Rapid Fluorescent-Antibody Method with Bromelase for Identification of Group A Streptococci

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This is a preliminary report on a rapid fluorescent-antibody staining method utilizing the enzyme Bromelase that effectively eliminates nonspecific staining of staphylococci. By this simple method, group A streptococci were accurately identified from 2- to 4-h broth cultures.

Many investigators (2, 3, 9, 10) have demonstrated that group A streptococci could be accurately identified by the use of fluorescent-antibody (FA) techniques. Some of these studies (3, 9, 10) were evaluations of FA smears made from 2- to 6-h broth cultures. Others (2, 10) reported that colonies picked from blood agar plates could also be accurately identified by the FA method. Excellent results with rapid FA staining methods have also been reported (6, 8). The use of a system incorporating broth cultures and a rapid staining method would make it possible for a diagnostic laboratory to accurately identify group A streptococci the same day the culture is collected.

It is well known that a major problem encountered in a broth culture method is the nonspecific FA staining of certain strains of staphylococci (1, 7). Various methods have been devised to control this problem. Treatment of the smears with enzymes such as papain (7) or trypsin (11) before staining, adsorption of the antiserum conjugate with staphylococci (13), or the addition of normal rabbit globulin (6, 13) appears to be the most frequently used method. Forsgren and Forsum (5) demonstrated that the nonspecific FA staining is due to an interaction between the Fc portion of immunoglobulin molecules and the protein A present in many strains of *Staphylococcus aureus*. Therefore, it would seem that enzyme treatment should be an effective way of eliminating the problem of protein A. The methods reported that use trypsin or papain require preparation of these solutions involving the addition of buffers and pH adjustment. This reagent preparation is bothersome and time-consuming for a busy hospital laboratory. A protease, bromelain, derived from pineapple was described by Pirofsky and Mangum (12) as useful to demonstrate erythrocyte antibodies in blood-banking procedures. This enzyme is available as a commercial enzyme product, Bromelase (Dade Division, American Hospital Supply Corp.).

The possibility of using a commercial enzyme product seemed to be worth investigating, since it would eliminate reagent preparation and would provide a product of uniform activity. Therefore, we incorporated the use of Bromelase with an FA staining procedure and found that it effectively eliminates nonspecific staining of staphylococci with no adverse effects on the staining of group A streptococci. Its effectiveness on staphylococci is apparently due to removing protein A from the surface of these organisms. This report describes the results obtained from the examination of 2- to 4-h broth cultures for group A streptococci by using a method of Bromelase treatment and rapid FA staining.

During a period of several weeks, 545 upper respiratory tract specimens from patients at St. Joseph's Hospital were cultured by inoculating 5% sheep blood agar (SBA) and 2-ml Todd-Hewitt broth tubes. After 2 to 4 h of incubation at 37°C, the broth cultures were examined for group A streptococci by FA staining. The SBA plates were incubated in a candle jar overnight at 35°C. Beta-hemolytic streptococci isolates were tested with a bacitracin "A" disk (BBL, Cockeysville, Md.). As recommended by Facklam (4), any zone of inhibition was considered positive. Precipitin grouping was done on all β -hemolytic streptococci not positive by FA broth smears and in cases of disagreement between FA results and the bacitracin test. The Rantz-Randall extraction method was used as described by the antisera manufacturer (BBL).

For FA staining, 2- to 4-h Todd-Hewitt broth cultures were centrifuged at 2,000 rpm. The supernatant was discarded, and the sediment was suspended in 1 ml of phosphate-buffered saline, pH 7.2 (FA buffer, Difco Laboratories, Detroit, Mich.). This buffered suspension was centrifuged as before, and the supernatant was discarded. One loopful of the sediment was transferred to a microscope slide and spread within a 1-cm circle. The smear was air-dried

and then heat-fixed by passing it through a flame. A drop of Bromelase was added to the fixed smear; the slide was placed in a moist chamber and allowed to stand at room temperature for 2 min. The slide was then rinsed under running tap water and blotted dry. A drop of diluted anti-group A fluorescein-conjugated serum (Sylvania Co. Inc., Millburn, N. J.) was added to the smear. The lot of antisera used was titrated for activity and was used at a working dilution of 1:40. The slide was placed in a moist chamber and incubated at 35°C for 5 min. The slide was then rinsed under running tap water and blotted dry. Positive and negative control slides were made with stock cultures of group A and G streptococci, respectively. A Bromelase activity control was made by using a stock culture of cross-reacting staphylococci. Two smears of this staphylococcus were made on one slide; one smear was treated with Bromelase, and the other smear was untreated. A drop of buffered glycerin, pH 7.2 (FA mounting fluid, Difco), was added to the stained area of the slides, and a no. 1 cover slip was applied.

Smears were examined by using the following equipment: Zeiss reflected-light fluorescence microscope with a 100-W tungsten-halogen illuminator, KP500 and KP490 excitor filters, LP528 barrier filter, LP510 chromatic reflector, planapochromat 40/1.0 oil objective, and a binocular head with a $\times 10$ ocular lens system. Brilliant yellow-green (4+) or bright yellow-green (3+) fluorescent cocci in these smears were interpreted as group A streptococci. The negative streptococci control and the Bromelase-treated staphylococci were either not visible or only very faintly stained (0 to 1+).

We found that the three false-negative FA broth specimens were positive when FA smears were made from colonies on SBA (Table 1). The failure to detect group A streptococci from smears of the original broth may have been due to making the FA smears before there was adequate growth. In an attempt to resolve the discrepancy of the five specimens that were FA broth positive and had no β -hemolytic streptococci on SBA, we restreaked the sediment from these onto SBA. Group A streptococci were isolated from one specimen but not from the other four. If selective SBA (containing neomycin-nalidixic acid) had been used for these restreaks, maybe more of these specimens would have been positive. Although *S. aureus* was isolated from approximately 7% of the specimens, there was no staining of staphylococci.

This study indicates that this rapid staining method utilizing Bromelase effectively eliminates nonspecific staining of staphylococci, so that group A streptococci can be accurately

TABLE 1. Identification of group A streptococci by Bromelase treatment of FA smears

Test smears	Results ^a
β -Hemolytic streptococci on SBA	119/545 (21.8)
No β -hemolytic streptococci on SBA, FA group A + ^b	5/545 (0.9)
β -Hemolytic streptococci on SBA, FA group A +	53/119 (44.5)
β -Hemolytic streptococci (group A) on SBA, FA group A - ^c	3/119 (2.5)

^a Numbers in parentheses are percentages.

^b One of five confirmed beta group A by cultural methods; four not confirmed.

^c Confirmed group A streptococci by serological methods.

identified from 2- to 4-h broth cultures. This early identification has an obvious advantage as an aid to improved patient care.

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