TURBILYTE® ASO

TURBIDIMETRIC IMMUNOASSAY FOR DETERMINATION OF ANTISTREPTOLYSIN 'O' IN HUMAN SERUM

SUMMARY

The group A, β-hemolytic streptococci produce various exotoxins such as Streptolysin O, Streptolysin S that can act as antigens. The affected individuals produce specific antibodies against Streptolysin 'O' that has clinical significance namely Antistreptolysin 'O'. Antistreptolysin 'O' can be detected 1 - 3 weeks after infection, attaining a maximum levels at around 3 - 6 weeks. Determination of these antibodies is very useful for the diagnosis of streptococcal infections and their relative effects such as rheumatic fever and acute glomerulonephritis.

REAGENT:

1. **TURBILYTE®-ASO** Activation buffer (R1): ready to use.
2. **TURBILYTE®-ASO** Latex Reagent (R2): Ready to use uniform suspension of polystyrene latex particles coated with stabilized Streptolysin 'O'.
3. **TURBILYTE®-ASO** Calibrator: Lyophilized preparation of ASO positive serum, that is equivalent to stated amount of ASO on IU/ml basis, when hydrated appropriately. The **TURBILYTE®-ASO** calibrator is traceable to the International standard for Antistreptolysin 'O' (97/662).

Each batch of reagents undergoes rigorous quality control at various stages of manufacture for its specificity, sensitivity, and performance.

REAGENT STORAGE AND STABILITY

1. Store the reagents at 2-8°C. DO NOT FREEZE.
2. The shelf life of the reagent, activation buffer and the calibrator is as per the expiry date mentioned on the respective vial labels.
3. The reconstituted **TURBILYTE®-ASO** calibrator is stable for 7 days at 2-8°C and 48 hours at 25-30°C (RT.).
4. The working reagent for **TURBILYTE®-ASO** can be prepared by mixing R2 and R1 in the ratio 1:10.
5. The mixed stability of the working reagent (R1+ R2) is 7 days when stored at 2-8°C.

PRINCIPLE

TURBILYTE®-ASO is a turbidimetric immunoassay for the determination of Antistreptolysin 'O' and is based on the principle of agglutination reaction. The test specimen is mixed with **TURBILYTE®-ASO** latex reagent (R2) and activation buffer (R1) and allowed to react. Presence of ASO in the test specimen results in formation of an insoluble complex resulting in an increase in turbidity, which is measured at wavelength 505-578 nm. The increase in turbidity corresponds to the concentration of ASO in the test specimen.

NOTE

1. In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use.
2. The reagents that are derived from human source have been tested for HBsAg and Anti-HIV antibodies and are found to be non-reactive. However handle the material as if infectious.
3. Reagents contain 0.095% Sodium Azide as preservative. Avoid contact with skin and mucosa. On disposal flush with large quantities of water.
4. The reagents can be damaged due to microbial contamination or on exposure to extreme temperatures. It is recommended that the performance of the reagents be verified using known controls periodically.
5. Gently mix the **TURBILYTE®-ASO** latex reagent well before use to disperse the latex particles uniformly to improve test performance.
6. The working reagent should be mixed gently.
7. Do not use vortex mixers for mixing. Gently mix the reagents and samples during test procedures.
8. As the reagents within lots have been matched, reagents from different lots must not be interchanged.
9. Calibrators of different manufacturers must not be used with **TURBILYTE®-ASO** reagents.
10. Though any semi automated analyzer with appropriate programming facility can be used, for best results it is recommended to use **Quantiamate** analyzer. Fully automated analyzers may be used, provided the reagent has been standardized on the system.
11. The procedures mentioned in this pack insert are based on a minimum reading volume of 500 µl (0.5 ml). In case of instruments where minimum volume required for reading absorbance is 1.0 ml, use double the quantity of reagents and samples mentioned in the test procedure.
12. Do not use damaged or leaking reagents.

SPECIMEN COLLECTION AND PREPARATION

No special preparation of the patient is required prior to specimen collection by approved techniques.
Only serum should be used for testing. Should a delay in testing occur, store the samples at 2 - 8°C. Samples can be stored for upto three days at 2 - 8°C, provided they are not contaminated. Do not use hemolysed, icteric, or highly turbid sera. Turbid or particulate serum samples must be clarified by centrifugation at 2000 rpm for 15 minutes. Use the clear supernatant for testing.

ADDITIONAL MATERIAL REQUIRED

Spectrophotometer with 505 to 578 nm wavelength filters and cuvette mode, stopwatch, well calibrated micropipettes, disposable tips, isotonic saline, particulate free distilled water, test-tube rack, incubator/ waterbath set at 37°C, optically clean disposable/glass **semi micro cuvettes**.
Note: Though any filter between the wavelengths 505-578 can be used, optimum results are obtained with 546 nm wavelength filter.

TEST PROCEDURE

Bring reagent and sample to room temperature before use.
Assay conditions;

Wavelength	546 nm
Reaction Temperature	37°C
Cuvette	1 cm path length

The **TURBILYTE®-ASO** calibrator must be reconstituted exactly with the stated amount of distilled water (refer vial label), wait for 10 minutes, gently swirl the vial till the solution attains homogeneity. Once reconstituted it is ready to use for ASO calibration. The Concentration (S) of ASO in the reconstituted calibrator is as mentioned at the end of this package insert.

Pipette into the cuvette:

	For calibration	For sample
R1	450 µl	450 µl
R2	50 µl	50 µl
Mix well and incubate for 5 minutes		
Calibrator	5 µl	-
Sample	-	5 µl
Mix well and read absorbance A1 at 10 seconds and A2 at 2 minutes.		

CALCULATIONS

1. Calculate ΔA:
   ΔA = (A2-A1).
2. Concentration of ASO (IU/ml) in sample = $\frac{\Delta A \text{ Sample}}{\Delta A \text{ Calibrator}}$ x Concentration (S) of calibrator

SPECIFIC PERFORMANCE CHARACTERISTICS

Linearity

The Linearity of **TURBILYTE®-ASO** is upto 800 IU/ml. The linearity limit depends on the sample to reagent ratio as well as the analyzer used. It will be higher by decreasing the sample volume, though the detection limit of the assay will be proportionately decreased.

Detection limit / Analytical Sensitivity

Detection limit: 50 IU/ml

The detection limit represents the lowest measurable ASO concentrations that can be distinguished from zero.

Prozone limit

No prozone effect was observed upto a concentration of 1250 IU/ml of ASO.

Precision

Intra-assay precision	n	Mean IU/ml	SD	CV(%)
Sample 1	20	213.6	6.05	2.8
Sample 2	20	361.6	10.37	2.9
Sample 3	20	562.8	17.83	3.2

Inter-assay precision	n	Mean IU/ml	SD	CV(%)
Sample 1	20	213.6	6.45	3.0
Sample 2	20	363.2	12.81	3.5
Sample 3	20	561.9	19.50	3.5

Interference

No interference was observed by Bilirubin upto 50 mg/dl Glucose upto 500 mg/dl, Haemoglobin upto 500 mg/dl, Triglycerides upto 1000 mg/dl and Albumin upto 10 g/dl.

REFERENCE VALUES

The reference values of ASO in normal population are < 200 IU/ml. Each laboratory should define its own reference range for relevant population.

REMARKS

(1) Usage of well-calibrated equipment and accessories and procedures is critical for achieving correct results. (2) Samples with values beyond the linearity limit have to be diluted with isotonic saline and retested. The values obtained must be multiplied with the dilution factor for calculating the result. (3) Markedly lipemic, hemolysed, and contaminated serum samples could produce erroneous ASO values. (4) Use of plasma rather than serum can result in erroneous ASO values. (5) Do not read results beyond two minutes. (6) It is recommended that results of the test should be correlated with clinical findings to arrive at final diagnosis.

WARRANTY

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

BIBLIOGRAPHY

(1) Todd W.W., (1934), J. Path and Bact., 39, 299-320. (2) Klein G.C., (1980), Manual of Clin. Immunol.., 7th Ed., 431. (3) Spaun J., Bentzon M. W., Larsen S.O., et. al., (1961), Bull. WHO, 24, 271-279. (4) Klein G. C., et. al., (1971), Appl. Microbiol., 21, 999. (5) Clinical Laboratory Diagnostics, Edited by Lothar Thomas, M.D., 1st Ed., 1998, TH-Books Verlagsgesellschaft mbH, Frankfurt, Germany. (6) Data on file: Tulip Diagnostics (P) Ltd.