KING ABDULAZIZ UNIVERSITY
FACULTY OF MEDICINE & ALLIED HEALTH
MEDICAL TECHNOLOGY PROGRAM

Laboratory Procedure Handout

Detection of Anti-streptolysin O (ASL)

Latex agglutination Assay for the detection of "ASO" antibodies In human serum or plasma

INTRODUCTION

Streptococcus, **classified either** by **haemolytic exotoxins** (**α, β, γ**) **or cell wall antigens** (**group A-Q**). **Group A, β-haemolytic** are the **most pathogenic**, **possessing capsules** (**M protein**) that **attach to mucous membranes** but **resist phagocytosis**, numerus **exotoxins** (**whence scarlet fever**), **indigestible cell walls causing severe cell-mediated reactions**, **antigens** that **cross-react** with **cardiac muscle** (**rheumatic fever**), and a **tendency** to **kidney-damaging immune complex**.

Streptococci, **produce extra cellular toxins** and **enzymes during growth**. Although the streptococci are found only in certain tissues, **some extra cellular metabolites can traverse cell membranes and enter the blood circulation**. **The metabolite concentration in blood is low and difficult to measure but antibodies formed in response to these antigens can be detected qualitatively**. **Streptolysin O is an extracellular metabolite of Group A, C, and G streptococci. The corresponding antibody, anti-streptolysin O (ASL), can be measured in the laboratory**.

Immunochemical assay of **specific antibodies** to **streptococcal metabolites provides valuable information about recent streptococcal infections** which is **useful** (**in conjunction with other clinical and physical findings**) **in establishing** a **diagnosis** of **acute rheumatic frver** and **post-streptococcal glomerulonephritis**. **Among** the **assays** of **antibodies** to the **various streptococcal exoenzymes, anti-streptolysin O** has **achieved dominance as a sensitive parameter since it has been elevated in about 80 to 85 % of the cases**. Moreover, **international standardization has only been possible for ASL up to now**.

Acute rheumatic fever has, to be sure, become infrequent in various regions; **however**, the **increase** in **mild** or **subclinical** cases **requires careful clarification** by **serodiagnosis**. **Rheumatic fever occurs only after Group A streptococcal infection** of the **upper respiratory tract**. However, the **ASL titer** also **increases after Group C** or **G streptococcal infections**. **Dnase B** and **hyaluronidase**, in **contrst**, are **secreted only** by **Group A streptococci so that detection** of **Anti-Dnase B and Anti-hyaluronidase is specific** for a **Group A streptococcal infection**. WHO recommends use ASL and anti-Dnase B for diagnosis of streptococcal infections. The presence of elevated ASL levels is of aid in the differential diagnosis of acute rheumatic fever and poststreptococcal glomerulonephritis. However, streptococcal skin infections often result in low ASL titers despite their association with glomerulomephritis.

Since there is **no reference point exists for the measured value with a single determination**, **conclusions can be drown only by repeating the test after 1 or 2 weeks and comparing the results**. **ASL can be assayed by the hemolysis inhibition, Ouchterlony immunodiffusion and latex agglutination tests among others**.

PRINCIPLE

The test is based upon immunologic reaction between anti-streptolysin O and streptolysin O coated on latex particles. An elevated anti-streptolysin O content leads to visible agglutination of the latex.

REAGENTS

A. Approximately 1% aqueous suspension of latex (polystyrene particles which are sensitized with streptolysin O, a purified protein preparation from a culture of β-haemolytic streptococci, group C.

B. ASL positive serum control (human) is a stabilized liquid human serum that contains at least 300 IU of anti-streptolysin O /ml.

C. ASL negative serum control (human) is a stabilized liquid human serum that contains less than 50 IU of anti-streptolysin O /ml.

- **The preservative in each reagent: Sodium azide, 1 g/l (max).**

SPECIMENS

Fresh (maximum storage, 8 days at +2 to +8 C) or frozen serum or plasma (collected using heparin or EDTA) sample are suitable. Samples can be stored frozen (at or below –25 C) up to 3 months if they are frozen within 24 hours of venipuncture and are not repeatedly thawed and re-frozen.

Serum samples must be completely clotted and contain no particulates nor traces of fibrin after centrifugation. Very lipaemic samples or samples which become turbid after thawing should be clarified by centrifugation (10 minutes at about 15000 x g) prior to assay.

METHOD

A. Qualitative "Slide" Test: 200 IU/ml discrimination level

1. Allow all reagents and patients samples to warm to room temperature.
2. Pipette 40 µl of **un**diluted patient sample onto separate fields on the slide. Also, place one (1) drop (approximately 40 µl) of the positive and the negative control serum on separate fields of the test slide.
3. Gently mix the contents of the latex suspension (reagent bottle, including the contents of the dropper). Fill dropper with well-mixed suspension and place 1 drop (about 40 µl) next to the sample drop on each field of the test slide. Thoroughly mix the two (2) drops in each field with a stirring rod, then continuously rotate the slide through several planes.
4. After 2 minutes, examine for agglutination.

***Distinct agglutination demonstrates an anti-streptolysin O content which exceeds 200 IU/ml ± 20%.**

B. <u>Qualitative "Slide" Test</u>: 100 IU/ml discrimination level

1. Allow all reagents and patient samples to warm to room temperature.

2. Pipette 80 µl of **<u>un</u>**diluted patient sample onto separate fields of the slide. Also, place one (1) drop (approximately 40 µl) of the positive and the negative control serum on separate fields of the test slide.

3. Gently mix the contents of the latex suspension (reagent bottle, including the dropper contents) . Fill dropper with well-mixed suspension and place one (1) drop (approximately 40 µl) next to the sample drop on each field of the test slide. Thoroughly mix the two (2) drops in each field with a stirring rod, then continuously rotate the slide through several planes.

4. After 4 minutes, examine for agglutination.

- **Distinct agglutination demonstrates an anti-streptolysin O content which exceeds 100 IU/ml ± 20%.**

C. <u>Semi-Quantitative Test</u>

The suspension of the latex which coated with streptolysin O can be used to estimate the anti-streptolysin O concentration. To do so, the patient sample is diluted according, for example, to the following scheme with isotonic sodium chloride solution:

- **Each dilution is tested as described under A.**

<u>Internal Quality Control</u>

The control sera must be included in each test series. ASL Control Serum, positive, must show distinct agglutination and Control serum, negative may not react (homogeneous suspension) when tested.

RESULTS

A. Qualitative Test: Discrimination Limit 200 IU/ml

Distinct agglutination identifies samples with an anti-streptolysin O content of > 200 IU/ml ± 20%.

- **Samples with an anti-streptolysin content below 200 IU/ml show no agglutination.**

B. Qualitative Test: Discrimination Limit 100 IU/ml

Distinct agglutination identifies samples with an anti-streptolysin O content of > 100 IU/ml ± 20%.

- **Samples with an anti-streptolysin content below 100 IU/ml show no agglutination.**
- **Samples which are negative in method A but positive in method B have an ASL content between 100 IU/ml and 200 IU/ml.**

C. Semi-Quantitative Test

The anti-streptolysin O content of the sample is based on the highest dilution showing distinct agglutination. The patient sample anti-streptolysin O content can be taken from the following table.

Dilution	ASL concentration ± 20%
1: 2	> 400 IU/ml
1: 4	> 800 IU/ml
1: 8	> 1600 IU/ml
1: 16	> 3200 IU/ml

- **Exact quantitation for reliable monitoring can be performed automated with the Behring Nephelometer reagents.**

LIMITATIONS & INTERFERENCES

Reading after more than 2 minutes (4 minutes in method B) can lead to false positive results. Very lipaemic sera/plasma can also cause non-specific reactions. Special attention should be paid to closing the suspension of the latex particles dropper bottle since otherwise evaporation can lead to flocculant formation. The suspension of latex should be stored in the refrigerator (+2 to + 8 C), Brought to room temperature before assay and , immediately before use, shaken well. Do not freeze the latex suspension reagents. Strength of the reactions may occur with slightly or markedly elevated concentrations. In cases of greatly increased ASL titer (more than 2000 IU/ml), agglutination may be inhibited because of antibody excess (prozone effect). When such high ASL concentration s are to be expected, the sample should be tested diluted. The results of this test should always be interpreted in the light of clinical and other laboratory findings. Negative results don not rule out the diagnosis of acute rheumatic fever or poststreptococcal glomerulonephritis.

REFERENCE VALUES

Since an immunological interaction of an organism with streptococcal exoenzymes occurs frequently, practically all sera demonstrate a certain ASL titer. This depend somewhat upon the patient age, the geographic locality, and the local frequency of streptococcal infections. Therefore, each laboratory should determine its own "normal rage". As an orientation point , 200 IU/ml is accepted internationally as the upper limit of the "normal range" since this value was seldom exceeded in persons without clinical symptoms among whom streptococcal infections were suspected. Normal values in pre-school age children lie mostly below 100 IU/ml. ASL concentrations increase with age in children, reaching a peak among school children, then falling thereafter.

- **A 2-fold or greater rise in ASL titer is considered significant in all age groups.**

SENSETIVITY

The sensitivity of the test is 200 IU/ml (± 20%) using 40 µl of sample and reading the results after 2 minutes. A sensitivity increases to 100 IU/ml (± 20 %) can be achieved by doubling the sample volume to 80 µl and increasing the reaction time to 4 minutes.

SPECIFICITY

The test is specific for antibodies to streptolysin O.

REFERENCES

1. **Playfair, J. H. L. Immunology at a Glance.**
2. **RapiTex ASL .**

DR. MUSTAFA HASAN LINJAWI