ASO (TURBIDIMETRY)

INTENDED USE

The ASO assay is intended for the in- vitro quantitative determination of Anti-streptolysin O in serum or Plasma.

CLINICAL SIGNIFICANCE

Streptolysin O (SLO) is a lethal, exocellular protein released by Group A Streptococcal bacteria. The release of SLO stimulates the production of anti-streptolysin O (ASO) antibodies to neutralise its haemolytic effect.

The ASO test is used to determine recent streptococcal infection and post streptococcal complications including rheumatic fever and glomerulonephritis. The presence and level of ASO antibodies in human serum directly reflects the extent and degree of infection. Elevated levels of ASO may also be present in other conditions including scarlet fever, acute rheumatoid arthritis, tonsillitis and various other streptococcal infections as well as in health carriers

METHOD AND PRINCIPLE

Latex particles coated with streptolysin O(SLO) are agglutinated when mixed with samples containing ASO.

The agglutination causes an absorbance change, dependent upon the ASO contents of the patient sample that can be quantified by comparison from a calibrator of known ASO concentration.

REAGENTCOMPOSITION

1	R1(Diluent): Tris buffer
2	R2(Latex Reagent): Latex particle coated with SLO
3	Calibrator

WARNINGS AND PRECAUTIONS

If the reagents became turbid or the absorbance of blank reagent is higher than 1.000, it means that the reagent is invalid and you should discard it.

Do not freeze; frozen Latex or Diluent could change the functionality of the test.

Components from human origin have been tested and found to be negative for the presence of HBsAg, HCV, and antibody to HIV (1/2). It is recommended to handle with caution.

REAGENT PREPARATION

Working reagent (WR): Mix R1 and R2 in 4:1 ratio.

STORAGE AND STABILITY OF REAGENT.

R1 ,R2 & Calibrator

The sealed reagents are stable till expiry date from manufacture date, when stored at 2-8ºC and protected from light.

Working reagent

Stable during 30 days at 2-8ºC. Shake gently the vial before use. Products must not be stored at room temperature for longer than 30 hours during use.

REAGENT DETERIORATION

Presence of turbidity or suspended particle in R1.

SPECIMEN COLLECTION AND STABILITY

Fresh serum, avoid use of hemolysed or lipemic samples. Samples are stable for 7 days at 2-8 deg c.

INTERFERENCES

Bilirrubin (20 mg/dL), lipemia (10g/L) and rheumatoid factors (600 IU/mL) do not interfere. Hemoglobin (≥ 10 g/L), interferes. Other substances may interfere.

CALIBRATION : Use Bioline ASO Calibrator, which is ready to use.

The calibration curve in the semi-automated analyzer is stable for 2 weeks, which a new curve must be generated. Re-calibrate when control results are out of specified tolerances, when using different lot of reagent and when the instrument is adjusted.

ASSAY PROCEDURE FOR SEMIAUTO ANALYZER

Bring the working reagent and the photometer (cuvette holder) to 37ºC.

Assay conditions:

Wavelength: . . . . . .546 nm (520-560)

Temperature: 37ºC

Working Reagent (µl)	1000
Calibrator or sample (µl)	10

Mix and aspirate into the analyzer, read the absorbance A1 after 5 seconds and absorbance A2 after 120 seconds of the sample addition.

CALCULATIONS

One point Calibration:

$$\frac{(A2-A1)\ \text{sample}}{(A2-A1)\ \text{Calibrator}} \times \text{Calibrator concentration} = \text{IU/mL}$$

QUALITY CONTROL

It is recommended to use Quality Controls to verify the performance of the assay.

Each Laboratory has to establish its own internal quality control scheme and procedures for corrective action if controls do not recover within the acceptable tolerance.

EXPECTED VALUES

It is recommended that each laboratory should establish its own reference values. The following value may be used as guideline.

Adults: < 200 IU/ml

Children (<5 years old) : <150 IU/ml

PERFORMANCE CHARACTERSTICS

Performance 1.Analytical Sensitivity

The analytical sensitivity of ASO assay is 20 IU/ml.

Precision

	Intra Run		Inter Run	
Control	Low	High	Low	High
n	20	20	20	20
Mean(IU/mL)	102	274	103	276
SD	2.86	4.66	2.96	4.94
CV (10%)	2.8%	1.7%	2.9%	1.8%

Linearity limit

Up to 800 IU/mL under the described assay conditions. If the concentration is greater than linearity (800 IU/mL), dilute the sample with normal saline and repeat the assay. Multiply the result with dilution factor.

Detection limit

Values less than 20 IU/mL give non-reproducible results.

Pro-zone effect:

No pro-zone effect was detected upon 3000IU/ml.

Accuracy:

Results obtained using this reagent (y) were compared to those obtained using a commercial reagent (x) with similar characteristics. 80 samples of different concentrations of ASO were assayed. The correlation coefficient (r) was 0.99 and the regression equation was $y = 1.042x + 7.231$.

INTERFERENCES

Bilirrubin (20 mg/dL), lipemia (10g/L) and rheumatoid factors (600 IU/mL) do not interfere. Hemoglobin (≥ 10 g/L), interferes. Other substances may interfere.

GENERAL TECHNICAL PARAMETERS.

Mode	FIXED TIME/INITIAL RATE/2-POINT KINETIC
Reaction	Ascending
Wavelength	546±20 nm
Blank	Distilled water
Sample Volume	10µL
Reagent Volume	1000µL
Delay Time	05 (Sec)
Read Time	120 (Sec)
Calibrator Value	Stated on the vial
Linearity limit	800 IU/ml
Unit	IU/ml

REFERENCES

1. Rantz LD, Dicaprio JM, Randall E, Am. J. Med. Sci, 24, 1952.
2. 2. Klien GC 1976, Manual of Clinical Immunology ASM, 264. th 3. Medical Bacteriology N.C. Day 6 edition P. 189. 204