JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1978, p. 725-728
0095-1137/78/0008-0725$02.00/0

Vol. 8, No. 6
Printed in U.S.A.

Serological Identification of Group A Streptococci from Throat Scrapings Before Culture

AZIZ EL KHOLY,[1] RICHARD FACKLAM,[2] GAMAL SABRI,[1] AND JIRI ROTTA[3]

Biomedical Research Center for Infectious Diseases, Cairo, Egypt[1]; Staphylococcus and Streptococcus Section, Bacteriology Division, Center for Disease Control, Atlanta, Georgia 30333[2]; and WHO Collaborating Centre for Reference and Research on Streptococci, Institute for Hygiene and Epidemiology, Prague 10, Czechoslovakia[3]

Received for publication 5 October 1978

The use of a microtechnique (modified nitrous acid extraction) to test samples from 150 school children and from patients with acute follicular tonsillitis has indicated that group A streptococci in the throat can be identified from tonsillar scrapings in 30 min. The results are comparable to the grouping results obtained by standard throat culture techniques and the Lancefield procedure for grouping. No cross-reaction with other bacteria or cellular material occurs. Study has also shown that the nitrous acid extraction yields three- to fourfold more polysaccharides than the Lancefield hot-HCl or Fuller formamide techniques. The use of the microtechnique leads to another 20-fold concentration of the antigen. Immune salting-out effect could be obtained with 1.00 M sodium acetate. Such molarity is too low to cause nonspecific salting out. It leads to a strong amplification of the precipitin reaction.

Some physicians are reluctant to take throat swabs from patients with upper respiratory tract infection because of the delays in getting reports on culture results. The serological grouping of beta-hemolytic streptococci requires a pure culture of organisms, and obtaining this often takes 48 h. For best results the fluorescent antibody technique of identifying streptococci from throat swabs requires a minimum of 4 h of incubation of the throat swab (6).

A technique that would make it possible to group these organisms serologically without culturing the throat swab would be a valuable tool for the rapid diagnosis of streptococcal pharyngitis and for the identification of group A streptococcus in general population surveys.

We have previously described the nitrous acid extraction technique (3). We have shown that small quantities of streptococcal growth can be extracted and that the precipitin reaction with such an extract is strongly amplified.

We describe a modification of the nitrous acid extraction technique that is adapted to use with either a single colony of streptococci from an agar plate or tonsillar scrapings, and we compare the amounts of group polysaccharide extracted by the modified nitrous acid, formamide, and hot-acid techniques.

MATERIALS AND METHODS

Tonsillar scrapings and throat cultures were taken from 150 school children aged 7 to 9 years and from 50 persons with clinical acute follicular tonsillitis who attended the outpatient clinic of Ain-Shams Medical School. Tonsillar scrapings were obtained with a blunt plastic spatula (3 mm wide by 15 mm long). The mouth was opened wide, the tongue was depressed, and the spatula was introduced. Each tonsil was swept twice from up to down by firm and moderate pressure. The spatula was then introduced in a Durham tube containing 20 μl of 2 M sodium nitrite. The scraping was released into the solution by gently agitating and rotating the spatula and knocking the bottom of the tube with it. No difficulty has been encountered in releasing material into the nitrite solution. The micro-nitrous acid extraction technique described below was used to extract these scrapings.

Strains. Four prototype strains of streptococci group A M6 (543/192/1) from the Rockefeller collection and wild strains A (3297), B (527), and C (2146) (isolated during throat culture of Egyptian school children in a rural area near Cairo) were used.

Sera. Group A immune sera provided by the Rockefeller University (R 1672/543/137/8) and locally prepared sera (6/78/3), (6/77/2), and (6/77/6) were used.

Modified nitrous acid extraction. Streptococcal cells (10^8) of an overnight growth were suspended in 0.2 ml of 2 M sodium nitrite, and 0.03 ml of glacial acetic acid was then added to the suspension. The reaction was allowed to proceed at room temperature for 15 min. Phenol red (0.25 ml) was added as indicator, and the pH of the suspension was adjusted with 10 N NaOH to a pH of 7.5. The total volume was brought to 0.5 ml with distilled water. The extract has 1 mol of sodium acetate per liter, produces 0.006 mol of nitrous acid per liter, and has a pH of 3.5.

Micro-nitrous acid extraction. A single strepto-

coccus colony from a blood agar plate or a tonsillar scraping from a patient was introduced into a Durham tube (3 by 30 mm) containing 20 μl of a 2 M sodium nitrite solution. Three microliters of glacial acetic acid was added. The reaction was allowed to proceed at room temperature for 15 min. Five microliters of phenol red indicator was then added, and the pH was adjusted to 7.5 with 10 N NaOH. The solution was brought to a final volume of 50 μl with distilled water.

Lancefield's HCl extraction (5) and Fuller's formamide extraction (4) were carried out according to standard techniques.

The precipitin test was performed with vaccine capillary tubes (1.2 to 1.5 mm, outside diameter). Approximately 5 mm of antiserum (10 μl) was first drawn into the tube, followed by the same amount of antigen, by capillary action. The tubes were inverted to allow the reagents to mix and then plunged into plasticine to hold them upright. Any precipitation that occurred within 15 min at room temperature was considered a positive reaction.

Rhamnose determination. The standard technique was used (1). However, the nitrous acid extract differs from the hot-HCl extract and the formamide extract because of its very strong oxidizing potential, which interferes with the formation of a cysteine-rhamnose complex. To avoid this masking effect, 0.5 ml of the crude nitrous extract was mixed with 1.0 ml of acid-alcohol solution in a ratio of 1:99 (vol/vol) and 8.0 ml of acetone for 15 min at 4°C. The precipitate was then collected by centrifugation at 1,000 × *g* for 15 min and reconstituted in 0.5 ml of normal saline. After neutralization with 1/5 N NaOH, the volume was brought up to 1.0 ml with normal saline. Rhamnose was then determined according to the standard technique (1). Such an extract will be referred to as the partially purified alcohol-acid extract.

RESULTS

Modified nitrous acid extraction. The amount of polysaccharides liberated from the cells by the nitrous acid extraction procedure is much higher than that released by the hot-HCl extraction method of Lancefield or by the formamide technique of Fuller. This is shown by the rhamnose determination in triplicate of the extract from 10^8 streptococcal cells by the different procedures shown in Table 1. The nitrous acid extracts had three to four times as much polysaccharide per milliliter of extract as the hot-acid or the formamide extracts.

The molarity of HNO_2 produced in the extraction procedure did not seem to affect significantly the amount of polysaccharides released under the conditions of the experiment. This was shown by the similarity of rhamnose content of the nitrous acid extract when 8 N HCl replaced the glacial acetic acid. In such an experiment, the molarity of HNO_2 produced was 200 times that obtained with glacial acetic acid. Moreover, the amounts of polysaccharide extracted with 0.02 N HCl and 0.05 N HCl were 148.70 and 125.00 μg of rhamnose, respectively.

TABLE 1. *Concentration of rhamnose in nitrous acid, hot-acid, and formamide extracts of group A streptococci*

Procedure	Rhamnose concn (μg/ml)
Nitrous acid[a]	
With 1 M glacial acetic acid	184.5
	214.99
	170.33
With 8 M HCl	170.9
	214.29
	230.77
Hot-HCl[b]	45.7
	71.43
Formamide[b]	50.0
	65.0

[a] Determinations were made in triplicate.
[b] Determinations were made in duplicate.

The modified nitrous acid extraction technique was used to show the qualitative amplification of the precipitin reaction. Each extract was diluted to contain 50 μg of rhamnose per ml. The amount of precipitate obtained with the nitrous acid extract of group A streptococcal strains measured 8 mm after 24 h at 4°C, whereas only 3 mm of precipitate formed with the Lancefield and the Fuller extracts containing the same amount of rhamnose. Moreover, the appearance of the precipitin reactions differed. The nitrous acid extract immune serum reaction appeared in small lumps of aggregated salted-out material, whereas the precipitin reaction was usually diffuse with the Fuller and Lancefield extracts.

The amplification of the precipitin reaction was further demonstrated by diluting nitrous acid, formamide, and hot-acid extracts with distilled water and sodium acetate. The three extracts were prepared from the same batch of streptococcal cells. Their polysaccharide contents were each adjusted to 50 μg of rhamnose per ml. The precipitin reaction of all extracts containing the sodium acetate diluent was strongly amplified. In nearly every instance, through the first three dilutions (1:2, 1:4, and 1:8) the amount of precipitate in the sodium acetate-diluted extract was twice that in the distilled water-diluted extract. The amplification is related to the amount of polysaccharide antigens, since the amplification decreased with increasing dilution of the polysaccharides in spite of an unchanging amount of sodium acetate.

The following experiment was undertaken to determine the amount of sodium acetate required to initiate a nonspecific salting-out effect.

Five different sera from nonimmunized rabbits (normal rabbit sera) and 10 different batches of immune rabbit sera were tested in capillary tubes against sodium acetate solutions of different molarities. The sodium acetate solutions were reacted with the different sera as if they were extracts. Equal amounts of sodium acetate and rabbit serum were drawn into the capillaries and allowed to react for 10 min. None of the antisera precipitated when 1 M sodium acetate was added. One antiserum to group A streptococci precipitated when 1.5 M and 1.25 M sodium acetate were added, and one of five normal and three of eight immune rabbit sera showed nonspecific salting out when 1.74 M sodium acetate was added.

The immune salting-out effect of sodium acetate did not seem to occur with a soluble antigen-antibody complex of group A polysaccharide. Solutions containing 10, 50, and 1,000 μg of pure *N*-acetyl glucosamine were prepared with distilled water, physiological saline, 1.5 M sodium acetate, and the nitrous acid extraction reagents. The pH was adjusted to 7.5. Each of the 12 solutions was reacted with three different group A antisera. No visible immune precipitin reaction occurred with any of the combinations of reactions.

Micro-nitrous acid extraction. From the previous experiment it has been shown that much larger amounts of polysaccharide are extracted from the cells by the nitrous acid extraction procedure than by the hot-HCl or the formamide extraction techniques. It has also been shown that the nitrous acid leads to amplification of the immune precipitin reaction in capillaries. On the basis of these two premises a microtechnique has been developed. The objective of the microtechnique is to extract an adequate quantity of polysaccharides from a single streptococcus colony or the scrapings from a patient's throat to produce a precipitating antigen-antibody complex. A second objective is to demonstrate that the insoluble antibody complex formed does initiate an appreciable degree of salting out to amplify the specific precipitin reaction. A third objective is to insure that enough extract is available for immune testing to allow four immune precipitin reactions in capillary tubes to be made, so as to test the extract for a single organism against the homologous sera of group A, B, C, and G strains.

By the micro-nitrous acid extraction technique we tested 30 strains of group A, B, C, and G streptococci taken from the Cairo Biomedical Research Center Culture Collection. Single colonies were picked up with a spatula, placed in sodium nitrite solution, and extracted. Strong homologous capillary precipitation reactions were observed. No cross-reactions were detected either between these groups of streptococci or with immune sera against *Neisseria* (groups A, B, C), *Haemophilus* (type b), or *Pneumococcus* (omni-serum).

An additional 50 stock strains of groups A, B, C, D, E, F, and G streptococci, taken from the Streptococcus Laboratory, Center for Disease Control culture collection, were extracted by the micro-nitrous acid extraction technique. Strong homologous, specific reactions were detected in all instances except for the group D strains. No reactions were observed between the micro-nitrous acid extracts of group D and homologous group D antiserum.

Application of the microtechnique to throat scrapings. The identification of streptococci by the throat scraping, micro-nitrous acid extraction method was compared with the identification of streptococci by the throat swab, culture confirmation technique, consisting of isolation, extraction, and serological grouping by the Lancefield procedure. Table 2 shows the results obtained on 200 patients. There was 96.5% (193 of 200) agreement between the two methods. The throat scraping, micro-nitrous acid technique failed to identify six patients with group A streptococci that were confirmed as having group A streptococci by the swab culture confirmation technique. Although the throat scraping, micro-nitrous acid technique identified only 82% of the group A streptococci in these patients, in the instances where the technique failed to identify group A streptococci there were only 15 colonies or less (an average of 6) on the agar plates.

DISCUSSION

The biochemical and immunological characteristics of the nitrous acid extraction procedure were studied. It was shown that nitrous acid releases a much higher amount of group carbo-

TABLE 2. *Number of identifications of group A streptococci by the throat scraping, micro-nitrous acid technique and throat swab, culture confirmation technique*

Throat-scraping technique	Throat swabs–culture confirmed[a]		
	Positive group A	Negative group A	Total
Positive group A	28	1	29
Negative group A	6	165	171
Total	34	166	200

[a] Throat swab culture confirmation technique: beta-hemolytic colonies were selected from blood agar plates, grown, extracted by the Lancefield procedure, and serogrouped by the standard capillary precipitin technique.

hydrate from beta-hemolytic streptococci and that the sodium acetate produced acts as a salting-out agent. With 1.0 mol of sodium acetate per liter, the insoluble antigen-antibody complex can initiate an immune salting-out effect which strongly amplifies the immune precipitin reaction. A microtechnique has been developed for the extraction of beta-hemolytic group-specific polysaccharides. Serological identification by immune precipitation in capillary tubes can be made in vitro from the extract of a single streptococcal colony. The technique has also proved to be potent and sensitive with tonsillar scrapings and compares adequately with the standard culture and extraction techniques. The greater than 96% correlation between the scraping, micro-nitrous acid technique and the standard culture technique is in the same range of accuracy as the fluorescent antibody and standard culture techniques (6).

If future studies show that the 96% correlation between the throat scraping, nitrous acid extraction technique and the standard culture technique holds true, it may be possible to replace the fluorescent antibody technique for identification of group A streptococci. Our data indicate that our technique is as sensitive as the fluorescent antibody technique, and it is more rapid. An immune precipitin reaction can be demonstrated 30 min after the scrapings are taken. In addition, none of the sophisticated equipment needed for the fluorescent antibody procedure is necessary for the identification of group A streptococci with the micro-nitrous acid technique. In theory, the patient could be asked to wait for the laboratory confirmation of the scraping to receive proper treatment.

Even if the throat swab is obtained, subculture of the beta-hemolytic streptococci is no longer necessary to identify the streptococcus as group A or non-group A. A single colony can be selected, extracted, and serogrouped within 20 min. With respect to time required, the micro-nitrous acid extraction technique compares favorably with the co-agglutination technique described by Edwards and Larson (2). Expensive complexed antiserum is not required for the micro-nitrous acid technique, as it is for the co-agglutination reaction.

Because of its simplicity, the scraping technique should make possible the rapid and early treatment of group A tonsillitis in private practice. The impact of the technique on population surveys of group A streptococci in developing countries will be enormous. For all practical purposes, a small kit of capillary glassware and some inexpensive reagents are all that is needed.

ACKNOWLEDGMENT

This work was supported by PL-480 grant no. 03-325-C from the Center for Disease Control.

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