Human Anti-Streptolysin-O Kit for use on SPAPLUS®

For *in vitro* diagnostic use

Product Code: LK189.S

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FDA (USA) Information
Analyte Name: Anti-Streptolysin O (ASO)
Complexity Cat.: Moderate

CE

1 INTENDED USE

This kit is designed for the quantitative *in vitro* measurement of human anti-Streptolysin-O (ASO) in serum using the SPAPLUS turbidimetric analyser.

2 SUMMARY AND EXPLANATION

Group A β-haemolytic streptococci produce a number of exotoxins which can act as antigens. One of these exotoxins, Streptolysin-O, leads to the production of specific antibodies in infected subjects increasing the serum concentration of ASO. This can be used to establish the degree of infection past or present, by β-haemolytic streptococci (ref. 1-7).

Measurement of ASO levels in serum, in conjunction with other laboratory and clinical findings, is an aid in the diagnosis of diseases caused by Streptococcal infections including rheumatic fever, scarlet fever, glomerulonephritis, tonsillitis, upper respiratory infections and pyoderma (ref. 1-7).

3 PRINCIPLE

The determination of soluble antibody concentration by turbidimetric methods involves the reaction with specific antigen to form insoluble complexes. When light is passed through the suspension formed a portion of the light is transmitted and focused onto a photodiode by an optical lens system. The amount of transmitted light is indirectly proportional to the specific antibody concentration in the test sample. Concentrations are automatically calculated by reference to a calibration curve stored within the instrument.

Latex-enhanced antigens: Certain antigen-antibody reactions do not form sufficiently large immune complexes to be detected turbidimetrically. If the antigen is coated onto latex particles of a suitable size, the light scattering ability of the immune complexes formed with antibody is enhanced sufficiently to enable turbidimetric detection.

4 REAGENTS

4.1 Latex reagent: Consisting of streptolysin-O coated onto polystyrene microparticles and is supplied in stabilised liquid form. The reagent contains 0.05% ProClin™, 0.099% sodium azide, 0.1% E-amino-n-caproic acid (EACA) and 0.01% benzamidine.

4.2 Calibrator and controls: These consist of pooled human serum and are supplied in stabilised liquid form. They contain 0.099% sodium azide, 0.1% EACA and 0.01% benzamidine as preservatives. The concentration of anti-streptolysin given on the quality control certificate has been obtained by comparison with the Siemens N Rheumatology Standard.

4.3 Reaction buffer: Containing 0.099% sodium azide as a preservative.

**ProClin™ is a trademark of Rohm and Haas Corp., Philadelphia, PA.*

5 CAUTION

All donors of human serum supplied in this kit have been serum tested and found negative for hepatitis B surface antigen (HBsAg) and antibodies to human immunodeficiency virus (HIV1 and HIV2) and hepatitis C virus. The assays used were either cleared by the FDA (USA) or cleared for *in vitro* diagnostic use in the EU (Directive 98/79/EC, Annex II); however, these tests cannot guarantee the absence of infective agents. Proper handling and disposal methods should be established as for all potentially infective material, including (but not limited to) users wearing suitable protective equipment and clothing at all times. Only personnel fully trained in such methods should be permitted to perform these procedures.

WARNING: This product contains sodium azide and ProClin 300 and must be handled with caution; suitable gloves and other protective clothing should be worn at all times when handling this product. Do not ingest or allow contact with the skin (particularly broken skin or open wounds) or mucous membranes. If contact does occur wash with a large volume of water and seek urgent medical advice. Explosive metal azides may be formed on prolonged contact of sodium azide with lead and copper plumbing; on disposal of reagent, flush with a large volume of water to prevent azide build up.

This product should only be used by suitably trained personnel for the purposes stated in the Intended Use. Strict adherence to these instructions is essential at all times. Results are likely to be invalid if parameters other than those stated in these instructions are used.

Reagents from different batch numbers of kits are **NOT** interchangeable. If large numbers of tests are performed care should be taken to ensure that all the reagents are from the same batch.

6 STORAGE AND STABILITY

The unopened kit should be stored at 2-8°C and can be used until the expiry date shown on the kit box label. DO NOT FREEZE. The *Human ASO Reagent, Reaction Buffer,* calibrators, and controls may be stored for up to three months after opening providing that they are capped to avoid evaporation and kept at 2-8°C in a refrigerator. *Human ASO Reagent* and *ASO Reaction Buffer* may be stored, uncapped, on the SPAPLUS analyser for up to 30 days, provided that the main power switch (located at the rear of the left hand panel) is left switched on.

7 SPECIMEN COLLECTION AND PREPARATION

Use fresh or deep frozen serum samples**.** Blood samples should be collected by venepuncture, allowed to clot naturally and the serum separated as soon as possible to prevent haemolysis. The serum may be stored at 20-25°C for 2 days or at 4-8°C for up to 8 days prior to assay, or for prolonged storage kept at -20°C or below (ref. 8). Repeated freezing and thawing should be avoided. Microbially contaminated, haemolysed and lipaemic serum and samples containing particulate matter should not be used.

8 METHODOLOGY

8.1 Materials Provided

8.1.1 1 x 100 Tests *Human ASO Reagent SPAPLUS*
8.1.2 1 x *Human ASO SPAPLUS Calibrator set 1-6* (6 x 1.0mL)
8.1.3 2 x 1.5mL *Human ASO SPAPLUS High Control*
8.1.4 2 x 1.5mL *Human ASO SPAPLUS Low Control*
8.1.5 1 x 100 Tests *ASO Reaction Buffer SPAPLUS*

8.2 Materials required but not provided

8.2.1 Equipment for collection and preparation of test samples e.g. sample tubes, centrifuge etc.
8.2.2 A fully operational and equipped SPAPLUS analyser.
8.2.3 Current analyser operating instructions: SPAPLUS Reference Guide, Insert Code FIN012.
8.2.4 Sample Diluent (99: Dil 1) Product Code: SN080.S

8.3 Reagent preparation

Before loading, gently mix by inversion ensuring no foam or bubbles are generated or remain on the surface as these may interfere with reagent aspiration.

8.4 Test procedure

The user should be familiar with the operation of the SPAPLUS analyser before attempting to carry out the test procedures. The analyser should be prepared for use according to the manufacturer's instructions and the assay protocol entered as described below.
For full details of analyser operation refer to the SPAPLUS Reference Guide (FIN012) supplied with the analyser.

8.4.1 Test parameters

Assay parameters are entered into item number 45.

Item Name 45 ASO
DATA INFORMATION
Units IU/mL
Decimals 3
ANALYSIS
Type End ▼
Main W.Length 1 340 ▼
Sub W.Length ▼
Method
CORR.
SLOPE INTER
Y = 1 X + 0
CALIBRATION
Type Logit 2 ▼
Standard
1 # 4 #
2 # 5 #
3 # 6 #
Auto Fill
NORMAL RANGE

	MALE LOW	MALE HIGH	FEMALE LOW	FEMALE HIGH
Serum	[ ]	[ ]	[ ]	[ ]
Urine	[ ]	[ ]	[ ]	[ ]
Plasma	[ ]	[ ]	[ ]	[ ]
CSF	[ ]	[ ]	[ ]	[ ]
Dialysis	[ ]	[ ]	[ ]	[ ]
Other	[ ]	[ ]	[ ]	[ ]

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Item Name 45 ASO
ASPIRATION
KIND ○ Single ● Double
VOLUME
SAMPLE 5
REAGENT1 VOL 170 µL
REAGENT2 VOL 40
Third mix ● **OFF** ○ **ON**
R1 Blank ● **Water – Blank**
MONITOR
0 LEVEL SPAN 1
SPAN 3.0
DATA PROCESS
READ

	START	END
MAIN	53	54
SUB	35	36

ABSORBANCE LIMIT
LOW -3.0
HIGH 3.0
FACTOR
Blank correction * ○ ON ● OFF
ENDPOINT LIMIT 2.0
LINEAR CHECK (%) 0
Reaction Check
CHECK POINT
LOW -3
HIGH 3
DILUTION
Diluent ● 99: Dil 1 ○ 100: Dil 2
Pre Dilution Rate 10 ▼
Auto Rerun Dilution Rate High 20 ▼
Auto Rerun Dilution Rate Low ▼
PROZONE CHECK
0

	START	END	LIMIT (%) Min dOD	
FIRST	[ ]	[ ]		
SECOND	[ ]	[ ]	[ ]	○ Low ● High
THIRD	[ ]	[ ]	[ ]	○ Low ● High

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*Automatically calculated

Item Name 45 ASO
Auto Rerun SW
● On ○ Off
Auto Rerun Range (Result)
● On ○ Off ● On ○ Off

	Lower	Higher
Serum	Cal 1 #	Cal 6 #
Urine		
Plasma		
CSF		
Dialysis		
Other		

Bottle Size (ml)

24 Items		36 Items	
Reagent1	60	Reagent1	0
Reagent2 R1	18.0	Reagent2 R1	0
Reagent2 R2	5.0	Reagent2 R2	0

Auto Rerun Condition (Absorbance)
Absorbance Range
Lower ○ On ● Off
Higher ● On ○ Off
Prozone Range ○ On ● Off

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The calibrator (Standard #) values are found in the Quality Control Certificate (SIN270.QC). Calibrator values on **Page 1** should be entered in ascending order, i.e. the lowest value first. The analyser will automatically calculate and enter the correct measuring ranges on item pages 3 and 4 providing the Autofill button is pressed after typing the value for calibrator 6 on page 1. View Item parameter pages 3 and 4 to ensure correct value entry.
* The Blank correction factor is automatically calculated by the instrument.

8.5 Measuring range

The approximate measuring range of the ASO assay is shown in the table below.

SPA PLUS analyser dilution	Approximate range (IU/mL)
1/1	5-80
1/10	**50-800**
1/20	100-1600

9 QUALITY CONTROL

9.1 At least two levels of appropriate control material should be tested a minimum of once a day. In addition, controls should be tested after calibration, with each new lot of reagent and after specific maintenance or troubleshooting steps described in the SPA PLUS Operation Manual.

9.2 Quality control testing should be performed in accordance with regular requirements and each laboratory's standard procedure. Should a control measurement be out of range when assayed with a stored curve the assay must be recalibrated. If on recalibration the control values measured with the new curve are still out of range, the instrument and the assay parameters should be checked before repeating the assay. If problems persist, refer to the local technical support organisation.

9.3 The concentrations of the controls provided are stated on the accompanying QC certificate (SIN270.QC). Sample results obtained should only be accepted if the control results are within ±15% of the concentration(s) stated.

10 LIMITATIONS

10.1 Turbidimetric assays are not suitable for measurement of highly lipaemic or haemolysed samples or samples containing high levels of circulating immune complexes (CICs) due to the unpredictable degree of non-specific scatter these sample types may generate. Unexpected results should be confirmed using an alternative assay method.

10.2 This assay has not been validated using paediatric samples.

10.3 Should a control measurement be out of range when assayed with a stored curve the assay must be recalibrated. If on recalibration the control values measured with the new curve are still out of range, the instrument and the assay parameters should be checked before repeating the assay. If problems persist, refer to supplier.

10.4 Diagnosis cannot be made and treatment must not be given on the basis of ASO measurements alone. Clinical history and other laboratory findings must be taken into account.

10.5 Variation in reagent temperature may affect results. Ensure that reagents are transferred directly from the refrigerator to the refrigerated reagent compartment of the analyser – do not allow to warm to room temperature.

11 EXPECTED VALUES

Adult serum range

The normal range provided below is for guidance only. It is strongly recommended that local ranges are generated.

Reference interval for ASO in serum: ≤200IU/mL (ref.9).

12 PERFORMANCE CHARACTERISTICS

12.1 Precision

A study was performed following CLSI *Evaluation of Precision Performance of Clinical Quantitative Measurement Methods; Approved Guideline* (CLSI Document EP5-A2)**.** The study was performed over 21 working days, with two runs per day. One user assessed three different samples using three different reagent lots on three analysers.

Human ASO Precision summary									
	Mean	Within run		Between run		Between day		Total	
	(IU/mL)	SD	CV %	SD	CV %	SD	CV %	SD	CV %
Serum 1	700.769	22.661	3.2	5.870	0.8	43.810	6.3	49.672	7.1
Serum 2	230.426	7.746	3.4	2.514	1.1	8.830	3.8	12.012	5.2
Serum 3	73.1039	2.503	3.4	1.432	2.0	5.950	8.1	6.612	9.0
Serum 4*	702.979	25.080	3.6	19.755	2.8	16.504	2.3	35.940	5.1
Serum 5**	63.427	0.915	1.4	0.886	1.4	3.092	4.9	3.344	5.3

*performed at 1/20 sample dilution.
**performed at 1/1 sample dilution.

12.2 Comparison

A correlation study was performed on 45 samples (using a variety of normal and clinical sera) using this kit on a SPA PLUS and an alternative commercially available human ASO assay. The study demonstrated excellent agreement with the following Passing & Bablok fit:

y = 1.01x + 0.15 (IU/mL) (y = SPA PLUS human ASO; x = alternative assay)

Correlation coefficient r = 0.982 (calculated by linear regression)

12.3 Limit of Quantitation

Based on *CLSI document EP17-A - Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline* the limit of quantitation for this assay is defined as the lowest point of the calibration curve i.e. 5 IU/mL based upon measuring neat sample.

12.4 Linearity

A linearity study was performed following CLSI (formerly NCCLS) *Evaluation of the Linearity of Quantitative Measurement Procedures* document EP6-A. One user assessed the linearity of a pool of high samples using one lot of reagent on one analyser. This gave a regression plot of y = 0.9689x + 2.042 (y = measured ASO concentration, x = theoretical concentration) over the range of 42.729 – 842.806IU/mL using the analyser's standard sample dilution

12.5 Interference

Interference by 1500 formazine turbidity units (FTU) of chyle, 200mg/L bilirubin, 5.0g/L haemoglobin has been determined to be below d_{max}, defined as the maximum level of interference considered acceptable (25IU/mL), at the minimum sample dilution (1/1).

	Bilirubin	Hb	Chyle
d_{obs} (IU/mL)	-0.529	-0.566	-2.799
d_{obs}, 95% CI (IU/mL)	-3.499 – 2.442	-3.536 – 2.404	-5.769 – 0.172

12.6 Antigen excess

No antigen excess was observed to a level of two times the top point of the assay; approximately 1600 IU/mL.

13 BIBLIOGRAPHY

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