Quantitative determination of anti-streptolysin O (ASO) Turbidimetric

IVD *For In-Vitro diagnostic and professional use only*

Store at 2-8°C.

Principle

The Latex particles coated with streptolysin O (SLO) are agglutinated when they react with samples that contain specific antibodies anti-estreptolysin O (ASO).
The latex particles agglutination is proportional to the concentration of ASO in the sample and can be measured by turbidimetry.

Clinical Significance

ASO is a group of specific antibodies developed against and exoenzyme produced by β-hemolytic 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.

Reagents

Diluent (R1)	Tris buffer 20 mmol/L, pH 8.2. Sodium azide 0.95 g/L. **Ready to use.**
Latex (R2)	Latex particles coated with estreptolysin O, pH 10. Sodium azide 0.95 g/L. **Ready to use. Mix gently the vial by inversion before use (See Note 4).**
ASO-CAL	Human serum. ASO concentration is stated on the label vial and it is traceable to the Reference Material anti-streptolisyn O 97/662 (NIBS). **Ready to use.**

Additional Equipment

- Thermostatic bath at 37ºC.
- Spectrophotometer or photometer thermostatable at 37°C a 540 ± 20 nm filters.

Preparation

Working reagent: Swirl the latex vial gently before use. Mix latex and diluent in a 1:5 ratio (i.e. 2ml latex reagent (R2) + 8 ml diluent (R1)) prior to use.

Storage and Stability

1. The reagents will remain stable until the expiration date printed on the label, when stored tightly closed at 2-8°C 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 °C. Shake gently the vial before use.
3. Reagent deterioration: presence of particles and turbidity. A hemolyzed or contaminated sample is not suitable for testing.

Precautions

- The reagents contain sodium azide 0.95 g/l. Avoid any contact with skin or mucous.
- The reagents from human donors have been given negative results to anti-HIV 1\2, HBsAg and anti-HCV. Handle cautiously is recommended.
- Wear protective clothing such as laboratory coats, disposable gloves and eye protection when specimens are being tested.
- The used test should be discarded according to local regulations.

Samples

Fresh serum. Stable for 7 days at 2-8°C and for 3 months at –20°C.
The samples with presence of fibrin should be centrifuged before testing.

Procedure

Preliminary Procedure

Prewarm the working reagent and photometer (cuvette holder) to 37°C.

Analytical Procedure

1. Using distilled water zero the instrument at 540 nm.
2. Pipette into a Cuvette:

Working Reagent (mL)	1.0
Calibrator or sample (µL)	10.0

3. Mix well and record the absorbance immediately (A1) and after 2 minutes (A2) of the sample addition.

Calculation

$$\frac{(A_2\text{-}A_1)\ \text{sample}}{(A_2\text{-}A_1)\ \text{Calibrator}} \times \text{calibrator concentration} = \text{IU/ml ASO}$$

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

Adults: up to 200 IU/mL.
Children (< 2 years): up to 150 IU/ml.
Children (School age): up to 250 IU/ml.
Each laboratory should establish its own reference range.

Performance Characteristics

- **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.

- **Detection limit:**

Values less than 12 IU/mL give non-reproducible results.

- **Analytical Sensitivity:**

0.8 mA/IU ASO/mL.

- **Prozone effect:**

Up to 4000 IU/mL

- **Precision:**

	Intra-assay (n=10)			Inter-assay (n=10)		
Mean (IU/mL)	161.7	411.3	593	161.7	411.3	593
CV %	5.2	4.3	1.8	4.6	4.3	3.7

- **Accuracy**

Results obtained with these reagents did not show systematic differences when compared commercial reagents of similar characteristics. Details of comparison are available on request.

- **Interferences**

Bilirubin (40 mg/dL), hemoglobin (12 g/L), Lipemia (10 g/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 meets the performance characteristic 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 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.

Bibliography

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REF	Catalogue Number		Temperature limit
IVD	*In Vitro* diagnostic medical device		Caution
Σ	Contains sufficient for <n> tests and Relative size		Consult instructions for use (IFU)
LOT	Batch code		Manufacturer
	Fragile, handle with care		Use-by date
	Manufacturer fax number		Do not use if package is damaged
	Manufacturer telephone number		Date of Manufacture
	Keep away from sunlight		Keep dry