Anti Streptolysin O (ASO) – Turbidimetric method

Instructions for Use (IFU)

IVD CE

ORDERING INFORMATION

	Code	Composition
OPEN KONELAB INDIKO	REF B78182269	n° 2 vials x 40 mL (R.A) n° 2 vials x 6 mL (R.B)
CHEMILAB	REF B82181011	n° 1 vials x 26 mL (R.A) n° 1 vials x 5 mL (R.B)
	REF B82181012	n° 2 vials x 33 mL (R.A) n° 1 vials x 13 mL (R.B)

INTENDED USE

Diagnostic immunoturbidimetric test the quantitative determination of Streptolysin O (ASO) antibodies in human serum and plasma. All results must be interpreted according with the clinical context. PROFESSIONAL USE ONLY.

CLINICAL SIGNIFICANCE

Anti-Streptolysin-O antibodies are produced by the body in order to neutralize Streptolysin-O, an oxygen-labile exotoxin secreted by some strains of group A β-haemolytic *Streptococcus pyogenes*.

Streptolysin-O belongs to the group of cytolytic toxins of bacterial origin which have the ability to lyse, in vitro, the red blood cells of numerous animal species.

The determination of the anti-streptolysin titer (TAS) is therefore very useful for the diagnosis, evaluation of the medical treatment and course of infections supported by the bacterium such as pharyngitis, tonsillitis, scarlet fever, glomerulonephritis and rheumatoid fever. The determination of the TAS also contributed to the evaluation of the endemic diffusion of streptococcus. In these cases, the single analytical data has little clinical significance, while the evaluation of the temporal variation of the antibody response assumes greater importance.

PRINCIPLE OF THE METHOD

Turbidimetric method latex enhanced. The latex particles were activated with purified Streptolysin-O by covalent bonding to increase the sensitivity and stability of the reagent. The suspension of sensitized particles, in the presence of anti-Streptolysin-O antibodies, agglutinates causing a turbidity, revealed spectrophotometrically, proportional to the concentration of TAS in the sample under analysis. The quantitative analysis is obtained by interpolation of the photometric data with those obtained with samples with known TAS titer.

Storage and Stability

= Storage temperature 2-8 °C

If stored at 2-8°C avoiding direct light, the intact reagents remain stable until the expiration date, printed on the label. Do not freeze. Slight variations in composition among batches will not affect test results.

Concentration

Reagente A			
		Conc.	**U.M.**
ASO-TAS Buffer	Glycine Buffer	150	mmol/L
	NaN_3	< 0.1	%
Reagente B			
Latex particle coated with purified Streptolysin O	NaN_3	< 0.1	%

Materials included in the kit

Reagent as described above.

Necessary materials not included in the kit

Controls and calibrators

PRECAUTIONS and WARNINGS

1. Reagents and waste materials shall be disposed of in accordance with Community waste provisions or national or regional provisions.
2. Reagents may contain non-active components such as preservatives and detergents. The total concentration of these components is below the limits set out in Regulation 1272/2008 EC and subsequent amendments and additions.
3. It is recommended that the reagent be handled in accordance with the rules of good laboratory practice and that appropriate personal protective equipment be used.
4. Do not use the reagent if it is visibly degraded (e.g. presence of corpuscles).
5. All human samples shall be handled and disposed of as potentially infectious material.
6. The kit should only be used by qualified and properly trained technical personnel.
7. Diagnoses shall be carried out exclusively by authorised and qualified personnel.
8. Comply with national directives on occupational safety and quality assurance.
9. Use equipment that complies with current regulations.

Reporting of serious incidents

Please inform the manufacturer (through your distributor) and the competent authority of the member state of the European Union in which the user and/or patient is established, of cases of serious incident that has occurred in relation to the device. For other jurisdictions, reports of serious incidents must be made in accordance with the regulatory requirements of the home Member State. By reporting serious incidents, you help provide more information about the safety of your in vitro medical diagnostic device.

PROCEDURE

Quality Control

Use the Sclavo Diagnostics Int. ASO - CRP - RF Control low B47182278 and High B47182279 for quality control purposes at least once a day. Repeat the analysis after calibration. Obtained values must be within the acceptability range.

Calibration

For calibration, use the Sclavo Diagnostics Int ASO Single Level Calibrator B47182291.

Traceability

For anti-Streptolysin O antibodies (ASO-TAS) the calibrators are traceable to the ASO International Reference Preparation.

SAMPLE

Sample types and storage

Serum or plasma obtained by normal medical techniques can be used. No special preparation of the patient is necessary. Test samples can be stored for 3 days at 2-8°C or 12 months if frozen. Defrost samples at room temperature and mix carefully by turning upside down before testing. Avoid repeated freezing and thawing of samples.

The samples does not require predilution before analysis.

Strongly lipemic samples or those which present a high degree of turbidity or precipitates must be clarified by centrifugation (10 min. at 15,000xg), before testing.

PREPARATION OF THE REAGENT

The Reagents are liquid, ready for use. After opening, the Reagents are stable until the expiry date if kept as indicated in "Storage and stability".

Automation

The kit can be used with all automatic analyzers that can meet the operating conditions of the reagent while maintaining the volumetric ratios R1 / R2 / C. Validated applications are available for Sclavo Konelab® - Indiko® and CHEMILAB instruments. Applications not approved by Sclavo Diagnostics do not guarantee the performance of the reagent and must therefore be approved under the responsibility of the user.

MANUAL METHOD

The kit, in Open format, can be used manually through the use of spectrophotometer or photometer with the following parameters:

Reaction conditions

Wavelength (primary):	600 nm
Temperature:	37°C.
Reaction	Fixed-Time

Dispense as follows for the blank, for each calibrator and unknown samples

	Volume (µL)
Buffer RA	225
Test Sample	3
Mix and incubate at 37°C for at least 1 minute, then add:	
ASO Reagent (RB)	40
Mix and incubate at 37°C. Read the absorbance (Abs-1) After 6 minutes read the absorbance (Abs-2)	

Note: **The reaction volumes can be varies proportioning without altering the results.**

Results

The concentration of ASO is obtained as following: generate the calibration curve with the ΔAbs values and concentration of the single calibrators. Calculate the analytical result expressed in "UI/mL", from the calibration curve. All samples with a an ASO concentration higher than the highest calibration point and/or giving a signal denoting an excess of antigen (in the automated instruments) must be diluted and retested. It is advisable to use doubling dilutions in saline.

Sclavo Diagnostics International
Loc. Pian dei Mori, via Po n° 26-28 • 53018 (SI) (Italy)
Phone +39 0577 390 41 • fax +39 0577 390 444
www.sclavodiagnostics.com

Control of the calibration curve
The calibration curve is valid for at least one month. However, its validity should be checked periodically using the SCLAVO Diagnostics Control ASO-CRP-RF Low (Ref. B47182278) and High (Ref. B47182279). The validity is confirmed if the values obtained are within the concentration range of the controls reported on the Instructions for use..

Automation
The results are automatically calculated by the analyzer based on the calibration line. The analyzer automatically performs calibration in accordance with the method protocol. The calibration line is calculated automatically by the different instruments.

REFERENCE RANGE
The typical reference range is ≤ 200 UI/mL. As sex, age, geographical location and other factors can influence the normal values found in the population, each laboratory should determine its own normal, medium and pathological values for its own population.

CHARACTERISTICS/PERFORMANCE
Analytical Range – Antigen excess
The analytical range was tested using a strongly positive sample and serial dilutions of this serum in saline solution. The method guarantees a correct response throughout the minimal detectable measurement range and the calibrator higher concentration. The present method does not show Antigen Excess until 1500 UI/mL.

Accuracy
Commercial control sera were tested with the present kit and the data obtained with the Konelab analyzer are reported in the table below (mean of three tests).

Level	Replicates	Mean (UI/mL)	DS	CV%
Low	25	83,241	1,6149	1,9
High	25	247,765	4,6279	1,9

The samples have TAS concentrations based on the Sclavo Diagnostics Int. Reference Preparation.

Specificity
The method is 100% specific for human Anti Streptolysin O antibodies.

Interferences

Interferent	Limit
Bilirubin	**50 mg/dL**
Ascorbic Acid	**50 mg/dL**
EDTA	**10 mM**
Haemoglobin	**500 mg/dL**
Sodium citrate	**1000 mg/dL**
Sodium Heparin	**40 mg/mL**
Triglycerides	**2%**
Rheumatoid Factor	**800 UI/mL**

Based on the great heterogeneity of potentially interfering substances and drugs, the results of this test, for diagnostic purposes, must always be evaluated jointly with the patient's medical history, clinical examinations and other findings of the medical examination.

Precision of the method
Precision of the analytical results was determined in terms of Repeatability and Total Precision according to the CLSI EP15-A2 protocol, analyzing commercial control sera. Obtained data are shown in the following table (95% confidence interval determinations).

Within-run Precision – Repeatability				
Level	**Replicates**	**Mean (mg/L)**	**DS**	**CV%**
Low	10	141,9	4,60	3,20
High	10	278,9	7,09	2,37
Total Precision (Within-lab Precision)				
Level	**Replicates**	**Mean (g/L)**	**DS**	**CV%**
Low	10	136,6	3,4610	2,54
High	10	273,7	8,7411	3,19

Limits of sensitivity
The Sensitivity limit has been measured using serial dilutions of a high concentrated sample. The smallest measurable concentration is 25 UI/mL.

Comparison between methods
The present method was compared with another commercially available, analyzing 99 human sera. Correlation data between the two methods are reported in the table below.

Parameter	Estimation
Intercept	17.008
Slope	1.1373
Correlation Coeff. (R)	0.9588

Symbols used in IFU and Packaging	
IVD In vitro diagnostic medical device vitro	Manufacturer
REF Catalogue Number	Instruction for use
LOT Lot Number	Temperature limitation
Expiration date	

REFERENCES

1. **Blombäck B. And Hanson Å**. (1979) Plasma Proteins. J. Kiley & Sons ed., Chichester
2. **Aguzzi F., Rezzani A., Nespoli L., Monafo V., Burgio G.R.** (1988) Anti-Streptolysin O Titer, Fifty-five Years after Todd: a reappraisal of its Clinical Significance. Boll. Ist. Sieroter. Milan. 67(2), 162-164
3. **Shahangian S., Agee K.A. And Dickinson R.P**. (1992);Concentration Dependencies of Immunoturbidimetric Dose-response Curves: Immunoturbidimetric Titer and Reactivity, and Relevance to Design of Turbidimetric Immunoassay. Clin. Chem. 38(6), 831-840
4. **Thomson D., Milford-Ward A., And Whicherj.T.** (1992) The value of Acute Phase Protein Measurements in Clinical Practice. Ann. Clin. Biochem. 29, 123-131.
5. **Whicher J.T., Price C.P. And Spencer K.** (1983). Immunonephelometric and Immunoturbidimetric Assay for Proteins. Crit. Rev. Clin. Lab. Sci 18(3), 213-260.
6. **Baudner S, Bienvenu J, Blirup-Jensen S, Carlstroem A, Johnson AM, Milford Ward A, et al.:(1993)** The certification of a matrix reference material for immunochemical measurement of 14 human serum proteins, CRM 470. EUR 15243 EN, 1993:1-186;
7. **Dati F, Schumann G, Thomas L, Aguzzi F, Baudner S, Bienvenu J, Blaabjerg O, Blirup-Jensen S, Carlström A, Petersen PH, Johnson AM, Milford-Ward A, Ritchie RF, Svendsen PJ, Whicher J.** (1996) Consensus of a group of professional societies and diagnostic companies on guidelines for interim reference ranges for 14 proteins in serum based on the standardization against the IFCC/BCR/CAP Reference Material (CRM 470). International Federation of Clinical Chemistry. Community Bureau of Reference of the Commission of the European Communities. College of American Pathologists. Eur J Clin Chem Clin Biochem. 6:517-20
8. **Clinical Laboratory Standards Institute (CLSI).** User Verification of Performance for Precision and Trueness; Approved Guideline – Second Edition. EP15-A2. Vol 25 N. 17
9. **Clinical Laboratory Standards Institute (CLSI).** Evaluation of Precision Performance of Quantitative Measurements Methods; Approved Guideline – Second Edition. EP05-A2. Vol 24 N. 25
10. **Clinical Laboratory Standards Institute (CLSI).** Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline – Third Edition. EP09-A3. Vol 33 N. 11
11. **Hahn RG, Knox LM, Forman TA**. (2005) Evaluation of poststreptococcal illness. Am Fam Physician. 71(10):1949-54.
12. **Dillon HC Jr, Reeves MS**. (1974) Streptococcal immune responses in nephritis after skin infections. Am J Med. 56(3):333-46.
13. **Renneberg J, Söderström M, Prellner K, Forsgren A, Christensen P** (1989) Age-related variations in anti-streptococcal antibody levels. Eur J Clin Microbiol Infect Dis. (9):792-5.

REVISION	DATE	CHANGE
Rev.A	03/2023	New Issue for IVDR Regulation (UE) 2017/746 compliance

Sclavo Diagnostics International
Loc. Pian dei Mori, via Po n° 26-28 • 53018 (SI) (Italy)
Phone +39 0577 390 41 • fax +39 0577 390 444
www.sclavodiagnostics.com