CENOGENICS
ACCULYSIN - O

SUMMARY AND TEST PRINCIPLE

Todd described in 1938, a test for measuring antibody response to recent **Group A streptococcal infection.[1] The group A, β-hemolytic streptococci** 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 and 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" because they are a by-product of the streptococci and lyse red blood cells. Todd later differentiated the streptolysins into two serologically identifiable lysins, Streptolysin 0 and Streptolysin S.

Streptolysin-O has antigenic activity which results in the production of antibodies known as antistreptolysin-O. The measurement of the antistreptolysin-O Titer (ASTO or ASO) level has led to a universally accepted diagnostic procedure important in streptococcal infections including rheumatic fever and glomerulonephritis.[3,4]

Infection with Group A streptococcus sensitizes the patient to streptolysin-O, causing the appearance in the patient's serum of a corresponding antibody, antistreptolysin-O. The most commonly used method for determining the concentration of streptolysin 0 antibodies is to perform a hemolytic inhibition test. Hemolysis of red cells by streptolysin-O can be inhibited by this antibody. The measurement of the serum level of antistreptolysin-O is called the Antistreptolysin-O Titration. For determination of this titration, a constant amount of streptolysin-O is added to decreasing amounts of serum; if the antistreptolysin present is sufficient to neutralize the antigen, no hemolysis occurs when red cells are subsequently added. A point is reached when antigen exceeds antibody, and the excess of streptolysin causes hemolysis of the added red cells. The antistreptolysin 0 titer is the reciprocal of the highest serum dilution that prevents hemolysis of the red cells.

However, it has been demonstrated that the antigen-antibody reaction is independent of whether the streptolysin 0 is in the reduced or oxidized state. This property has enabled the development of a slide agglutination test for the qualitative and semiquantitative determination of Antistreptolysin-O in the serum of patients with group A streptococcal infections.

TEST PRINCIPLE

CENOGENICS ACCULYSIN-O is a polystyrene latex reagent coated with streptolysin-O. In the presence of homologous antibodies, the coated latex particles will agglutinate to give characteristic patterns which can be seen after three minutes. The slide test reaction is compared with the positive and negative controls provided.

MATERIALS PROVIDED IN THE TEST KIT

ACCULYSIN-O Latex Reagent: Polstyrene latex particles, coated with streptolysin-O and suspended in a buffered solution.

ASO Positive Control: Stabilized, diluted human serum containing antistreptolysin-O and reactive with the latex reagent.

ASO Negative Control: Stabilized, diluted human serum non reactive with latex reagent.

Disposable pipets
Black glass slide
Instructions

MATERIALS REQUIRED BUT NOT PROVIDED
Isotonic saline (0.85% sodium chloride).
High intensity lamp or fluorescent light source
Test tubes for dilution

REAGENT PREPARATION
Reagents in the ACCULYSIN-O Kit are ready to use.

STORAGE
Store all reagents at 2°- 8° C.

PRECAUTIONS
1. The source material from which the positive and negative controls were derived was tested and found to be non-reactive for HVB and HIV when tested with licensed reagents. No known test method can offer assurance that products derived from human blood will not transmit infectious agents.

CAUTION: HANDLE AS IF CAPABLE OF TRANSMITTING INFECTIOUS DISEASE.

2. For In Vitro diagnostic use only.
3. Do not mix components from different manufacturers or kits.
4. Work with one reagent bottle at a time. Avoid interchanging caps among kit components.
5. Reagents in this kit contain sodium azide as a preservative. This material is known to form explosive mixtures in the presence of lead compounds. Use copious amounts of water to rinse glass slides or test tubes after completion of the test procedures.
6. When transferring each serum sample, always use a new dispensing tube or pipet tip.
7. Do not use reagents after the stated expiration date.

TEST PROCEDURE

QUALITATIVE TEST
1. Allow reagents and controls to reach room temperature.

2. Insert the disposable pipet provided into the serum sample to be tested. Squeeze the pipet between the thumb and the forefinger, release pressure. This will allow the serum to fill the tip.

3. Hold the disposable pipet perpendicularly over the circle of the black background glass slide and squeeze to release one free-falling drop of patient serum. This will deliver 0.05ml of serum.

4. Mix the ACCULYSIN 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 entire circle.

6. Rock slide for three minutes. Observe for agglutination while holding the slide under a high intensity lamp or fluorescent light.

RESULTS

POSITIVE RESULT:

Agglutination after three minutes indicates a content of antistreptolysin-O in the serum equal to or greater than 200 IU/ml.

NEGATIVE RESULT:

A smooth homogeneous suspension of the antigen serum mixture is indicative of a negative reaction.

SEMI-QUANTITATIVE TEST

1. Allow reagent and controls to reach room temperature.

2. Dilute patient serum in isotonic saline (0.85% sodium chloride) as follows:

TUBE	SERUM(ml)	SALINE(ml)	DILUTION	IU/ml
1	0.5	0	1:1	200
2	0.5	0.5	1:2	400
3	0.5 from tube 2	0:5	1:4	800
4	0.5 from tube 3	0.5	1:8	1600
5	0.5 from tube 4	0.5	1:16	3200
6	0.5 from tube 5	0.5	1:32	6400

3. Using the disposable pipet as described in the screening procedure, place a drop of each one of the serum dilutions on the black glass slide.

4. Mix the ACCULYSIN 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 three minutes. Observe for agglutination while holding the slide under a high intensity lamp or fluorescent light.

INTERPRETATION OF RESULTS

The highest serum dilution showing a definite agglutination pattern is considered the titer end point.

PERFORMANCE AND LIMITATIONS OF THE TEST

The results of ACCULYSIN-O test, as with other serological procedures, should not be used as the sole diagnostic criterion for the presence or absence of the disease state. The antistreptolysin O titer of normal individuals may vary widely. After acute streptococcal infection, the titer begins to rise in about two weeks and increases to a maximum level in perhaps four to six weeks. It remains high for a more or less prolonged period and then decreases. Even in nonrheumatic fever cases, patients who have had a recent acute Group A Streptococcal infection may have high titers of 166 Todd units or even 2500 units. In rheumatic fever patients, the titer is elevated in about 90% of cases. Persistence of titers 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-O can vary with age, season of the year and geographical area. 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.

Titers 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

1. Todd, E.W., J. Exp. Med., 55:267, 1932.

2. Rantz, L.A. and Randall, E., Proc. Soc. Exp. Biol. and Med. 59:22, 1945

3. Davidsohn, I., and Henery, J.B., Todd-Stanford Clinical Diagnosis by Laboratory Methods, 14th ED. W.B. Saunders Co. Phila., 1969.

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