Diagnostic Automation/Cortez Diagnostics, Inc.

IMMUNO DIAGNOSTICS

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ANTISTREPTOLYSIN O (ASO) LATEX TEST KIT

REF 330-50

IVD See External Label 2°C 8°C Σ 50 Tests

INTENDED USE

For the qualitative and semi-quantitative measurement of antibodies to streptococcal exoenzymes in human serum.

SUMMARY AND EXPLANATION

The group A β-hemolytic streptococci produce various toxins that can act as antigens. One of these exotoxins Streptolysin O was discovered by Todd in 1932.[1]

A person infected with group A β-hemolytic streptococci produces specific antibodies against these exotoxins, one of which is antistreptolysin O (ASO). The quantity of this antibody in a patient's serum will establish the degree of infection due to the β-hemolytic streptococcal.[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 quantitative test for the determination of the ASO by agglutination of latex particles on slide.[2]

ASSAY PRINCIPLE

ASO test method is based on an immunological reaction between streptococcal exoenzymes bound to biologically inert latex particles and streptococcal antibodies in the test sample. The reagent has been adjusted in the way that presence of an ASO titer of 200 IU/mL or higher in the serum gives a visible agglutination of the latex particles without previous sample dilution.

REAGENTS

1. ASO *Latex Reagent*: A suspension of polystyrene particles coated with streptococcal exoenzymes. **MIX WELL BEFORE USING.**
2. ASO *Positive Control*: A stabilized human serum containing at least 200 IU/mL of ASO reactive with the test reagent. Ready for use; do not dilute.
3. ASO *Negative Control*: A stabilized human serum containing less than 200 IU/mL of ASO non-reactive with the test reagent. Ready for use; do not dilute.
4. Glycine-Saline Buffer (20x) pH = 8.2 ± 0.1. A diluent containing 0.1 M glycine and 0.15 M NaCl. Dilute buffer according to instructions on the label. All reagents contain 0.1% (w/v) sodium azide as a preservative. Store all reagents at 2 - 8°C. **DO NOT FREEZE.**

WARNINGS AND PRECAUTIONS

1. Reagents containing sodium azide may combine with copper and lead plumbing to form highly explosive metal azides. Dispose of reagents by flushing with large amounts of water to prevent azide build-up.
2. For *in vitro* diagnostic use.
3. Positive and negative controls predated using human sera found negative for hepatitis B surface antigen (HBsAg) and HIV, however, handle controls as if potentially infectious.

REAGENT STORAGE AND STABILITY

1. Reagents are stable until stated expiration date on bottle label when stored refrigerated (2 - 8°C).
2. **DO NOT FREEZE.**
3. The ASO Latex Reagent, once shaken, must be uniform without visible clumping. When stored refrigerated, a slight sedimentation may occur and should be considered normal.
4. Do not use the latex reagent or controls if they become contaminated.

SPECIMEN COLLECTION AND STORAGE

1. Use fresh serum collected by centrifuging clotted blood.
2. If the test cannot be carried out on the same day, the serum may be stored between 2 - 8°C for no longer than 48 hours after collection. For longer periods the sample must be frozen.
3. As in all serological tests, hemolytic or contaminated serum must not be used.
4. ***DO NOT USE PLASMA.***

MATERIALS AND COMPONENTS

Materials provided with the test kit

1. ASO Latex Reagent.
2. ASO Positive Control.
3. ASO Negative Control.
4. Glycine - Saline Buffer.
5. Glass Slide.
6. Stir Sticks.

Materials required but not provided

1. Timer.
2. Test Tubes, Rack
3. Serological Pipettes

ASSAY PROCEDURE

***Qualitative Test*:**

1. Bring reagents and specimens to room temperature before use.
2. Place one drop (50 µl) of ASO Positive Control on field #1 of the glass slide. Place one drop (50 µl) of the ASO Negative Control on field #2 of the glass slide. Use pipette to deliver 1 drop (50µl) of undiluted test serum sample to field #3. Continue likewise with additional unknowns.
3. Gently resuspend the ASO Latex Reagent and add one drop to each test field.
4. Mix well with the flat end of the stir stick. Gently rock the glass slide for two (2) minutes and read immediately under direct light.

Semi-quantitative Test:

1. Set up at least five test tubes: 1:2, 1:4, 1:8, 1:16, 1:32, etc. and dilute samples according to dilution factors on each test tube with diluted glycine-saline solution (see table below). *NOTE*: glycine-saline solution has to be diluted with distilled water before use.

Dilutions	1:2	1:4	1:8
Sample Serum	100 µl	--	--
Glycine-Saline	100 µl	100 µl	100 µl
	→	100 µl	→ 100 µl

2. Place one drop each of positive and negative controls onto the glass slide rings. Place one drop of each dilution on successive fields of the glass slide.
3. Gently resuspend the ASO Latex Reagent and add one drop to each test field.
4. Mix well with the flat end of the stir stick. Gently rock the glass slide for two (2) minutes and read immediately under direct light.

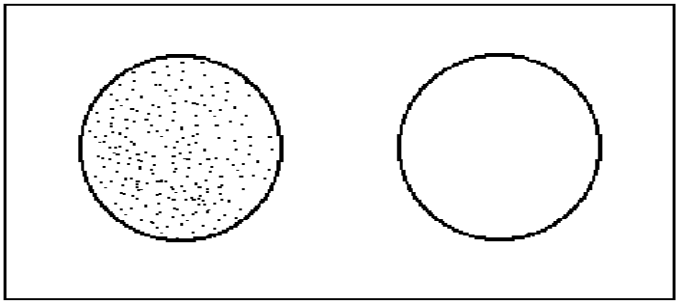
QUALITY CONTROL

1. Positive and negative controls should be included in each test batch.
2. Acceptable performance is indicated when a uniform milky suspension with no agglutination is observed with the ASO Negative Control and agglutination with large aggregates is observed with the ASO Positive Control.

RESULTS

Qualitative Test:

1. *Negative reaction*: Uniform milky suspension with no agglutination as observed with the ASO Negative Control.
2. *Positive reaction*: Any observable agglutination in the reaction mixture. A positive reaction indicates that the concentration of ASO in the specimen is equal or greater than 200 IU/mL. The specimen reaction should be compared to the ASO Negative Control (Fig. 1).



Positive Negative

Figure 1.

Semi-quantitative Test:

A positive reaction is indicated by any observable agglutination in the reaction mixture. Record the last dilution showing a positive reaction. Concentration of ASO can be determined by multiplying the last positive dilution factor of the sample with the concentration of the positive control (200 IU/ml).

The titer of the serum is the reciprocal of the highest dilution, which exhibits a positive reaction.

IU/ml of sample = Conc. of positive control x reciprocal

DILUTION	RECIPROCAL	IU/ml
1/2	2	400
1/4	4	800
1/8	8	1600
etc.		

LIMITATIONS

1. Results should be read two (2) minutes after the mixing of the reagent on the glass slide. A reading obtained after this period of time may be incorrect.
2. Existence of prozone at high titers has not been encountered.

EXPECTED VALUES

1. Although normal values can vary with age, season of the year and geographical area[2], the "upper limit of normal" ASO 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. In any case, the average can be established at less than 200 IU/ml.
2. 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[1].
3. Following acute streptococcal infection, the ASO titer will usually rise after one week, increasing to a maximum level within 3 to 5 weeks and usually returning to the pre-infection levels in approximately 6 to 12 months[2].

PERFORMANCE CHARACTERISTICS

1. ASO Reagent was evaluated on a total of 70 samples from hospital patients. The qualitative test was evaluated by comparison with a commercially available latex agglutination test. This study demonstrated a 90% agreement between these tests. The discrepant results were obtained in samples with titers near the limit of sensitivity of the reagents. There were no discrepancies with titers higher than 250 IU/ml.
2. A panel of 10 positive serum samples was tested on three consecutive days using the quantitative technique. The results of the study indicated that ASO Reagent has 100% precision. The error of repeated estimations were expected to be only one doubling dilution.

REFERENCES

1. Todd. E.W., J. *Exp. Med.* 55:267-280, (1932).
2. Klein. O.C. Immune Response to Streptococcal Infection, *Manual of Clinical Immunology*, American Society of Microbiology, (1974).
3. Kalbak, K., *The State Serum Institute*. Denmark, (1947).
4. Klein, G.C., et al., *Applied Microbiology*. 19:60 (1970).
5. Klein, G.C., et al. *Applied Microbiology*, 21:999 (1971).
6. Rantz, L.A. et al. *Proc. Soc. Exp. Biol. and Med.* 59:22 (1945).
7. Hodge, B.K. *Exp. Med.* 58:277 (1933).

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