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Procedure	Rheumajet ASO - NCCLS
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Prepared by	Date Adopted	Supersedes Procedure #

Review Date	Revision Date	Signature

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PRINCIPLE:

The **Rheumajet ASO** latex reagent is a suspension of polystyrene latex particles of uniform size coated with streptolysin-O. Latex particles allow visual observation of the antigen-antibody reaction. When a serum containing antistreptolysin-O is mixed with the Rheumajet ASO latex reagent, the uniform appearance of the latex suspension will convert to a clear agglutination. This change occurs because the antistreptolysin-O present in the serum reacts with the streptolysin-O coated latex particles forming a web between them.

Note: When Rheumajet ASO latex is mixed with neat serum, if the serum contains 200 IU/mL or greater of antistreptolysin-O, a clear agglutination will appear.

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SPECIMEN

Type: Use fresh serum. **Do not use plasma.**

Handling Conditions: If the test cannot be performed on the same day of sample collection, the serum may be stored between 2° and 8° C for no longer than 8 days after collection. For longer periods the samples must be frozen (-20°C). It is not necessary to inactivate the serum. As in all serological tests, hemolytic or contaminated serum must not be used.

EQUIPMENT AND MATERIALS

Materials Required:

- Normal saline (0.9% NaCl for semi-quantitative technique)
- Serological or automatic pipettes
- Timer
- Stirrers
- Rotator

Materials Provided:

- Rheumajet ASO latex reagent
- Positive control, pre-diluted
- Negative control, pre-diluted
- Disposable slides

Storage Requirements:

Reagents and controls should be stored at 2-8° C.
DO NOT FREEZE.

QUALITY CONTROL

1. Prior to each set of determinations, the latex reagents should be tested with each run using the positive and negative controls provided in the kit.
2. Both controls should be used following steps 4 through 8 of the Qualitative procedure. Do not dilute the controls prior to use.
3. The reaction between the positive control and the reagent should show clear agglutination, different from the uniform appearance of the negative control. If no agglutination takes place, the test should be repeated; if there is still no agglutination the kit should be discarded and Biokit Technical Service should be contacted.

PROCEDURE - QUALITATIVE (Stepwise)**A. 200 IU/mL dilution level:**

1. Allow reagents and controls to reach room temperature (20-30° C).
2. Gently shake the reagent vial to disperse and suspend the latex particles. Vigorous shaking should be avoided.
3. Use neat serum.
4. Place 0.05 mL of neat serum in one section of disposable slide.
5. Place a drop of reagent next to the drop of serum.
6. Mix both drops together using a stirrer covering the entire surface of the slide section.
7. Gently rotate the slide for two minutes manually or on a rotary shaker set at 80 - 100 rpm.
8. Look for the presence or absence of agglutination after the two minute time period.

PROCEDURE - SEMI-QUANTITATIVE (Stepwise)

A. For samples positive at the 200 IU/mL dilution.

1. Allow reagents and samples to reach room temperature (20-30° C).
2. Place 0.05 mL of normal saline on slide sections 2 through 6.
3. Using an automatic pipette, place 0.05 mL of neat serum on slide sections 1 and 2
4. Using the same pipette, take in and release the serum and the normal saline in section 2 several times until well mixed.
5. Take 0.05 mL of the mixture made on section 2 and transfer it to section 3.

Section	1	2	3	4	5	6
Saline uL	-	50	50	50	50	50
Serum uL	50	50	-	-	-	-
Mix and Transfer			50→	50→	50→	50→
Dilution	1:1	1:2	1:4	1:8	1:16	1:32
International Units/mL	200	400	800	1600	3200	6400

50→
Discard

6. Repeat steps 4 and 5 to obtain a thorough mixing of reagents, transferring 0.05 mL from section 3 to section 4 and so on, in succession, through section 6 discarding the last 0.05 mL.
7. Gently shake the vial of latex reagent, placing a drop on sections 1 through 6 of the slide containing the different serum solutions.
8. Mix both drops with a stirrer covering the whole surface of the slide section.
9. Gently rotate the slide two minutes manually or on a rotary shaker set at 80-100 rpm.
10. Look for the presence or absence of agglutination after two minutes.

Procedure – Qualitative (stepwise)**B. 100 IU/mL dilution level**

1. Allow reagents and controls to reach room temperature.
2. Gently shake the reagent vial to disperse and suspend the latex particles. Vigorous shaking should be avoided.
3. Place 100 ul of the serum onto one section of the disposable slide.
4. Place one drop of reagent next to the drop of serum.
5. Mix both drops with a stirrer covering the whole surface of the slide section.
6. Gently rotate the slide for 4 minutes manually or on a rotary shaker set at
7. 80-100 rpm.

REPORTING RESULTS:**Qualitative: 200 IU/mL detection level**

The presence of agglutination indicates a titer of antistreptolysin-O equal to or greater than 200 IU/mL. The absence of agglutination indicates a titer of antistreptolysin-O less than 200 IU/mL.

Qualitative: 100 IU/mL detection level

The presence of agglutination indicates a titer of antistreptolysin in the serum equal to or greater than 100 IU/mL. The absence of agglutination indicates a content of antistreptolysin in the serum less than 100 IU/mL.

Semi-quantitative: The approximate titer corresponds to the highest serum dilution that still presents a clearly visible agglutination.

Limitations: Results should be read two minutes after the mixing of the reagents on the slide. A reading obtained after this period of time may be incorrect. Existence of prozone at high titers has not been encountered.

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Expected Values: Although normal values can vary with age, season of the year and geographical area, the “upper limit of normal” for antistreptolysin-O titers in 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. Because of this variation, titers above the upper limits may be indicative of a streptococcal infection, the antistreptolysin-O titer will usually rise after one week, increasing to a maximum level within three to five weeks and usually decreases to the pre-infection levels in approximately six to twelve months.