Evaluation of the Culturette Brand Ten-Minute Group A Strep ID Technique

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Received 30 December 1983/Accepted 20 March 1984

A direct extraction of the antigens of group A beta-hemolytic streptococci from 557 throat swabs was performed by a new microtechnique of the nitrous acid extraction method with the Culturette Brand Ten-Minute Strep ID technique from Marion Scientific, Division of Marion Laboratories, Inc., Kansas City, Mo. This group A latex reagent kit contains the reagents for the micronitrous acid extraction of throat swabs and does not require a centrifugation step in its protocol. There was a 99.3% (553 of 557) total agreement between the direct nitrous acid extraction-latex agglutination method and the standard culture method. The direct extraction method yielded an identification of 95.1% (76 of 82) of the group A streptococci identified by the standard method. Throat swabs used for standard culture may also be extracted with nitrous acid for the detection of group A antigen. A 5-min nitrous acid extraction destroys the viability of bacteria associated with normal throat flora as well as group A streptococci and Mycobacterium tuberculosis. This highly rapid method is simple to perform and requires no costly instrumentation. Accordingly, it would be most applicable in a hospital laboratory as well as in a physician's office.

A direct serogrouping method for group A (5, 9) and group B (8) streptococci from swab specimens by a nitrous acid extraction-coagglutination method was recently shown to be a highly rapid method. This procedure yields a 77 to 78% agreement for group A streptococci from throat swabs (9) and 92.0% agreement for group B streptococci from urine specimens (8) when compared with results obtained from a standard culture method. This direct extraction method, including the examination of results by coagglutination, requires about 20 to 25 min (8, 9).

Recently, a latex agglutination kit for the identification of group A streptococci directly from throat swabs was made available to be examined for its potential application in clinical use. This latex reagent kit, the Culturette Brand Ten-Minute Group A Strept ID (Marion Scientific, Division of Marion Laboratories, Inc., Kansas City, Mo.), contains the reagents required for the micronitrous acid extraction of throat swabs and does not require a centrifugation step in its protocol.

The purpose of this investigation was to evaluate this kit in regard to its sensitivity, accuracy, and suitability for the direct serogrouping of group A streptococci from throat swabs. The procedure was compared with a standard throat swab culture confirmation method, in which serological grouping was achieved with the Meritec-Strep coagglutination reagents (Meridian Diagnostics, Inc., Cincinnati, Ohio) with overnight broth suspensions.

MATERIALS AND METHODS

Swab specimens. Pharyngeal cultures were obtained in duplicate by simultaneously rubbing two Dacron-tipped swabs (Culturette II; Marion Scientific) over the throats of 557 patients.

Standard culture method. One of the paired swabs was rolled on a portion of a Columbia agar plate containing 5% sheep blood (Scott Laboratories, Fiskeville, R.I.). The inoculum was further distributed by streaking with a loop to obtain isolated colonies. The plates were then incubated at 35°C under anaerobic conditions in Gaspak (BBL Microbiology Systems, Cockeysville, Md.) units or Bio-Bags (type A; Marion Scientific) for 12 to 18 h. These plates were then observed for the presence of beta-hemolytic and catalase activities (4). The primary isolates of the beta-hemolytic streptococci were serogrouped from extracts of overnight broth cultures inoculated with a single colony of group A streptococci with the Meritec-Strep beta-hemolytic grouping coagglutination reagents (Meridian Diagnostics). The coagglutination procedure was performed generally in the manner suggested by the manufacturer. Briefly, drops of the overnight culture were respectively mixed with group A, B, C, F, and G coagglutination reagents. A clear glass slide was used and was rocked for 1 min and examined for coagglutination with transillumination against a dark background.

Direct-swab extraction method. The second of the paired swabs was placed in a microtube, provided in the Culturette Brand Ten-Minute Group A Strept ID kit, after 1 drop each of extraction reagents 1 and 2 were added. The swab was ground into the bottom of the microtube. The swab was then rolled against the wall of the microtube to express liquid from the swab and back into the microtube.

After the swab was incubated for 5 min at room temperature, 2 drops of extraction reagent 3 were added. The swab was rolled and pressed against the walls of the microtube again to express fluid. The swab was then permitted to remain in the microtube for about 10 s so that the maximum amount of liquid was absorbed back into the swab.

Identification of group A streptococci by latex agglutination. The swab, after having been placed in the microtube and extracted, was briefly rolled on two circular areas of the glass slide provided in the kit. This maneuver released extraction fluid onto the slide. One drop of the latex group A detection reagent contained in the Culturette Brand Ten-Minute Group A Strept ID kit was next added to one of these inoculated circular areas, and one drop of the negative control reagent was added to the other circular area. The slide was then rocked for 2 to 3 min and examined for agglutination of the latex particles. A positive control rea-

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gent was also available in the kit and was employed on a daily basis to test the activity of the group A detection reagent.

In another set of experiments, the effectiveness of the nitrous acid extraction procedure associated with the kit and the swab extraction method requiring a centrifugation step and the reagents prepared in our laboratory (8, 9) was compared. For this experiment, three swabs per patient were employed, respectively, for standard culture, nitrous acid extraction with the reagents and microtube from the kit and nitrous acid extraction with the laboratory-prepared extraction reagents and the associated centrifugation step (7). The group A latex reagent from the Culturette Brand Ten-Minute Group A Strep ID kit was employed in the detection of group A streptococci from all the extracted throat swabs in this study.

The effectiveness of nitrous acid extraction on throat swabs previously used for culture was examined. For this area of the investigation, 30 swabs previously shown to contain group A streptococci by the standard culture method were each extracted with the nitrous acid reagents and microtubes contained in the Culturette kit. These “used” swabs were not extracted and examined for the presence of group A antigen until 3 weeks after their respective receipt and culture in the laboratory. Accordingly, these swabs were maintained after culture in the swab sheaths at room temperature until their extraction was performed. The Culturette Brand Ten-Minute Group A Strept ID latex reagents were again employed for the detection of this serogroup antigen from these swabs.

The effect of nitrous acid extraction on the viability of group A streptococci, normal throat flora, and Mycobacterium tuberculosis was investigated. Throat flora from swabs cultured on sheep blood agar plates were compared with the growth of bacteria from the nitrous acid extracts derived from paired swabs extracted with the kit. Three strains of M. tuberculosis and one strain of H37RV were grown in Middlebrook 7H9 broth (Difco Laboratories, Detroit, Mich.) for 3 weeks and yielded approximately 10^8 cells per ml. Swabs were placed in these broths and extracted for 5 min with nitrous acid. One drop of extract from each swab was spread, respectively inoculated on slants of Grant modified Lowenstein-Jensen agar (Difco). The slants were incubated in a 35°C carbon dioxide incubator, and the presence of growth was examined through 6 weeks.

**RESULTS**

There was a 99.3% (553 of 557) total agreement between the direct nitrous acid extraction-latex agglutination method and the standard culture method. The direct extraction method yielded an identification of 95.1% (78 of 82) of the group A streptococci identified by the standard method. The four negative direct tests were associated with the isolation of one to eight colonies of group A streptococci per primary culture plate. The agglutination responses generally were observed to occur within 1 to 2 min. The identification of the group A streptococci by the direct method was achieved within the 12 to 15 min required for both the extraction and the examination of the latex agglutination response.

Of the 557 paired swabs examined by culture and direct extraction for group A streptococci, no cross agglutination responses were observed with 15 swabs containing group B streptococci, 8 swabs containing group C streptococci, 5 swabs containing group F streptococci, and 3 swabs containing group G streptococci.

Twenty throat swabs taken in triplicate, containing group A streptococci as previously determined by the standard culture method, were employed in an investigation to compare the method with the extraction kit that employs a microtube and the method with our laboratory-prepared extraction reagents with its associated centrifuge step as to any potential differences in their respective sensitivity. On primary culture, all the swabs yielded about 40 to 100 colonies per plate. The group A antigen was detected when both extraction methods were respectively applied to the remaining paired swabs. Furthermore, the relative agglutination response was similar with the extraction fluid derived from the paired swabs.

Thirty throat swabs, previously used for primary isolation of group A streptococci, were extracted for the group antigen. All of these swabs released detectable serogroup A antigen after nitrous acid extraction with the kit reagents.

Twenty-five nitrous acid extracts of throat swabs were cultured on sheep blood agar plates. Standard culture of paired unextracted swabs revealed bacterial flora consistent with normal throat specimens. Nine of these throat swabs yielded group A streptococci and one contained group B streptococci. Nitrous acid extraction of the second of these paired swabs did not yield viable bacterial growth after 6 days of incubation. Furthermore, the nitrous acid-treated strains of M. tuberculosis examined in this investigation did not yield growth through 6 weeks of observation.

**DISCUSSION**

Latex agglutination testing has previously been shown to be useful for the serogrouping of beta-hemolytic streptococci from primary isolation plates (6). The present investigation is the first that has demonstrated that latex agglutination can also be successfully employed for the direct detection of group A streptococci from nitrous acid extracts of throat swabs. Previously, the use of a coagglutination reagent with direct microrinsacid extractions of throat swabs yielded a 77 to 78% agreement of group A streptococcal identification by a standard culture procedure (5, 8). In contrast, the present investigation by the Culturette Brand Ten-Minute Group A Strept ID yielded a 95.1% agreement for the identification of group A streptococci from throat swab specimens.

An examination for specificity to group A streptococci by the Culturette latex reagent was further enhanced by the fact that cross agglutination responses were never observed with swabs containing non-group A beta-hemolytic streptococci.

The method described in this report is unique in that a centrifugation step, as has been reported in previous investigations (5, 8, 9), was not required. Accordingly, the kit permits the extraction of throat swabs with the use of microtubes. The extraction steps are relatively simple to perform. The time for an extraction of a swab through the examination of the nitrous acid extract for an agglutination response ranges from only 12 to 15 min. This also is in contrast to the previously reported nitrous acid-coagglutination methods that required about 20 to 25 min (5, 8, 9). The time difference appears to be directly related to the use of a microtube for the direct extraction as well as to the absence of a need of a centrifugation step for the sedimentation of bacteria.

Recently, a commercially available extraction-latex agglutination kit was made available (Hyson, Westcott and Dunning, Baltimore, Md.). This kit makes use of enzymatic extraction. In contrast to the present investigation and the previous reports that describe the nitrous acid extraction (2, 3), the enzymatic extraction procedure requires over 60 min
from receipt of swab sample until the examination of the extract for group A streptococci. Accordingly, enzymatic extraction of colonies of beta-hemolytic streptococci for the determination of their respective group antigen is generally reported to be performed with a 1-h incubation period [1, 10].

Recently, a method has been reported that employed a 45-min enzymatic extraction of throat swabs for the detection of group A antigen [7]. The procedure employed a laboratory-prepared coagglutination reagent for the detection of these streptococci. The extracts, however, then had to be boiled for 2 min to suppress nonspecific agglutination. In contrast, nitrous acid extracts of throat swabs have not been reported to yield nonspecific agglutination responses [5, 8, 9].

The Culturette Brand Ten-Minute Group A Strep ID method and the extraction method of our laboratory were equally effective in yielding detectable group A streptococcal antigen with the group A latex detection reagent. Accordingly, the higher sensitivity of detection reported in this investigation in comparison to previous reports [5, 8, 9] may be related, in part, to the nature of the antibody to group A carbohydrate incorporated on the respective carrier particle.

This investigation has demonstrated that if a throat swab is first used in the preparation of primary isolation plates, it may also be effectively employed for the detection of group A streptococci when extracted with nitrous acid. Since the latter observation was based on the results from only 30 swabs, the dependency of utilizing one swab for both culture and nitrous acid extraction may, at this time, be viewed with caution until more used swabs are examined in future investigations.

The use of the standard culture method in conjunction with the direct extraction method for group A streptococci may be warranted by some physicians in instances in which quantitation of bacterial flora is required and in which information is required as to the presence of microorganisms other than group A streptococci that may be associated with pharyngitis.

Our data further emphasize that nitrous acid extraction is a highly efficient means to yield the streptococcal group A carbohydrate from clinical material. It has been demonstrated previously that nitrous acid extracts can yield three to four times as much polysaccharide per milliliter of extract as hot acid or formamide extracts [2].

The directions given in the microtiter acid extraction-group A identification kit state that on completion of an extraction-agglutination procedure, the extract and slide with associated reactants may be disposed in a laboratory waste disposal container. Our study disclosed that a 5-min nitrous acid extraction destroys the viability of bacteria associated with normal throat flora as well as group A streptococci and M. tuberculosis. Although the effect of nitrous acid extraction on the viability of various fungi and viruses was not examined, the investigation has shown that the extraction fluid is of a minimum biohazard factor.

In summary, the Culturette Brand Ten-Minute Group A Strep ID kit appears to provide a highly rapid and sensitive means to identify group A streptococci directly from throat swabs. The method is simple to perform and requires no costly instrumentation. Accordingly, it would be most applicable in a hospital laboratory as well as in a physician’s office.

LITERATURE CITED


