Evaluation of a New Latex Agglutination Test for Detection of Streptolysin O Antibodies

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Acute- and convalescent-phase serum specimens were collected from 50 patients with group A streptococcal pharyngitis. The anti-streptolysin O (ASO) titer for each serum specimen was determined by using both the standard neutralization assay and the latex agglutination (LA) test (Rheumagen ASO; Biokit Inc., New Britain, Conn.). When the ASO titers derived by the two methods were compared, the correlation coefficient was 0.93. When the ability of the LA test to demonstrate a significant ASO titer rise (±2 dilutions) was compared with that of the standard neutralization assay, the LA test had a sensitivity of 91%, a specificity of 86%, a positive predictive value of 83%, and a negative predictive value of 92%. Triplicate LA test determinations were performed on a subset of 31 serum specimens, and for 29 (94%), the repeated ASO titers were all within 1 dilution of each other; the width of the 95% confidence interval for the triplicate measurements of each serum specimen was ±32.8 IU. We found the Rheumagen ASO to be a simple, rapid LA procedure for measuring ASO titers that produces results that are highly reproducible, show little lot-to-lot variability, and are comparable to the ASO titers obtained with the standard neutralization assay.

The measurement of antibodies to streptococcal extracellular antigens has been used by clinicians to help support the diagnosis of one of the nonsuppurative complications of group A beta-hemolytic streptococcal (GABHS) infections: acute rheumatic fever or acute poststreptococcal glomerulonephritis. Measurement of these antibodies has also been used by investigators studying the epidemiology of GABHS infections, as well as the epidemiology and pathogenesis of their nonsuppurative sequelae. Since the original description of the anti-streptolysin O (ASO) procedure by Todd in 1932 (18), this test has been the most widely used for determination of antibodies to streptococcal extracellular antigens. In many clinical laboratories, it is the only streptococcal antibody test available. However, the neutralization assay that is most often used to determine ASO titers is a relatively complicated procedure requiring a well-equipped laboratory. The neutralization assay also requires the use of rabbit erythrocytes that are unstable on storage.

The purpose of this investigation was to compare a new, simple latex agglutination (LA) method for measuring ASO titers with the standard neutralization procedure.

MATERIALS AND METHODS

During the winter and spring of 1984 and 1985, children seen in a private pediatric office (M.F.R.) with clinical findings suggestive of GABHS pharyngitis were enrolled in an investigation of this disease after informed written consent had been obtained. The details of the study protocol are presented elsewhere (3). Briefly, individuals had throat cultures performed, and serum specimens were obtained at the first visit and again at a follow-up visit approximately 4 weeks later. All patients with a positive throat culture for GABHS were treated with an appropriate course of antibiotics. Sera were stored at −70°C, and both acute- and convalescent-phase serum specimens from each subject were tested simultaneously for ASO antibodies by using the microdilution procedure for the neutralization assay (4) with ASO reference control serum and streptolysin O reagent obtained from Difco Laboratories (Detroit, Mich.). Titers were reported in Todd units.

The ASO titer for each serum specimen was also determined by using a new LA test (Rheumagen ASO; Biokit Inc., New Britain, Conn.). In place of the dilution scheme suggested by the manufacturer (1:200, 1:400, 1:800, etc.), the following dilutions of serum with normal saline were made in an attempt to approximate the neutralization ASO dilution scheme: 1:50, 1:100, 1:150, 1:200, etc. A drop (0.050 ml) of each dilution was placed in one section of a disposable slide. A drop of the latex reagent was placed next to the drop of diluted serum, and the drops were then mixed. The slide was gently rotated manually for 3 min, and the presence or absence of agglutination was then determined. The acute- and convalescent-phase serum specimens from each patient were tested simultaneously, along with appropriate positive and negative controls. Titers were reported in international units. One international unit equals 1.04 Todd units (16). A significant rise in antibody titer for either ASO assay was defined as a rise of 2 dilution increments or more from acute- to convalescent-phase serum specimens. The dilution scheme for the neutralization ASO is constructed so that a significant rise (2 dilutions or greater) in antibody titer represents exactly a ≥0.2-log rise, while for the LA test, by using the dilution scheme derived for this study, a significant rise (2 dilutions or greater) in antibody titer represents approximately a ≥0.2-log rise.

For a subset of 31 serum specimens, the LA test was performed on three different occasions and the ASO titers derived were compared as a measure of the reproducibility of the assay. For another subset of 25 serum specimens, each specimen was simultaneously tested with three different lots of Rheumagen reagent (1-4588, H-3689, and C-3089) and the ASO titers derived were compared as a measure of lot-to-lot variability.

The data were analyzed by using Student’s paired t test, linear regression analysis, and Hoyt’s method (7), which uses an analysis of variance approach to assess reliability.

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RESULTS

Acute- and convalescent-phase serum specimens were available from 50 individuals (mean age, 9.9 years; range, 2 to 20 years), all of whom had acute pharyngitis and GABHS isolated from their upper respiratory tracts. When the ASO titers of these 100 serum specimens as determined by the LA test were compared with the corresponding ASO titers as determined by the neutralization assay, the correlation coefficient was 0.93 (Fig. 1) and Student’s paired t test showed no significant difference ($P > 0.05$). For only 11 of the 100 (11%) serum specimens was there more than a 1 dilution discrepancy between the ASO titer as determined by the LA test and the ASO titer as determined by the neutralization assay.

By using the titers as determined by the neutralization assay as the “gold standard,” the sensitivity, specificity, and predictive values of the LA test in demonstrating a significant rise in ASO titers were determined (Table 1). The LA test had a sensitivity of 91%, a specificity of 86%, a positive predictive value of 83%, and a negative predictive value of 92%. Of the four false-positive LA results, three did have a rise in neutralization assay titers but of only 1 dilution in magnitude. In addition, both of the patients with false-negative LA results did have a rise in LA titers but of only 1 dilution in magnitude.

For 29 of the 31 (94%) serum specimens that were run in triplicate by using the LA test, the ASO titers were all within 1 dilution (50 IU) of each other. In addition, for these triplicate measurements of each serum specimen, Hoyt’s $r = 0.98$ and the width of the 95% confidence interval was ±32.8 IU, which is less than 1 dilution. For 21 of the 25 (84%) serum specimens that were tested simultaneously with three different lots of Rheumagen reagent, the ASO titers obtained were identical. For 4 of the 25 (16%) serum specimens, the ASO titer obtained with one lot (not always the same lot) was 1 dilution higher than the titer obtained with the other two lots.

![FIG. 1. Correlation between ASO titers obtained with Rheumagen LA test and standard neutralization assay.](image)

**TABLE 1.** Significant rise in ASO titer

<table>
<thead>
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<th>Rise in Rheumagen LA test</th>
<th>No. with rise by standard neutralization assay</th>
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<td></td>
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DISCUSSION

Streptolysin O (SO) is an oxygen-labile hemolysin that is one of a variety of extracellular products elaborated by group A streptococci. SO elicits an antibody response in the host during a GABHS infection. This antibody response (ASO) can be used to confirm the diagnosis of a GABHS infection or the diagnosis of one of its nonsuppurative sequelae.

In the initial report of the ASO procedure, Todd (18) demonstrated the presence of ASO antibodies in the sera of patients with various streptococcal infections by neutralizing SO with serial amounts of sera of the patients. The excess, unneutralized SO was then revealed by adding erythrocytes to the system as an indicator. The endpoint was the highest dilution of serum having no hemolysis, with the ASO titer expressed in Todd units, which are equivalent to the reciprocal of the dilution. This technique was subsequently modified by Rantz and Randall (12), and later a microtitration procedure developed by Edwards (4) was introduced.

Approximately 80% of patients who have had a group A streptococcal infection have a demonstrable antibody response to a single streptococcal extracellular antigen. If one looks for antibody responses to multiple streptococcal extracellular antigens, the percentage approaches 95% (1). However, most laboratories are unable to perform multiple streptococcal antibody assays and rely solely on the ASO determination. Unfortunately, the standard neutralization assay used to determine ASO titers is a relatively expensive, time-consuming, and complicated procedure requiring a well-equipped laboratory. In fact, the American Heart Association’s revised Jones criteria of 1965 for the diagnosis of acute rheumatic fever (17), which required evidence of a prior streptococcal infection, were not initially accepted by the WHO Expert Committee on Prevention of Rheumatic Fever because there were often insufficient laboratory resources in developing countries to provide the necessary serologic tests (21).

Over the years a number of attempts have been made to develop simplified, rapid tests for measuring either multiple or single streptococcal antibody titers. The Streptozyme test (Wampole Laboratories, Cranbury, N.J.) is a hemagglutination procedure that is said to detect antibodies to five different streptococcal extracellular enzymes. However, a number of studies have demonstrated problems with the use of the Streptozyme test (6, 10, 11). The WHO has concluded that this test is insufficiently reliable for assaying streptococcal antibodies and recommends that it no longer be used (20).

In 1978, Ricci and co-workers (15) introduced a simplified neutralization assay for measuring ASO antibodies in whole blood rather than serum, using the patient’s own erythrocytes as the indicator. This procedure was based on the fact that the capacity of SO to hemolyze erythrocytes, but not to bind specific antibodies, is lost when sulfhydryl groups are oxidized. The results with this procedure showed a good correlation with the results when the standard neutralization assay was used, but, despite the fact that this technique was later modified to allow for automation (14), the procedure has never gained wide acceptance. In 1986, Reitano and co-workers (13) reported on the use of an enzyme-linked immunosorbent assay for measuring ASO antibodies, and in 1988, Umeda and co-workers (19) described a neutralization assay that used carboxyfluorescein-entrapped liposomes instead of rabbit erythrocytes. While both of these procedures showed a good correlation with the standard method, neither
represented a major advance with respect to simplification of the ASO procedure. There have been several earlier attempts at developing a LA test for measuring ASO antibodies. The Leap Strept (Organon Teknika, Malvern, Pa.) is a liposome-enhanced LA test which measures not only ASO but anti-DNase B antibodies as well. However, when compared with the standard neutralization assay for ASO antibodies, the Leap Strept was judged to have insufficient sensitivity and could not be recommended as a useful test for screening for ASO antibodies (8, 9). The Check-Spectra Kit (Mercia Diagnostic Ltd., Great Britain) is another LA test for determining ASO antibody titers (2). When used to screen serum specimens with a cutoff of ≥200 IU for positivity, this test had a sensitivity of 100% and a specificity of 74% when compared with the standard neutralization assay. However, when specific titers, as determined by the two methods, were compared, the correlation was poor. Finally, the Rapi Tex ASL (Behring, Hounslow, England) is another new LA test for measuring ASO antibodies. The data comparing the Rapi Tex ASL with the standard neutralization assay look promising but are, at this time, very limited (3).

We have examined the Rheumagen ASO LA test for the measurement of ASO antibodies and have found it to be a simple procedure that requires no special equipment or expertise. The Rheumagen ASO can be used as either a qualitative or quantitative test, takes only several minutes to complete, and has a shelf life of up to one year. In addition, with as little as 225 μL of serum, titers ranging from 50 to 900 IU can be determined. Fingerstick samples would, therefore, be adequate for the performance of this test. We found that the ASO titers measured by the Rheumagen ASO were highly reproducible, showed little lot-to-lot variability, and correlated very closely with the ASO titers measured by the standard neutralization procedure. We also found that the ability of the Rheumagen ASO to demonstrate a significant rise in ASO titer was comparable to that of the standard neutralization procedure. Much of the discrepancy between the two procedures in this regard appeared to be attributable to differences in the dilution schemes. If these findings are corroborated by additional investigations, the Rheumagen ASO LA test could replace the neutralization assay as the procedure of choice for the determination of ASO antibodies. Ideally, LA tests for other streptococcal antibodies would be developed so that in the future one could simultaneously perform LA tests for multiple streptococcal antibody assays.

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LITERATURE CITED