ASO

Turbilatex Method

Intended Use:
a - Streptolysin-O (ASO) Test reagent/kit is a medical device intended for the screening of a-Streptolysin-O (ASO) in human specimen. It is intended for *in vitro* diagnostics use by trained healthcare professionals in IVD laboratory only and not near patients.

Clinical Significance
SLO is a toxic immunogenic exoenzyme produced by β-heamolitic Streptococci of groups A, C and G. Measuring the ASO antibodies are useful for the diagnostic of rheumatoid fever, acute glomerulonephritis and streptococcal infections. Rheumatic fever is an inflammatory disease affecting connective tissue from several parts of human body as skin, heart, joints etc… and acute glomerulonephritis is a renal infection that affects mainly to renal glommerulus.

Principle
The ASO-Turbilatex is a quantitative turbidimetric test for the measurement of ASO in human serum or plasma.
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.

Reagent Composition

R1 Diluent	Tris buffer 20 mmol/L, pH 8.2.
R2 Latex	Latex particles coated withstreptolysin O,pH10.0
ASO Calibrator	Human serum ASO. Concentration is stated on vial label. and it is traceable to the Reference Material antistreptolysin O 97/662 (NIBS).

Precautions
Components from human origin have been tested and found to be negative for the presence of HBsAg, HCV, and antibody to HIV (1/2). However handle cautiously as potentially infectious.

Calibration
Use ASRITHA ASO Calibrator Only.
The sensitivity of the assay and the target value of the calibrator have been standardized against the ASO International Standard from NIBSC 97/662(NIBS)

Preparation
ASO Calibrator: **Ready to use.**

Working Reagent:
Swirl the latex vial before use. Mix Latex and Diluent in a 1:4 ratio (i.e 2 ml R2 + 8 ml R1) prior to use. Working Reagent is stable during 20 days at 2-8^{0}C

Stability and Storage
1. The reagents will remain stable until the expiration date printed on the label, when stored tightly closed at 2-8ºC and contaminations are prevented during their use. Do not use the reagents after the expiration date.
2. Working reagent is stable during 20 days at 2-8 ºC. Shake gently the vial before use.
3. **Reagent deterioration:** Presence of particles and turbidity.

Additional Equipment
- Thermostatic bath at 37ºC.
- Spectrophotometer or photometer thermostatable at 37ºC with a 540 ± 20 nm filter.

Samples
Fresh serum. Stable for 7 days at 2-8ºC or 3 months at –20ºC.Samples with presence of fibrin should be centrifuged beforetesting. Hemolyzed or contaminated samples are not suitablefor testing.

Assay Parameters

Mode	Two Point / Fixed Time
Reaction	Ascending
Wavelength	540 nm (530-550)
Blank With	Distilled Water
Sample Volume	5 µl
Reagent Volume	400 µl : 100 µl
Delay Time	10 (Sec)
Read Time	120 (Sec)
Calibrator Conc.	See Vial Label
Linearity Limit	Up to 800 IU/ml
Unit	IU/ml

Procedure
1. Bring the reagents and the photometer (cuvette holder) to 37ºC.
2. Assay conditions:
   Wavelength: 540 nm (530-550)
   Temperature: 37°C
   Cuvette light path: 1 cm
3. Adjust the instrument to zero with distilled water.
4. Pipette into a cuvette:

Addition Sequence	Test
R1-Diluent	400 µl
R2-Latex	100 µl
Calibrator or sample	5 µl

5. Mix and read the absorbance immediately Abs(A1) and after 2 minutes Abs (A2) of the sample addition.

Calculation

$$\text{ASO (IU/ml)} = \frac{\text{Abs (A2-A1)sample}}{\text{Abs (A2-A1) calibrator}} \times \text{Calibrator conc.}$$

Quality Control
Control sera are recommended to monitor the performance of manual and automated assay procedures. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

Reference Values

Normal values up to 200 IU/mL (adults) and 100 IU/mL (children < 5 years old).

Each laboratory should establish its own reference range.

Performance Characteristics

1. Linearity limit: Up to 800 IU/mL, under the described assay conditions. Samples with higher concentrations, should be diluted 1/5 in NaCl 9 g/L and retested again. The linearity limit depends on the sample-reagent ratio, as well the analyzer used. It will be higher by decreasing the sample volume, although the sensitivity of the test will be proportionally decreased.
2. Detection limit: Values less than 12 IU/ml givenon-reproducible results.
3. Prozone effect: No prozone effect was detected up to 4000 IU/ml.
4. Sensitivity: 0.8 mA. IU/ml.
5. Accuracy:Results obtained with this reagent did not show systematic differences when compared with commercial reagents of similar characteristics. Details of comparison are available on request
6. Interferences:Bilirubin (40 mg/dl), hemoglobin (12 g/L) lipemia (10g/L) and Rheumatoid factors (800 IU/ml), do not interfere. Other substances may interfere.

Notes

1. This method may be used with different instruments. Any application to an instrument should be validated to demonstrate that results meet the performance characteristics of the method. It is recommended to validate periodically the instrument. Contact to the distributor for any question on the application method.
2. The linearity limit depends on the sample/reagent ratio, as well as the analyzer used. It will be higher by decreasing the sample volume, although the sensitivity of the test will be proportionally decreased.
3. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.
4. For automatic instruments, avoid the presence of bubbles in the reagents that may interfere with the assay results

References

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2. Haffejee I, Quarterly Journal of Medicine, New series 84; 305:641 (1992)
3. Kassem AS et al. Pediatric Annals. 21 : 853 (1992).
4. Bisno DL. N Engl J Med 325 : 783 (1991).
5. Wannamaker LW. Circulation. 21: 598 (1960).
6. Klein GC et al. Appl Microbiol. 21: 758 (1971).
7. Young DS. Effects of drugs on clinical laboratory tests. 3th ed. AACC Press (1997).

Pakaging

R1 - Diluent: 1 x 40ml

R2 - Latex : 1 x 10ml

ASO- CAL:1 x 1ml

For *in vitro* Diagnostic Use Only