Intended Use

Rapid test for the qualitative and semiquantitative determination of antistreptolysin-O in serum by agglutination of latex particles on slide. Measurement of antistreptolysin-O in serum aids in the diagnosis of group A streptococcal infections.

Summary

The group A ß-hemolytic streptococci produces various toxins that can act as antigens. One of these exotoxins is streptolysin-O that was discovered by Todd in 1932.[1]
A person infected with group A ß-hemolytic streptococci produces specific antibodies against these exotoxins, one of which is antistreptolysin-O. An elevated level of antistreptolysin-O is an indication of a recent infection with group A ß-hemolytic streptococci and can be an aid in the diagnosis of acute rheumatic fever and post-streptococcal glomerulonephritis.[2]
The usual procedure for the determination of the antistreptolysin titer is based on the inhibitory effect that the patient's serum produces on the hemolytic power of a pretitrated and reduced streptolysin-O.[2-6] However the antigen-antibody reaction occurs independently of the hemolytic activity of streptolysin-O.[7] This property enables the establishment of a qualitative and semiquantitative test for the determination of the antistreptolysin-O by agglutination of latex particles on slide.

Principle

The Sure-Vue® ASO reagent is a suspension of polystyrene latex particles of uniform size coated with recombinant streptolysin-O. Latex particles allow visual observation of the antigen-antibody reaction. If the reaction takes place, due to the presence of antistreptolysin-O in the serum, the latex suspension changes its uniform appearance and a clear agglutination becomes evident. This change occurs because the antistreptolysin-O present in the serum reacts with the streptolysin-O coated to the latex particles, starting the formation of a web between them.
When the latex reagent is mixed with the serum, if the serum contains abnormally high levels of antistreptolysin-O, a clear agglutination will appear. Results are expressed in International Units of antistreptolysin-O per mL (IU/mL) based on the WHO International Standard for antistreptolysin-O.[8]

Equipment and Materials

Reagents

a) **Latex reagent:**
Suspension of polystyrene latex particles coated with recombinant streptolysin-O in a buffer.
Contains sodium azide 0.1%.

b) **Positive control:**
Diluted rabbit serum containing more than 200 IU/mL of antistreptolysin-O. Ready to use.
Contains sodium azide 0.1%.

c) **Negative control:**
Diluted human serum containing less than 100 IU/mL of antistreptolysin-O. Ready to use.
Contains sodium azide 0.1%.

Available packaging

Kit 50 tests, Cat. No. 23 038000.
Contains: 1 x 2.5 mL reagent, 1 x 1 mL positive control, 1 x 1 mL negative control and 9 disposable slides with 6 sections each.

Material required but not provided

- Normal saline (0.9% NaCl, only for semiquantitative test).
- Automatic pipettes.
- Disposable stirrers.
- Rotator.
- Timer.

Precautions

Sure-Vue® ASO is intended for IN VITRO diagnostic use.
The reagents in this kit contain sodium azide as a preservative. Sodium azide has been reported to form lead or copper azide in laboratory plumbing which may explode on percussion. Flush drain with water thoroughly after disposing of fluids containing sodium azide.
Each donor unit used in the preparation of the negative control of this kit was tested by an FDA approved method for the presence of HIV 1/2 and HCV antibodies as well as for hepatitis B surface antigen and found to be negative.
WARNING: POTENTIALLY BIOHAZARDOUS MATERIAL.

Storage and Stability

The reagents will remain stable through the expiration date shown on the label, if stored between 2-8°C. Do not freeze. The reagents can be damaged by improper handling, especially temperature extremes. Checking with the positive and negative controls provided will permit detection of reagents deterioration.
The reagents should not be used after the expiration date shown on the label.
The latex reagent, once shaken, must be uniform without visible clumping. When stored a slight sedimentation may occur and should be considered normal.
Do not use reagents if they become contaminated.
The reagent dropper dispenses drops of 50 µL ± 10%. The dropper must be held perpendicular to the slide surface and a single drop allowed to fall. Do not use another dropper without previously checking the volume of the drop.

Specimen Collection

Use fresh serum collected by centrifuging clotted blood. If the test cannot be performed on the same day, the serum may be stored between 2-8°C for no longer than 8 days after collection. For longer storage, store samples frozen (-20°C).
It is not necessary to inactivate the serum.
As in all serological tests, hemolytic, lipemic or turbid sera may cause incorrect results and should not be used.
Do not use plasma.

Quality Control

Control of the latex reagent:

- Before performing a set of determinations it is advisable to check the latex reagent with each of the controls, positive and negative, included in the kit.
- Both controls should be used following the steps outlined in the QUALITATIVE TEST.
- The reaction between the positive control and the reagent should show a clear agglutination, different from the uniform appearance of the negative control. If no agglutination takes place, the test should be repeated, and the kit discarded if there is no positive reaction.

It is required that external quality control be performed every 30 days, with new shipments or lot number changes between reagent kits.

If the expected control reactions are not observed, repeat the control tests to determine the root cause of the failure. If control failures are repeated please notify department lead.

Send to Reference Laboratory for testing if in-house method is unavailable due to unexpected control reactions.

Procedure-Qualitative (Stepwise)

200 IU/mL detection level

- Allow reagents and samples to reach room temperature (20-30°C).
- Gently shake the reagent vial to disperse and suspend the latex particles in the buffer solution. Vigorous shaking should be avoided.
- Place 50 µL of the serum onto one section of the disposable slide.
- Place one drop of reagent next to the drop of serum.
- Mix both drops with a stirrer covering the whole surface of the slide section.
- Gently rotate the slide for **2 minutes** manually or on a rotary shaker set at 80-100 rpm.
- Look for the presence or absence of agglutination after the aforementioned period of time.

Reporting Result

Positive

The presence of agglutination indicates a content of antistreptolysin-O in the serum equal to or greater than 200 IU/mL.

Negative

The absence of agglutination indicates a content of antistreptolysin-O in the serum of less than 200 IU/mL.

Expected Values

Although normal values can vary with age, season of the year and geographical area,[2] the «upper limit of normal» antistreptolysin-O titers for preschool children is less than 100 IU/mL, and in school age children or young adults is usually between 166 and 250 IU/mL.[5] In any case, the average can be established at less than 200 IU/mL.

Because of this variation, titers above the upper limits may be indicative of a streptococcal infection, but only a two dilution rise in titer between acute and convalescent stage specimens should be considered significant.[2] Following acute streptococcal infection, the antistreptolysin-O titer will usually rise after one week, increasing to a maximum level within 3 to 5 weeks and usually returning to the preinfection levels in approximately 6 to 12 months.[2]

Limitations of the Procedure

- Reading of the results after more than 2 minutes (4 minutes at the 100 IU/mL detection level) may give false positive results.
- The strength of agglutination is not necessarily indicative of relative antistreptolysin-O concentration. When antistreptolysin-O concentration exceeds 1500 IU/mL, (750 IU/mL in the 100 IU/mL detection level), weak reactions may occur due to antibody excess. If concentrations higher than 2000 IU/mL are suspected (1000 IU/mL in the 100 IU/mL detection level), samples should be tested diluted.
- An elevated antistreptolysin-O titer is used as a laboratory aid in the detection of group A streptococcal infections and their sequellae, acute rheumatic fever and post-streptococcal glomerulonephritis. Although a rise in the antistreptolysin-O titer is noted in 80 to 85% of patients, the diagnosis should not be excluded because of a negative test.[2]

Performance Characteristics

Sure-Vue® ASO was evaluated (200 IU/mL detection level) by comparison with a commercially available latex test. A total of 170 samples from hospital patients were tested following the qualitative technique. This study demonstrated a 95.9% agreement between the tests (sensitivity 96.7% and specificity 95.4%). Discrepancies were resolved with another commercially available latex test, and the obtained sensitivity was 98.4% and the specificity 98.1%, with an overall agreement of 98.2%
Three different people tested double dilutions of a strong sample on five different days, twice every day. The results of the study indicate that **Sure-Vue® ASO** in-house reproducibility (within one dilution) was 100%.

References

1. Todd, E.W. Antigenic streptococcal hemolysin. J. Exp. Med. 55: 267-280, 1932.

2. Klein, G.C. Immune response to streptococcal infection. In: Manual of Clinical Immunology. Chapter 33. American Society for Microbiology. Washington, D.C. 1976.

3. Kalbak, K. The antistreptolysin reaction. The State Serum Institute. Denmark, 1947.

4. Klein, G.C., Baker, C.N. and Moody, M.D. Comparison of antistreptolysin-O latex screening test with the antistreptolysin-O hemolytic test. Applied Microbiology. 19: 60-61, 1970.

5. Klein, G.C., Baker, C.N. and Jones, W.L. «Upper limits of normal» antistreptolysin-O and antideoxyribonuclease B titers. Applied Microbiology. 21: 999-1001, 1971.

6. Rantz, L.A. and Randall, E. A modification of the technic for determination of the antistreptolysin titer. Proc. Soc. Exp. Biol. and Med. 59: 22-25, 1945.

7. Hodge, B.E. and Swift, H.F. Varying hemolytic and constant combining capacity of streptolysins; Influence on testing for antistreptolysins. J. Exp. Med. 58: 277-287, 1933.

8. Spaun, J., Bentzon, M.W., Larsen, S.O. and Hewitt, L.F. International standard for antistreptolysin-O. Bull. WHO. 24: 271-279, 1961.

9. Biosafety in Microbiological and Biomedical Laboratories. CDC/NIH manual, 5th Edition, 2007.

Effective date

Effective date for this procedure:_____

Author

Compiled by Fisher HealthCare

Revised by**:** Ana Petru, MT (ASCP)

Designee Authorized for annual Review

See Annual Procedure manual Review Policy.

LIS REPORTING: Sure-Vue® ASO

The following directions for LIS reporting of the antistreptolysin-O screen should be followed with each patient run:

1. Create worksheet
   a. worksheet name “HASOSC”
   b. worksheet name “HQC” to enter ONLY Quality Control results (DO NOT CREATE FOR PATIENT ONLY)
2. Following testing; Process worksheet
3. Enter both control values for ASO test via worksheet “HQC”
4. Enter patient results via worksheet “HASOSC”
5. Print and Close worksheet
6. Place worksheet in center workstation area in Special Chemistry.

****FOLLOWING TESTING: STORE ALL SPECIMENS IN SEROLOGY RACK IN JEWET T100 REFRIGERATOR IN SPECIAL CHEMISTRY****