because a physician confronted with a patient with an infection of the throat has to make an early therapeutic decision and clinical diagnosis of streptococcal pharyngitis is unreliable (5, 6, 13), many physicians take the safer method of administering antimicrobial therapy rather than taking a culture and waiting for the results. This situation is clearly undesirable for many reasons, and many attempts have been made to improve it: The nitrous acid antigen extraction method from throat samples, with further detection by precipitation or coagglutination, has given good results (4, 12). However, the procedure of throat scrapings is very uncomfortable for the patients, and the manipulations required for antigen extraction are too laborious for routine application. The detection of soluble antigen in clinical samples by agglutination of latex particles sensitized with specific antibodies also has yielded good results (3), but these strongly depend on the quality of the sample, which requires the patient to gargle with phosphate-buffered saline (PBS) or a nutrient broth. Small children are generally incapable of fulfilling this requirement, and the commercially available reagents for this procedure are expensive.

Many laboratories use the *Streptomyces* sp. lytic extract-coagglutination technique for serological identification of isolated colonies of beta-hemolytic streptococci, which gives grouping results in less than 1 h (1, 11). We attempted to determine whether this procedure could be reliably applied to the detection of group A streptococcal antigen extracted directly from throat swabs under normal conditions in routine culturing. A description of the procedure and an analysis of the results follow.

MATERIALS AND METHODS

Patients and throat samples. Samples were taken with swabs from the throats of 538 children between 6 months and 7 years of age and were sent routinely to the laboratory for culture. No special instructions were given to the physicians taking the samples. Most of the swabs used in this trial were the Transwab (MW 170; Medical Wire Equipment Co., Cleveland, Ohio) with clear tranpsort media. A few were the Hospiswab (MW 130; Medical Wire Equipment Co.), coated with bovine serum albumin and without transport media.

Preparation and evaluation of lytic extract. The lytic extract was obtained from cultures of the Maxted strain of *Streptomyces griseus* (ATCC 13440) as described in Cowan and Steel (2). The fluid obtained was clarified by centrifugation at 4°C and stored at −70°C in small glass tubes (65 by 10 mm) in amounts of 0.3 or

0.4 ml per tube until used. A simple test was designed to evaluate the potency of this lytic extract. The sediment of a 24-h culture of *Streptococcus pyogenes* ATCC 19615 in 4 ml of Todd-Hewitt broth (Difco Laboratories, Detroit, Mich.) was emulsified in 1 ml of PBS, pH 7.2. This emulsion was homogeneized in a Vortex Mixer, and 25 μl was added to a tube containing 0.3 ml of the lytic extract. This tube was incubated for 45 min at 56°C in a water bath, after which twofold dilutions were made in PBS and 50 μl of each dilution was mixed with 25 μl of a group A-specific coagglutination reagent on a glass slide. Group A streptococcal antigen was detected up to a 1:512 dilution after 8 min of agitation of the plate. No drop in activity was detected after storage. A high potency of this lytic extract was shown to be essential. When the lytic capacity was intentionally reduced with adequate dilutions in PBS, false-negative results were obtained in known cases of group A streptococcal pharyngitis.

Coagglutination reagents. Coagglutination reagents were prepared as described by Carlson and McCarthy (1). A specific group A reagent (Coag-A) was prepared with a group A streptococcal antiserum (Difco) and a suspension of the Cowan I strain of *Staphylococcus aureus* ATCC 12598 (Pansorbin; Calbiochem-Behring). As a control, the same staphylococcal strain coated with normal rabbit serum (Coag-NRS) was also prepared. These reagents were stored at 4°C.

RAD test procedure. Upon arrival in the laboratory, the swabs were first seeded routinely on a sheep blood agar plate, which was incubated for 18 h at 37°C in 5% CO_2 and examined for the presence of beta-hemolytic colonies. These were identified and typed by the lytic extract-coagglutination procedure (11). Once the swab was seeded on the plate, it was introduced into a tube containing either 0.4 or 0.3 ml of the lytic extract, respectively, depending on whether the swab was dry or contained transport media. Before this step, excess transport medium was removed from the swab, rubbing it against the rim of the transport tube, to avoid later interference when coagglutination was read. The tube was then incubated for 45 min at 56°C in a water bath. After this incubation, swabs were removed from the tubes, pressing and rotating the cotton tips against the walls to release the fluid, and then were discarded. The tubes were then heated for 2 min at a boiling-water bath. This step was found to be necessary to remove nonspecific agglutinating factor from the mixture, with no lost of specific antigen concentration. The mixture was then centrifuged at 3,500 rpm for 5 min, and the clear supernatant was then ready for the rapid antigen detection (RAD) test. A 50-μl portion of this clear fluid was deposited in each of two contiguous wells of a transparent glass plate, in which 24 throat swabs could be simultaneously tested. To one of these two wells, 25 μl of the Coag-A reagent was added, whereas the other, which served as a control, received 25 μl of the Coag-NRS reagent. The contents of the two wells were briefly mixed with the tip of a toothpick and then mixing was continued, rotating the plate by hand. Agglutination was readily visible with proper illumination. A positive test was defined as presenting a rapidly or slowly progressing agglutination pattern appearing in the well containing the Coag-A reagent, with no, or only minimal, nonspecific agglutination in the control well. Results were read with the naked eye. Observation was never prolonged beyond 8 min.

TABLE 1. Comparison of results obtained by culture and the RAD test in 538 throat samples for detection of group A streptococci

No. of samples	Results with:	
	Culture	RAD test
480	−	−
49	+	+
6	+	−
3	−	+

RESULTS

The results of 538 tests performed by blindly comparing the findings of cultures and the RAD technique are summarized in Table 1. In 480 cases, both tests were negative, and in 49 cases both tests were positive. It is interesting to note that in 7 (14%) of these 49 cases in which results of both techniques coincided, cultures were initially and erroneously read as negative. When these plates were reexamined in view of the discrepancy, group A beta-hemolytic streptococci were recovered after a second careful reading or by subculture. In all cases, either the quality of the blood agar media or the streaking technique of the sample on the plate was not considered optimum; therefore, we do not consider these seven cases to represent true discrepancies in the results.

In three throat swabs, taken from children with acute pharyngitis, the RAD test gave strongly positive results, whereas the cultures were negative even after careful reisolation. These three cases were considered to give truly discrepant results. In six cases, colonies of group A beta-hemolytic streptococci were found on the culture plates, whereas the RAD test gave negative results. In all cases, the number of colonies on the plate was <10. Five samples were from children who were not acutely ill, but who were under routine study for their allergic complaints. The other sample was from a child with acute pharyngitis. These six cases were considered to be false-negative results of the RAD test.

As a rule, the intensity of coagglutination correlated well with the number of colonies present on the blood-agar plate. However, in five cases, strong reactions were observed, appearing during the first minute, with <10 colonies present on the plate.

In four cases of known infection with group A streptococci, an extra swab (in two cases with transport media and in two cases without it) was taken from the patients and left on a shelf in the laboratory for 3 weeks. The RAD test performed after this time remained positive. To our further surprise, even dry swabs maintained viable group A streptococci.

DISCUSSION

The culture of throat swabs and the further identification of beta-hemolytic colonies by any of the various procedures now used (bacitracin, fluorescence, coagglutination, latex agglutination, counterimmunoelectrophoresis), although very satisfactory for the definition of cases of group A streptococcal pharyngitis in 24 to 48 h, do not satisfy the needs of physician confronted with an acute case of throat infection. In this and in many other infectious diseases, accurate and more rapid means of defining etiological agents are clearly needed.

The procedure presented here, an RAD test, explores and amplifies the possibilities of enzymatic digestion and coagglutination for diagnosis of group A streptococcal pharyngitis before culture. The reagents needed for performance of the test can be prepared in a normally equipped laboratory at low cost. From 1 liter of growth medium of the Maxted strain of *Streptomyces griseus*, sufficient lytic extract was obtained for approximately 1,000 tests. A 1-ml amount of the suspension of *Staphylococcus aureus* and 0.1 ml of antiserum, finally diluted in 10 ml of PBS, provided sufficient coagglutination reagent for 400 tests. Moreover, all reagents are extremely stable, with minimal care, for long periods of time. As previously mentioned, the physicians taking the samples from the patients were unaware of the laboratory trial being performed, so the specimens were taken as usual. This is an important practical advantage when compared with the special requirements for sampling in other similar tests (3, 4).

The three cases in which culture was negative and the RAD test was strongly positive are difficult to interpret, especially since the three patients were suffering from acute pharyngitis in which group A streptococci would be expected to be found in abundance. A faulty culture procedure might be the explanation. However, cultures from other throat swabs performed on plates of the same batch of sheep blood agar were positive on the same days. Perhaps the use of a selective blood agar medium might have avoided these culture-negative results (7, 9, 10). Another possible explanation of these discordant results might be that the streptococci responsible for these acute infections were of those mutant nonhemolytic strains normally not detected by culture and which have been described as responsible for small epidemics (8). Also, the possibility of false-positive results of the RAD test cannot be ruled out.

Of the six false-negative results with the RAD test, only one was from a symptomatic patient; the other five were from carriers. Perhaps the sensitivity of the RAD test in detecting streptococcal antigen requires more bacteria than are normally found in a silent carrier and even in some pharyngitis cases. That only one swab was used to perform both tests and that the plates were seeded first could have given an advantage in number to the culture plates in the case of throat swabs with small amounts of bacteria present.

The number of positve cases included in this trial is probably insufficient to define any differences in sensitivity between culture and the RAD test. However, if the above results prove to be confirmed in successive trials in various environments, we feel that this procedure will be a very helpful addition to the diagnosis of streptococcal pharyngitis, one which can provide accurate results in approximately 1 h, at very low cast, with minimal requirements for laboratory equipment, and without any need of changing the routine procedures for taking samples.

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