CONCISE COMMUNICATION

False-Positive Rapid Antigen Detection Test Results: Reduced Specificity in the Absence of Group A Streptococci in the Upper Respiratory Tract

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Rapid antigen detection tests (ADTs) are widely promoted for the laboratory identification of group A streptococci (GAS) in the upper respiratory tract (URT) of patients with pharyngitis and also have been found to be useful in identifying GAS in impetigo [1, 2]. Although debate persists about their sensitivity, most investigators consider ADTs to be highly specific. Nevertheless, reports continue to describe false-positive ADT results (positive ADT result, negative culture result). This study examined culture results from 522 adult patients with acute pharyngitis and a positive ADT result; unexpectedly, 15% had throat culture results negative for GAS. Normal bacterial flora from 30% of these “negative” culture results produced a positive ADT result. An ADT-reactive organism belonging to the Streptococcus milleri group was isolated from 1 culture; it carried group A carbohydrate antigen. Although S. milleri previously has been associated with false-positive ADT reactions, the frequency of this phenomenon is unknown. The present, large study suggests that false-positive ADT reactions may be more common than previously reported, an observation that must be precisely explained and epidemiologically and clinically defined.

Patients and Methods

Study population. Throat swabs from 522 patients (mean and median age, 26 years) who presented with symptoms of acute-onset pharyngitis and/or tonsillitis and who were presumptively diagnosed as having Streptococcus pyogenes in their URTs—on the basis of a positive ADT result—were examined. None of the patients had received a systemic antibiotic within 3 days or a “long-acting” antibiotic within 4 weeks of enrollment. These patients were enrolled from January through March 1998, from 27 clinics located in 25 states. All enrolled patients received appropriate antibiotic therapy. No patients developed sequelae of GAS infection.

Culture methods. After presumptive confirmation of GAS pharyngitis, on the basis of a positive ADT result, throat cultures were obtained by using a Bacti-Swab culture kit (Remel) and were sent to our laboratory by overnight courier. On receipt, the throat swabs were plated on trypticase soy agar with 5% sheep blood (DiMed). During plating, the agar was stabbed with the loop to produce a growth environment with reduced oxygen tension, to enhance detection of poorly hemolytic GAS strains. The plates were incubated aerobically at 36°C for 18–24 h. Beta hemolytic streptococci were isolated from all positive cultures, were grown in broth, and were frozen for future serological group verification. Negative plates were held at room temperature (~20°C) and were reexamined at 48 and 72 h. In addition, all negative cultures were replated, using the original swab (stored at 4°C), and then were incubated in 5% CO₂ for 24 h. If the original plate was overgrown with staphylococci or gram-negative bacteria, the swab was replaced on a 5% sheep blood agar plate containing 1 mg/mL crystal violet or on Columbia CNA agar (DiMed) containing 5% sheep blood and was incubated aerobically. All plates were carefully examined for colonies with GAS morphology with a 3.5× magnifying lens. A patient was considered to have a negative throat culture result only if all the agar plates were negative for GAS.

Serogrouping. Serological grouping was done by the classical Lancefield method, using antisera prepared in our reference laboratory [4].
**ADT.** The rapid ADT (TestPack Plus; Abbott Laboratories) was done initially at the patient’s primary care site by trained laboratory personnel, following instructions in the package insert. This kit, like most others, depends on detection of group A carbohydrate antigen [3]. For this study, a false-positive ADT result was defined as a positive ADT reaction with a throat culture that was negative for GAS (*S. pyogenes*).

“Negative” culture plates from patients previously determined to have a positive ADT result were examined carefully, to determine whether the ADT reaction might be due to the presence of either true GAS with atypical morphological or hemolytic properties or, alternatively, non-GAS organisms that reacted in the ADT. A swab was streaked once across the confluent growth area of the original agar plate and then was tested with the ADT. Cultures giving positive ADT reactions were carefully reexamined, to isolate the specific ADT reactive organism(s). Up to 20 morphologically distinct colonies from each negative culture plate were isolated, were subcultured, and were tested for ADT reactivity.

**Streptococcus milleri** identification. Identification of *S. milleri* group organisms was made by phenotypic characteristics (colony morphology and caramel odor) [5] and was confirmed by routine biochemical tests [6].

### Results

Of the 522 cultures obtained from patients with a positive ADT result, 77 (15%) had negative GAS culture results. The culture plates from 76 (99%) of 77 were subsequently tested for a reaction with the ADT; 23 (30%) of 76 gave an unequivocal positive reaction.

An organism producing a positive ADT reaction belonging to the *S. milleri* group of bacteria (*Streptococcus intermedius*) was identified from one of these GAS-negative plates. This organism had the typical tiny colony morphology and caramel odor of *S. milleri* [5]. It was nonhemolytic on original isolation but became beta hemolytic after passage on sheep blood agar. This organism carried group A carbohydrate antigen, as determined by classical Lancefield serogrouping [4]. *S. milleri* group bacteria were isolated from a total of 14 of these GAS-negative culture results. Only the one described above, however, gave a positive ADT reaction in pure culture. Although mixed flora subcultures of the 23 ADT reactive cultures continued to give positive ADT reactions, we were unable to isolate and identify the specific responsible organism(s) from the remaining 22 cultures.

### Discussion

The finding that fully 15% of patients initially diagnosed with pharyngitis associated with GAS on the basis of a positive ADT result were, in fact, culture negative was unexpected and somewhat disturbing. Published reports have suggested that the primary concern with ADTs has been false-negative results, especially when small numbers of streptococci are present. The reported rate of false-positive results usually has been quite low [3].

There are several possible explanations for our findings other than “failure” of the ADT. It is possible that the GAS on the original swab died before being received in our laboratory, because of either delays in shipping or effects of possible freezing and thawing during winter transport. To evaluate these possibilities, we compared data from the present study with a study conducted 1 year earlier with 538 pediatric-aged patients. That study used the same ADT, culture transport swab kit, and culture protocol and also was conducted during the winter months. That study yielded only 4.1% (22/538) positive ADT results with a concomitant negative plate, which is less than one-third the rate seen in the present study.

We also compared how long it took the swabs that yielded positive culture results and those that gave negative culture results to reach our laboratory. In the present study, 97.5% of the positive cultures and 96.8% of the negative cultures were received within 2 days. These transit times are shorter than those achieved in the previous study, in which 83% of the positive cultures and 77% of the negative cultures were received within 2 days. Delays in transit apparently were not responsible for the higher than expected negative culture rate. These observations suggest that loss of viability during transport does not adequately explain the high rate of negative culture results.

In addition, repeated subcultures of mixed flora from these GAS-negative plates continued to give a positive ADT result, which is strong supportive evidence that nonviable GAS were not responsible for these reactions.

It is also possible that the swab used for the ADT and the one used for the culture were not comparable because of sampling errors. Even 2 swabs taken simultaneously can differ 10% of the time [7]; however, using a virtually identical culture protocol, the previous study yielded only 4.1% positive ADT results with a concomitant negative plate, which suggests that swab-to-swab discordance is not significantly responsible for our results.

Some investigators have hypothesized that false-positive ADT reactions can be explained by residual GAS carbohydrate antigen in the throat after killing of the organism with antibiotics. This explanation appears to be unlikely, however, because individuals who recently received antibiotic therapy were excluded from enrollment. One study found that GAS antigen does not usually persist in the URT >24 h after antibiotic therapy [8]. It is interesting to note that the study reported positive ADT reactions in 5% (4/85) of patients with negative GAS culture results 14–16 days after antibiotic therapy [8]; this is compatible with the possibility that these reactions were due to non-*S. pyogenes* ADT “reactive” organisms.

In spite of the described ease of performance of ADTs, there is a learning curve associated with their use [1]. With this particular ADT and its internal controls, however, it is more likely that procedural errors would result in a false-negative, not a false-positive, reaction.
Instances of a positive ADT result but a negative throat culture result have been attributed to a lack of sensitivity of the traditional blood agar plate culture [9]. The observations reported here—that many GAS "negative" cultures appear to contain ADT reactive organisms that are not S. pyogenes—however, suggests that that explanation may be incomplete. Lack of culture sensitivity would most likely be due to atypical morphologic or hemolytic GAS variants or to nutritional variants of GAS that are unable to grow sufficiently for culture recognition. GAS variants that lack the ability to produce hemolysis on standard blood agar plates have been associated with several GAS outbreaks [10, 11]. One recent report describes a patient infected with a nonhemolytic GAS; the ADT result was incorrectly considered to be false positive because of the lack of beta hemolysis on the associated culture plate [12]. In spite of altered hemolytic properties, the GAS colonies themselves retain typical group A morphology [11]. Because every negative culture and all suspect colonies were subcultured and tested for group A reaction, we do not believe that the false-positive reactions encountered were the result of nonhemolytic or morphologic variants of GAS.

Nutritional variants of GAS that have been identified on the basis of their occurrence as satellite colonies around colonies of specific normal flora species have been described elsewhere [13]. In the present study, all plates were examined for "satellite colonies"; none was observed. Furthermore, all negative cultures were replated and were incubated in 5% CO₂, an environment that enhances growth of some nutritional variants. Only 1 additional positive culture was detected by use of CO₂, and it grew normally on subculture.

A single report of a false-positive ADT reaction due to a group A carbohydrate carrying S. milleri group organism has been published elsewhere [14]. Although it has been known for many years that some strains in the S. milleri group contain group A carbohydrate antigen [15], we are not aware of any published data defining their prevalence. Our data suggest that false-positive ADT reactions may be more frequent in the clinical setting than was previously recognized.

Why is the apparent rate of false-positive reactions observed in this study so much higher than that observed in other reports? One theoretical explanation may be the age of the study population. Most studies of streptococcal pharyngitis are carried out in children. The present study was carried out in an older population (mean age, 26 years). It is possible that potentially cross-reactive organisms are more prevalent in this age group. To confirm this explanation, however, further studies will be needed.

Because all the commonly used ADTs depend on detection of the group A carbohydrate moiety, it is possible that these test kits result in more false-positive detection of group A antigen than has been recognized. It also is reasonable to expect that those test kits with the highest sensitivity are more likely to detect these non-S. pyogenes ADT reactive organisms. It is important to further identify and define the prevalence of these cross-reactive organisms in the population to clarify this observation.

Acknowledgments

We thank Patricia Ferrieri, Director of the Clinical Microbiology Laboratory, Fairview University Medical Center, Minneapolis, and the laboratory staff for characterizing the Streptococcus milleri group organism.

References