Clinical use and interpretation of group A streptococcal antibody tests: a practical approach for the pediatrician or primary care physician

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OBJECTIVES

1. Recall the different serologic group A streptococcal antibody tests available and understand their diagnostic potential.
2. Identify the indications for performing group A streptococcal antibody tests and select the appropriate serologic test for a given clinical situation.
3. Describe when not to perform a group A streptococcal antibody test.
4. Interpret the results of group A streptococcal antibody tests.
5. Describe the concept of “upper limit of normal” in reference to streptococcal antibody tests and the importance of obtaining a “rise in titer” vs. a “single titer.”

Group A beta-hemolytic streptococci (Streptococcus pyogenes) commonly infect the throat and the skin of children. An estimated 4 to 5 million cases of group A streptococcal infection occur in the United States alone each year. It is extremely common in children, especially between the ages of 5 and 12 years. What sets group A streptococcal infections apart from other common childhood ailments is the potential for resulting in serious sequelae like acute rheumatic fever and acute glomerulonephritis after the streptococcal infection has resolved.

The incidence of pharyngeal infection is greater during the winter and spring months. Although classic signs and symptoms of group A streptococcal pharyngitis are fever, sore throat with pharyngeal erythema, tonsillar swelling and exudate and anterior cervical adenitis, these are not always present.\(^1\) In addition a positive throat culture for group A streptococci cannot distinguish between the perplexing streptococcal upper respiratory tract “carrier” state and an acute infection.\(^2\) Such carriers may even asymptptomatically harbor the organism in their upper respiratory tract.\(^3\) These individuals are not truly infected, and they appear to have a much lower risk of developing complications of group A streptococcal infections.\(^4\) Carriers do not by definition experience a rise in streptococcal antibody titer when acute and convalescent sera are compared.\(^3\) Often during episodes of upper respiratory tract illness, nasopharyngeal cultures obtained from these streptococcal carriers confirm a viral etiology for their upper respiratory symptoms.\(^5\)

Superficial skin infections such as impetigo and pyoderma caused by group A streptococci more often occur in warmer climates but are seen in all geographic areas. Other group A streptococcal infections like bacteremia, deep tissue infections and toxic shock syn-
drome also occur. A resurgence of serious group A streptococcal infections and their sequelae has occurred during the past 15 years.6, 7 Sporadic outbreaks and individual cases of rheumatic fever are still being reported in the United States.8

Such data have emphasized the importance of accurate clinical diagnosis, often requiring laboratory confirmation of the preceding group A streptococcal infection. The diagnosis of group A streptococcal infections is often made by clinical observation and then confirmed by rapid antigen tests or by isolation of group A streptococci from the site of infection. However, when one is considering the diagnosis of poststreptococcal nonsuppurative sequelae like acute rheumatic fever or poststreptococcal glomerulonephritis, it is not always possible to obtain an adequate clinical history or to recover the organism. In such cases the presence of a host immune response is the only evidence of the recent infection that remains. Measurement of antibodies to specific streptococcal antigens is therefore necessary to confirm the diagnosis of the preceding group A streptococcal infection.

This discussion reviews for the clinician available streptococcal antibody tests and provides guidance for their interpretation. It is meant to be a practical clinical guide; references will provide more in depth information.

THE IMMUNE RESPONSE TO GROUP A STREPTOCOCCAL INFECTIONS

The group A streptococcus produces both cellular and extracellular antigens, which stimulate the production of specific antibodies in the infected patient. The most common clinically utilized antigens and the corresponding antibody tests are listed in Table 1. The extracellular antigens are products released by group A Streptococcus, whereas the cellular (or somatic) antigens form an integral part of the organism and are usually exposed on its surface. Figure 1 depicts streptococcal cell surface structures and secreted products that often induce a measurable immune response in the human host and can be used clinically.

General principles. There are certain general principles that may be applied to enhance understanding of the immune response to streptococcal antigens. Most authorities believe that a rising titer from acute to convalescent is a more accurate reflection of a previous streptococcal infection than only a single titer.9 The factors that influence the streptococcal immune response are summarized in Table 2. The age of the patient is very important in determining streptococcal antibody concentrations in a given population. Children between the ages of 6 and 15 years have the highest group A streptococcal antibody titers compared with very young children and older adults.10, 11 This is influenced by the more frequent experience of this segment of the population with group A streptococcal infections. Therefore when attempting to define a "normal" streptococcal antibody value, the age of the patient must be taken into consideration. In those geo-

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**TABLE 1.** The most common clinically useful group A streptococcal antibody tests

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extracellular</td>
<td></td>
</tr>
<tr>
<td>Streptolysin O</td>
<td>Anti-streptolysin O</td>
</tr>
<tr>
<td>Deoxyribonuclease B</td>
<td>Anti-DNase B</td>
</tr>
<tr>
<td>DNase B</td>
<td>Anti-streptokinase</td>
</tr>
<tr>
<td>Streptokinase</td>
<td>Anti-streptococcal hyaluronidase*</td>
</tr>
<tr>
<td>Hyaluronidase</td>
<td>Anti-DNase B</td>
</tr>
<tr>
<td>Nicotinamide adenine</td>
<td>Anti-NADase*</td>
</tr>
<tr>
<td>dinucleotidase (NADase)</td>
<td></td>
</tr>
<tr>
<td>Somatic/cellular</td>
<td></td>
</tr>
<tr>
<td>Type-specific M-protein</td>
<td>Type-specific M antibody*</td>
</tr>
<tr>
<td>Group A carbohydrate</td>
<td>Anti-A carbohydrate*</td>
</tr>
</tbody>
</table>

* Not commercially available, but currently used in research and reference laboratories.
TABLE 2. Factors that can influence group A streptococcal immune response

<table>
<thead>
<tr>
<th>Specific Influences</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of patient</td>
<td>Titers maximum between ages 6 and 12 yr.</td>
</tr>
<tr>
<td>Population involved</td>
<td>Populations with endemic streptococcal infections have higher titers.</td>
</tr>
<tr>
<td>Site of infection</td>
<td>There is a feeble ASO response after streptococcal skin infections,</td>
</tr>
<tr>
<td></td>
<td>compared with streptococcal upper respiratory infections.</td>
</tr>
<tr>
<td>Season of the year</td>
<td>Titers are higher during winter and early spring months.</td>
</tr>
<tr>
<td>Prompt antibiotic treatment</td>
<td>Reduces the antibody response.</td>
</tr>
</tbody>
</table>

*See also Table 6.

graphic regions where the incidence of streptococcal infections is high, streptococcal antibody titers are also correspondingly high. Thus for different populations there may be significant differences in antibody titers even within the same age group. Because streptococcal pharyngitis occurs more commonly during winter and early spring, it is not surprising that streptococcal antibody titers are higher during those seasons.

The response after streptococcal pyoderma or impetigo has been shown to be both qualitatively and quantitatively different from that after throat infection. Other factors that influence the kinetics of this response are antibiotic or corticosteroid administration. Studies have shown that prompt antibiotic therapy after streptococcal infection can reduce (but not abolish) the immune response to streptococcal antigens (both somatic and extracellular antigens) and thus result in a lower titer. Persistence of the organism or reinfection may result in either sustained titers or reduction in the rate of decline. Because the dynamics of the various streptococcal antibody responses differ from each other, the time after infection that the serum is obtained from the patient will influence which antibody will show a maximum response. However, only 80 to 85% of patients with rheumatic fever show a response to any single antigen, but if antibody responses to multiple antigens are looked for, this approaches 92 to 98%. Hence performing more than one streptococcal antibody test will increase the chance of confirming a true previous streptococcal infection (Fig. 2).

Determination of antibody titers in patients with uncomplicated streptococcal upper respiratory tract infection is rarely, if ever, indicated.

Is there a universally applicable “normal” streptococcal antibody titer? The answer is no! The distribution of antibody titers varies depending on the population, age and geographic location. Because rheumatic fever and poststreptococcal glomerulonephritis are nonsuppurative sequelae of group A Streptococcus infection and there is a latent period between the streptococcal infection and onset of disease, serum taken at disease onset may really be “convalescent”; a rising titer may not be demonstrated. Hence an “upper limit of normal” (ULN) value is useful when acute and convalescent sera are not available. For purposes of uniformity the ULN is defined as that titer exceeded by 20% of a normal population. Ideally ULN streptococcal antibody titers should be established in every population and for each age group.

For example Table 3 shows age-stratified mean values of anti-streptolysin O (ASO) and anti-DNase B in children residing in the United States between the ages of 2 and 12 years. Previous studies have shown that the ULN for the ages 13 to 15 years was somewhat lower (as might be expected) for both ASO and Anti-DNase B titers. It should be emphasized as a practical clinical measure that the recommended ULN in package inserts of commercially available kits are often inaccurate (usually too low) because they often are established with sera from either inadequately defined populations or from adults.

The anti-streptolysin O test. Of the two hemolysins produced by group A streptococci, streptolysin S and streptolysin O, only the latter is antigenic. Streptolysin O is a cytolytic toxin that is produced by group A streptococci. Its biologic properties include hemolysis of erythrocytes and other eukaryotic cells; it may also damage leukocytes. The antibody produced by the human host against this toxin, ASO, is the most widely used and the most standardized of the group A streptococcal antibody tests available. This antibody does not have a protective role in the host.

In patients with group A streptococcal infections, the ASO response can usually be demonstrated after 1 week with maximal response being reached 3 to 6 weeks after the infection. The decline in ASO titer after uncomplicated infections is less well-defined; it may begin 6 to 8 weeks after the infection, although in some patients the titer may remain elevated for indefinite periods. The significance of this persistence is not well-understood. The decline in ASO has not been...
carefully studied in normal children with uncomplicated infections and remains a cause for confusion among clinicians. Most information regarding dynamics of ASO response comes from studies conducted in patients with rheumatic fever. In one study patients were follow up to 1 year after an attack of acute rheumatic fever; 16% of the patients still had elevated ASO titers at the end of 1 year in the absence of documented recurrent infections.

The highest ASO titers are seen in children between the ages of 6 and 15 years. The site of infection, whether upper respiratory tract or skin, plays a role in determining an ASO response. Controlled epidemiologic studies have shown that the ASO response is generally brisk after a streptococcal upper respiratory tract infection but is relatively feeble after group A streptococcal impetigo or pyoderma. This is explained by the fact that free cholesterol present in the skin binds to the streptolysin O molecule and thus decreases the antigenicity of streptolysin O. The presence of nonspecific lipoprotein inhibitors in the sera of patients with chronic liver disease may result in spuriously high ASO titers. The same is true for sera that are contaminated by bacteria before the test is performed. Bacterial esterases cleave free cholesterol from cholesterol esters present in serum. The free cholesterol can bind to the streptolysin O reagent used in the assay, resulting in inhibition of streptolysin O-induced hemolysis and a subsequent falsely elevated ASO reading. One laboratory “trick” that can be used to determine whether a falsely elevated titer is a result of free cholesterol in serum is to extract the serum with chloroform, which will remove the free cholesterol. The serum can then be retested, and usually the titer will fall remarkably, verifying the clinician’s suspicion. Falsely elevated ASO titers may also be seen in patients with multiple myeloma or hypergammaglobulinemia and in those individuals whose sera contain a high concentration of rheumatoid factor.

It must be kept in mind that group C and G beta-hemolytic streptococci (which are less frequently associated with clinical pharyngitis) also produce streptolysin O, and hence an elevated ASO titer may be seen after infections with these serologic groups as well.

The classic ASO test is a neutralization assay based on the fact that specific anti-streptolysin O antibodies in the serum neutralize the hemolytic activity of the streptolysin O toxin when tested in the presence of erythrocytes. The end point is taken as the highest dilution of the patient’s serum showing no hemolysis, i.e., inhibition of toxin activity by ASO antibody. The reciprocal of this dilution is expressed as Todd units or International units (depending on whether the streptolysin O reagent used is the Todd standard or the WHO international standard). A standard ASO reference serum is available at the State Serum Institute, Copenhagen, Denmark. More recently latex agglutination tests and nephelometric techniques have become commercially available. The latter two techniques have not been as well-standardized as the classical technique. The reproducibility and therefore the increase in titer required to demonstrate a significant rise from acute to convalescent titers has not been carefully defined for the nephelometric technique.

Table 4 lists the different types of assays for measuring antistreptolysin O.

The anti-DNase B test. Group A streptococci produce four DNases designated A, B, C and D. After group A streptococcal infection, the human host produces the most consistent immune response against DNase B. Anti-DNase B concentrations usually begin to rise by the second week after infection. Peak values occur at 6 to 8 weeks (which is later than ASO), and they tend to remain elevated for somewhat longer periods than the ASO titer.

The influences on the immune response to streptococcal DNase B are similar to ASO with respect to population, age, geographic location and season of the year. Unlike ASO, however, infection of the skin results in a brisk anti-DNase B response. The anti-DNase B titer does not appear to be influenced by liver disease, myeloma or conditions resulting in hypercholesterolemic or hypergammaglobulinemic sera. A false negative anti-DNase B titer can be seen in acute hemorrhagic pancreatitis when an elevated serum DNase concentration is encountered. The classic anti-DNase B assay is a neutralization assay based on the fact that antibodies to DNase B neutralize the enzymatic activity of DNase B, preventing it from depolymerizing DNA that has been coupled to an indicator dye. Presently there is no standardized universal reference serum available as there is for the ASO test. Other less rigorously evaluated and standardized assay techniques have become available. The available types of assays are shown in Table 5.

The anti-DNase B test is more reliable than ASO for confirming evidence of a preceding group A streptococ-

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### TABLE 3. Age-stratified values of ASO and anti-DNase B in children between ages 2 and 12 yr

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Geometric Mean Titer</th>
<th>ULN</th>
<th>Geometric Mean Titer</th>
<th>ULN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ASO</td>
<td></td>
<td>Anti-DNase B</td>
<td>B</td>
</tr>
<tr>
<td>2</td>
<td>52</td>
<td>160</td>
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<tr>
<td>12</td>
<td>320</td>
<td>320</td>
<td>219</td>
<td>480</td>
</tr>
</tbody>
</table>

* Modified with permission from Reference 11.
cal skin infection; this is especially useful in patients with suspected glomerulonephritis after pyoderma. The anti-DNase B test is also used in patients with suspected rheumatic fever who show no ASO response as an additional antibody test. As previously stated the use of an additional test increases the probability of demonstrating a rise in titer and confirming a previous streptococcal infection.

The characteristics of the ASO and anti-DNase B tests are compared in Table 6.

**Other group A streptococcal extracellular antibody tests.** Anti-streptococcal hyaluronidase is an enzyme neutralization test in which streptococcal hyaluronidase, elaborated by the capsule, is used as the antigen. Streptokinase is also an immunogenic enzyme that catalyzes the conversion of plasminogen to plasmin and thus has fibrinolytic properties. The enzyme nicotinamide adenine dinucleotidase (NADase) has leukocytotoxic effects. However, anti-streptococcal hyaluronidase, anti-streptokinase and anti-NADase are currently not commonly used for determination of group A streptococcal infection, because they are technically difficult to perform and are not commercially available. These tests are often available only in reference or research laboratories. Reviews of these tests are available.

The Streptozyme multienzyme agglutination test gained popularity several decades ago as a screening test. This is a hemagglutination test based on agglutination of erythrocytes coated with a nonstandardized mixture of several streptococcal extracellular antigens. A batch-to-batch variation has been shown for the reagent, and the antigens present have not been precisely defined. The major drawback is that it has been
reported to produce variable results with inconsistent specificity. Several studies do not recommend this test for confirming a previous group A streptococcal infection.33, 34

Group A streptococcal somatic antigens. The group A carbohydrate is present in the cell wall of the group A streptococcus and is responsible for the group-specific reactivity on which the Lancefield serologic grouping classification of streptococci is based. Studies of sera of patients with rheumatic fever and glomerulonephritis revealed that titers of anti-A carbohydrate peaked at 1 to 3 weeks after the acute infection and declined to normal levels 6 to 12 months later in most patients. However, in those patients with rheumatic valvular disease, antibody values persisted for 8 years or more.35 This evidence has been used to support the hypothesis that this might be one of the antigens associated with antigenic mimicry and the development of rheumatic valvular heart disease. Additional studies showed that this antibody response was vigorous in patients with rheumatic valvular disease (as opposed to nonrheumatic cardiac valve disease).36 However, this test is most used in the research or reference laboratory and has limited clinical use, given that reagents are not commercially available.

The M protein is a somatic antigen extending from the cell membrane of the group A streptococcus that has been used to categorize the organism into different serotypes (Fig. 1) Measurement of type-specific antibody against the M protein of group A streptococci is not used clinically as a diagnostic test, given that presently there are >100 known types (either serotypes or types based on sequencing of the emm gene) with distinctly different M proteins. These antibodies may appear slowly over a period of several months; this represents a primary immune response rather than a secondary response. The immunity to M protein is protective against group A streptococcal infections; hence type-specific M antibody has been used in studies of M protein vaccines.37 Detection of these antibodies is seldom, if ever, used clinically.

**CONCLUSIONS**

Obtaining acute and convalescent sera showing a rise in antibody titer is definitive proof of a preceding streptococcal infection. However, because it is not always easy or feasible to obtain paired sera it must be emphasized that the occurrence of a single isolated titer that is high is also evidence of a previous streptococcal infection. Together with the clinical findings the tests may support the diagnosis of acute rheumatic fever and post-streptococcal glomerulonephritis. It cannot be emphasized too strongly that the use of streptococcal antibodies is not indicated in the management of routine uncomplicated streptococcal infections.

**REFERENCES**


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Multiple Choice Questions

1. Anti-streptolysin O (ASO) and anti-DNase B titers are highest in:
   a. Infants, because of maternally derived streptococcal antibodies.
   b. Children between the ages of 6 and 15 years because of frequent exposure to group A streptococcal infections.
   c. Adults, because their humoral immune system is more active.
   d. There is no distinct age associated with high streptococcal antibody titers.

2. Obtaining an ASO titer is not indicated in the routine management of acute uncomplicated pharyngitis because:
   a. Infection with group B and C beta-hemolytic streptococci also results in a high ASO titer.
   b. There is no standardized ASO assay available.
   c. Evidence of an antecedent group A streptococcal infection provided by an elevated ASO titer is useful only when considering a diagnosis of rheumatic fever or poststreptococcal glomerulonephritis.
   d. An ASO titer is not as informative as an anti-DNase B titer.

3. A 10-year-old boy with aching knee joints has an ASO titer of 150 units which is interpreted by the package insert in the commercial kit as an elevated ASO. The physician should:
   a. Disregard the interpretation as false and reassure the patient and his parents that this is a normal value.
   b. Start oral penicillin V 250 mg twice a day for 10 days or one intramuscular dose of 1.2 megaunits of benzathine penicillin G immediately.
   c. Repeat the acute ASO titer and obtain a convalescent serum to see whether there is an increasing titer and continue close clinical observation.
   d. Obtain an echocardiogram to diagnose rheumatic heart disease.
4. Pyoderma caused by *Streptococcus pyogenes* does not elicit a strong anti-DNase B response. This is because:
   a. Free cholesterol present in the skin interferes with the action of streptococcal DNase B.
   b. Skin infections in general do not produce a vigorous humoral immune response.
   c. Group A streptococcal DNase B secreted by the organism in pyodermal lesions does in fact produce a strong surface IgA response, but is not sufficiently found in the serum where anti-DNase B is frequently measured.
   d. The above statement is false. The ASO response is not prominent after streptococcal skin infection because free cholesterol in the skin modifies the antigenicity of streptolysin O.

5. When interpreting an ASO test, the physician should pay close attention to the type of ASO assay by which the test is performed. This statement is:
   a. True, because only the classic hemolysin inhibition method is adequately standardized and the reference values for the other assays are based on the classic method with inadequate comparison studies.
   b. True, because the ASO titer varies with each type of assay and it is crucial to take into account the degree of variation because a high ASO titer is diagnostic of acute rheumatic fever and poststreptococcal glomerulonephritis.
   c. False, because most available ASO assays are well-standardized and accurate.
   d. False, because all ASO assays nowadays are performed by the nephelometric method, which is rapid, reliable, cost-effective and accurate.
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2. a b c d
3. a b c d
4. a b c d
5. a b c d
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