



ASO (Antistreptolysin O) Immunoturbidity Method

INTENDED USE:

The ASO - Immunoturbidity method is a turbidimetric test for the quantitative determination of ASO in human serum or plasma.

INTRODUCTION:

Anti-streptolysin O (ASO) is intended for *in-vitro* quantitative determination of Anti-streptolysin O in human serum & plasma. Anti-Streptolysin O (ASO) is the antibody produced in response to streptolysin O, an antigen produced by Lancefield group A streptococci. The World Health Organisation recommends the use of ASO to aid the diagnosis of streptococcal infections. ASO titers are elevated in the sera 80 to 85% of patients with rheumatic fever and in 95% of patients with acute glomerulonephritis. Raised ASO levels can also occur in other conditions such as scarlet fever, acute rheumatic arthritis, tonsillitis and various other streptococcal infections.

PRINCIPLE:

The reagent consists of a suspension of latex particles of homogeneous size sensitized with anti-ASO, capable of aggregation in the presence of ASO. This aggregation process produces an increase in the size of the latex particles which in turn produces an increase in the absorbance of the system.

KIT CONTENTS:

Reagent 1 : ASO Buffer.

Reagent 2 : ASO Latex.

Reagent 3 : ASO Calibrator.

Product Insert

PREPARATION OF WORKING REAGENT:

Working reagent preparation mix gently 4 part of Reagent-1 (ASO Buffer) with 1 Part of Reagent-2 (ASO Latex) and avoid foaming.

Stability of working reagent is 4 days at 2-8°C.

STORAGE AND STABILITY:

The reagents when stored at 2-8°C are stable up to expiry date mentioned on the label. The reagent is stable for 10 days onboard the analyser at 2-10°C. Protect from light and avoid contamination.

SPECIMEN COLLECTION AND STORAGE:

Fresh sera stored at 2-8°C for no longer than 48 hours. It is necessary to freeze the sample when the assay is to be carried out after that period of the time. Discard contaminated or haemolyzed sera.

PRECAUTIONS:

1. Storage conditions as mentioned on the kit to be adhered.
2. Do not freeze or expose the reagents to higher temperature as it may affect the performance of the kit.
3. Before the assay bring all the reagents to room temperature.
4. After use store the kit contents immediately at 2-8°C.
5. Avoid contamination of the reagent during assay process.
6. Use clean glassware free from dust or debris.

PLOTTING OF MULTI-POINT CURVE:

The Immunoturbidity ASO is based on Non-Linear Reactions, hence, it is strongly recommended to run multi-standard mode to plot the multi-curve to have better accuracy and precise result.

Serial Dilution step

| Tube | 1 st | 2 nd | 3 rd | 4 th | 5 th |
|-------------------|-----------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Calibrator | 100 µl | 50 µl from 1 st Tube | 50 µl from 2 nd Tube | 50 µl from 3 rd Tube | 50 µl from 4 th Tube |
| Normal Saline | 0 | 50 µl | 50 µl | 50 µl | 50 µl |
| Ratio of Dilution | Neat | 1/2 | 1/4 | 1/8 | 1/16 |

PROCEDURE (Automated):

Refer to specific instrument application instructions.

TEST PROCEDURE (Manual):

Wavelength : 540 nm

Temperature : 37°C

Cuvette : 1 cm

Pipette into clean dry test tubes labelled Calibrator (C) and Test (T) as follows:

| Reagent | Calibrator (C) | Test (T) |
|-----------------|----------------|----------|
| Working Reagent | 1000 µl | 1000 µl |
| Calibrator | 10 µl | — |
| Sample | — | 10 µl |

Mix well, after about 10 sec. (37°C) read the absorbance A1 of the test (T) and calibrator (C) against air or water. After exactly 120 secs. read the absorbance A2 of the test (T) and Calibrator (C). Calculate $\Delta A/\text{min}$. (A2- A1) for the test and calibrator.

CALCULATION:

ASO concentration (IU/ml) = $\Delta A(T) / \Delta A (C) \times \text{calibrator concentration}$

NORMAL VALUES:

Upto 200 IU/ml

**It is recommended for each laboratory to establish its own reference ranges for local population.

QUALITY CONTROL:

To ensure adequate quality control, each run should include assayed normal and abnormal controls. If commercial controls are not available it is recommended that known value samples be aliquoted, frozen and used as controls.

PERFORMANCE CHARACTERISTICS:

1. **Sensitivity / Limit of Quantitation:** 10 IU/ml
2. **Linearity :** Upto 800 IU/ml.
Samples that give higher concentration should be diluted in saline Nacl 0.9% (1-4) and the final result have to be multiplied by 5.
3. **Specificity /Interferences**
No interference was observed by Bilirubin (170 umol/l), Haemoglobin (5 g/L), Triglycerides (2.28 mmol/L), RF (210 IU/ml), other drugs and substances may interfere in the test.

REFERENCE:

- 1) Manual of clinical lab Inmuno.ED by Rose, N.R., Friedman, H., Fahey, J.L., 3a ed. 336-339.
- 2) Wannamalter, L.W., Ayoub E.M.(1960). Circulation, 21,614.
- 3) Klein, G.C., Baker C.N., Jones, W.C. (1971). Appl. Microbiol. V21(6).999-1001.
- 4) Winkles, J., Lunec, J., Deverill, I. (1987). Clin, 33 (5), 685-689.
- 5) Winkles, J.W., Lunec, J., Gray, L. (1989). Clin Chem. 35 (2). 303 - 307.
- 6) Young OS. Effects of drugs on clinical laboratory tests. 5 ed. AACC Press.

APPLICATIONS:

Input parameter for semiauto/auto analyzers are given below:-

| Method | Fixed Time (2-Point) |
|------------------------|-----------------------|
| Wavelength | 540 nm |
| Zero Setting | Distilled Water |
| Temperature Setting | 37°C |
| Incubation Temperature | 37°C |
| Incubation Time | — |
| Delay Time | 10 secs |
| Read Time | 120 secs |
| No. of Reading | 2 |
| Interval Time | — |
| Sample Volume | 10 µl |
| Reagent Volume | 1000 µl |
| Concentration | Refer Calibrator vial |
| Units | IU/ml |
| Factor | — |
| Reaction Slope | Increasing |
| Linearity | 800 IU/ml |