The greatest dilution of test sample is a stabilized buffered solution prepared from a stabilized human serum containing antibodies to Streptolysin 0. Before use shake dispensed bottle gently to achieve a homogeneous suspension of particles. The latex reagent has been produced so that agglutination will take place only when the level of antibodies to Streptolysin 0 is greater than 200IU/ml, a level determined by Todd. The ASO latex reagent, when used at the dilution supplied, will be reactive.

The toxins have been named 'STREPTOLYSINS' and the infected patient responds by producing antibodies known as Antistreptolysin-O. The measurement of the antistreptolysin 0 Titer (ASO) level has led to the identification of a serum component which the patient responds to recent group, a streptococcal infection 1. The measurement of Anti-Streptolysin-O antibodies in serum is a rapid, reliable and specific test of recent group A, B-Hemolytic Streptococcal infection including Rheumatic Fever and Glomerulonephritis.

The Akucheck-ASO Latex Reagent is a stabilized buffered suspension of polystyrene latex particles coated with Streptolysin 0. Before use shake dispensing bottle gently to achieve a homogeneous suspension of particles. The latex reagent has been produced so that agglutination will take place only when the level of antibodies to Streptolysin 0 is greater than 200IU/ml, a level determined by Todd. The ASO latex reagent, when used at the dilution supplied, will be reactive.

**INTENDED USE**

The Akucheck-ASO Test is a rapid latex agglutination test designed for the qualitative and semi-quantitative measurement of Antistreptolysin-0 antibodies in serum.

**SUMMARY**

Todd describes in 1938 a test for measuring antibody response to recent group, a streptococcal infection 1. The group A, B-Hemolytic Streptococcal produces an exotoxin, Streptolysin-O, that has the capacity to hemolyze red blood cells of various species. Todd’s test technique was later modified by Rantz & Randall 2. Many strains of Streptococci organisms produce both toxins and antigenic substances to which the infected patient responds by producing antibodies. The toxins have been named ‘STREPTOLYSINS’ and the infected patient responds by producing antibodies known as Antistreptolysin-O.

**PRINCIPLE OF TEST**

The AKUCHECK-ASO is a stabilized buffered suspension of polystyrene latex particles that have been coated with Streptolysin O 0. When the latex reagent is mixed with a serum containing antibodies to Streptolysin O, agglutination occurs. The latex reagent has been produced so that agglutination will take place only when the level of antibodies to Streptolysin 0 is greater than 200IU/ml, a level determined to be indicative of disease by epidemiological and clinical studies. The sera having titers of between 200IU/ml and 3500IU/ml will be reactive.

**REAGENTS AND MATERIALS PROVIDED**

1. Akucheck-ASO Latex Reagent is a stabilized buffered suspension of polystyrene latex particles coated with Streptolysin 0. Before use shake dispensing bottle gently to achieve a homogeneous suspension of particles.
2. Positive Control is prepared from a stabilized human serum pool containing more than 2000IU/ml of Antistreptolysin 0, in a color-coded dispensing bottle.
3. Negative Control is prepared from a stabilized human serum pool, non-reactive for Antistreptolysin 0, in a color-coded dispensing bottle.
4. Disposable plastic pipette
5. 6-ring glass slide
6. Package insert
All components contain 0.1% sodium azide as preservative.

**MATERIALS REQUIRED BUT NOT PROVIDED**

1. Marking pen
2. Test tubes 12×7.5 mm
3. Centrifuge
4. Timer
5. Pipettes for sample dilution
6. 0.9% saline solution
7. High intensity light source

**PRECAUTIONS**

All human blood components used to prepare controls have been tested for Hepatitis B surface antigen (HBsAG) and HTLV-III antibodies by an FDA approved procedure and found to be non-reactive. No known test method for HBsAG or HTLV-III antibodies offers total assurance that a human derived product will not transmit hepatitis or HTLV-III virus. The user is therefore cautioned to handle reagents as if being capable of transmitting these diseases.

The reagents in each kit are matched. Reagents from different kits must not be interchanged or pooled. If the kit does not yield expected results when controls are tested, the kit should be discarded. Mix the reagents well before use. Use clean equipment. Traces of detergent or dried reagents on the glass slide may adversely affect test performance and results.

**STORAGE AND STABILITY**

Store at 2° - 8°C. Do not freeze.

Avoid extended exposure of reagents to elevated temperatures.

**SPECIMEN COLLECTION AND HANDLING**

Serum is specimen of choice and should be separated immediately after complete clot retraction. If testing is to be prolonged in excess of 24 hours, serum should be frozen.

**PROCEDURE A: QUALITATIVE**

1. Bring all reagents and serum samples to room temperature.
2. Insert the disposable pipet into the serum sample to be tested. Squeeze the pipet between the thumb and the forefinger and release pressure. This will allow the serum to fill the tip.
3. Hold the disposable pipet perpendicularly over the circle of the glass slide and squeeze to release one free-falling drop of patient serum. This will deliver 0.03ml of serum.
4. Mix the ASO Latex Reagent gently but thoroughly. Using the dropper provided, deliver one drop of the latex to the drop of serum.
5. Using the other end of the disposable pipet, mix the serum sample and latex and spread over the entire circle.
6. Rock slide for 2 minutes. Observe for agglutination while holding the slide under a high intensity lamp or fluorescent light.

**RESULTS**

Agglutination after 2 minutes is a positive result and indicates a content of Anti-streptolysin-0 in the serum equal to or greater than 200 IU/ml. A smooth homogeneous suspension of the antigen serum mixture is indicative of a negative reaction.

**PROCEDURE B: SEMI-QUANTITATIVE**

1. Allow reagent and controls to reach room temperature.
2. Dilute patient serum in normal saline solution as follows:

<table>
<thead>
<tr>
<th>Tube</th>
<th>Serum</th>
<th>Saline(mL)</th>
<th>Dil</th>
<th>IU/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5</td>
<td>0</td>
<td>1:2</td>
<td>400</td>
</tr>
<tr>
<td>2</td>
<td>0.5</td>
<td>0.5</td>
<td>1:2</td>
<td>800</td>
</tr>
<tr>
<td>3</td>
<td>0.5</td>
<td>0</td>
<td>1:2</td>
<td>800</td>
</tr>
<tr>
<td>4</td>
<td>0.5</td>
<td>0.5</td>
<td>1:2</td>
<td>400</td>
</tr>
<tr>
<td>5</td>
<td>0.5</td>
<td>0.5</td>
<td>1:16</td>
<td>3200</td>
</tr>
<tr>
<td>6</td>
<td>0.5</td>
<td>0.5</td>
<td>1:32</td>
<td>6400</td>
</tr>
</tbody>
</table>

3. Using the disposable pipet as described in the screening procedure, place a drop of each one of the serum dilutions on the glass slide.
4. Mix the ASO latex reagent gently but thoroughly. Using the dropper provided deliver a drop of the latex reagent to each drop of serum dilutions.
5. Using the flat end of the disposable pipet mix the serum dilution and the reagent and spread over the entire circle.
6. Rock the slide for 2 minutes. Observe for agglutination while holding the slide under a high intensity lamp or fluorescent light.

**SUMMARY**

The highest serum dilution showing a definite agglutination pattern is considered the titer end point. The sensitivity of the AKUCHECK-ASO reagent has been standardized.
against WHO standards so that positive reactions will be obtained when samples with titers between 200IU/ml and 3500IU/ml are assayed. AKUCHECK-ASO will not agglutinate in the presence of samples containing less than 200IU/ml of Antistreptolysin 0.

QUALITY CONTROL PROCEDURE

A positive control will produce, usually within 1 minute gross agglutinated flocs against a clear background.

A negative control will produce, usually no agglutination at all. It should be used for a basis for comparison. The relative degree of smoothness of the latex reagent itself should be considered -and incorporated in reading the results.

If the indicated results using the positive and negative controls are not obtained, the ASO test kit should not be used.

LIMITATIONS OF THE PROCEDURE

1. The result of ASO reagent test, as with other serological procedures, should not be used as a sole diagnostic criterion for the presence or absence of the disease state.

2. The Antistreptolysin-0 Titer of normal individuals may vary widely. After acute strepococcal infection, the titer begin to rise in about 2 weeks and increases to a maximum level in about 4-6 weeks. It remains high for a more or less pro-longed period and then decreases.

Even in non-rheumatic fever cases, patients who have had a recent acute Group-A Streptococcal infection may have high Titer of 166 Todd Units or even 2500 Units. In rheumatic fever patients, the titer is elevated in about 90% of cases. Persistence of titer of 500 units or more, especially after antibiotic therapy, is now considered by some to be evidence of rheumatic fever. It is for such reason that a test on a single specimen has relative value, and that a test on successive serum samples taken at intervals of 10 to 14 days may be of great value. As an exclusion test, when successive serum samples show constantly low values (under 100 units), the antistreptolysin titration is considered as fairly good evidence that the patient does not have rheumatic fever and did not have a recent acute Group-A Streptococcal infection. A low titer of perhaps 50 units could be obtained initially on a sample of serum from a rheumatic fever case. However, sera taken subsequently generally show rising titers thus indicating recent infection.

Similarly, falling titers are at least suggestive of recovery. Production of the antibody, Antistreptolysin-0 and Rheumatic Fever symptoms are considered to be results of the pathogenic process, but are independent of each other. Titer levels and severity of symptoms are not necessarily parallel and changes may proceed at different rates.

EXPECTED VALUES

Normal values of Antistreptolysin-0 can vary with age, season of the year and geographical area. A detectable level of Antistreptolysin-0 is usually regarded as the normal upper limit since less than 15-20% of healthy individuals demonstrate titers greater than 200 IU/ml when their sera are assayed. In most newborns the titer is initially greater than that of the mother due to maternally acquired IgG but the newborn level fall sharply during the first few weeks of life. Normal ASO levels for preschool children are generally less than 100IU/ml but the levels rise with age, peaking in school age and decreasing in adulthood. The normal value, in school children and young adults, however, is between 166 and 250 IU/ml. The average has been established at less than 200 IU/ml.

Increases in ASO titer generally occur one (1) to (4) weeks after the onset of infection with B-hemolytic streptococci Group A. As the infection subsides, the titer declines and returns to normal levels within six months. If the titer does not decrease, a recurrent or chronic infection may exist. Elevated ASO titers may be associated with ankylosing spondylitis, glomerulonephritis, scarlet fever, and tonsillitis. Increased ASO levels are generally not found in sera of patients with rheumatoid arthritis except during acute episodes.

Extremely low levels of ASO have been observed in the blood samples of patients with nephrotic syndrome and antibody deficiency syndrome.

Titters above 200 IU/ml may be indicative of Streptococcal infection but only a two dilution rise in titer between acute and convalescent stage specimens should be considered significant.

REFERENCES:

4. Freeman, S.O., Clinical Immunology, Harper and Row, New York, 1971

WARRANTY

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