Human IgA1 Kit for use on SPAPLUS®

For *in vitro* diagnostic use

Product Code: NK087.S

Product manufactured by:
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FDA (USA) Information:
Analyte Name: Immunoglobulins A subclasses
Complexity Category: Moderate

CE

1 INTENDED USE

This kit is intended for quantifying human IgA subclass 1 (IgA1) in serum using the Binding Site SPAPLUS turbidimetric analyser. Measurement of this immunoglobulin aids in the diagnosis of abnormal protein metabolism and the body's lack of ability to resist infectious agents. The test result is to be used in conjunction with other clinical and laboratory findings.

2 SUMMARY AND EXPLANATION

Two classes of IgA have been identified in humans: IgA1, which accounts for 80-90% of total serum IgA, and IgA2 which is the major subclass in secretions such as milk. The two subclasses appear to be regulated independently. Antigenic sites on the IgA subclasses are responsible for the anaphylactic transfusion reactions experienced by some patients totally deficient in either IgA1 or IgA2. After repeated transfusion such patients may produce antibodies to these antigens. IgA subclasses are of further importance in that certain pathogenic microorganisms, including *Haemophilus influenzae*, are capable of enzymic cleavage of IgA1, leading to partial inactivation of this subclass. Recurrent sinopulmonary infections may therefore be related to deficiency of IgA2 which is resistant to these organisms (refs 1, 2 & 3).

3 PRINCIPLE

Evaluating the concentration of a soluble antigen by turbidimetry involves the addition of the test sample to a solution containing the appropriate antibody in a reaction vessel or cuvette. A beam of light is passed through the cuvette and, as the antigen-antibody reaction proceeds, the light passing through the cuvette is scattered increasingly as insoluble immune complexes are formed. Light scatter is monitored by measuring the decrease in intensity of the incident beam of light. The antibody in the cuvette is in excess so the amount of immune complex formed is proportional to the antigen concentration. A series of calibrators of known antigen concentration are assayed initially to produce a calibration curve of measured light scatter versus antigen concentration. Samples of unknown antigen concentration can then be assayed and the results read from the calibration curve.

4 REAGENTS

4.1 **Human IgA1 Antiserum:** This is supplied in stabilised liquid form. Preservatives: 0.099% sodium azide, 0.1% E-amino-n-caproic acid (EACA), 1mM ethylenediamine-tetraacetic acid (EDTA), 0.5% BSA 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 IgA1 given on the quality control certificate has been obtained by comparison with the DA470k international reference material.

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

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 approved 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 gloves, protective equipment and clothing at all times.** Only personnel fully trained in such methods should be permitted to perform these procedures.

This product contains sodium azide and must be handled with caution. 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 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 IgA1 Antiserum, Reaction Buffer, Calibrators and Controls may be stored for up to three months after opening provided that they are capped to avoid evaporation and kept at 2-8°C in a refrigerator. The Human IgA1 Antiserum and Reaction Buffer may be stored at 8-12°C 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 2-8°C for up to 48 hours prior to assay, or for prolonged storage kept at -20°C or below. 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 50 Tests *Human IgA1 Antiserum SPAPLUS*
8.1.2 1 x *Human IgA1 SPAPLUS Calibrator set 1-6* (6 x 1.0mL)
8.1.3 2 x 1.2mL *Human IgA Subclass SPAPLUS High Control*
8.1.4 2 x 1.2mL *Human IgA Subclass SPAPLUS Low Control*
8.1.5 1 x 50 Tests *IgA1 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 11.

Item Name 11 IgA1
DATA INFORMATION
Units mg/L
Decimals 1

ANALYSIS
Type End ▼

Main W.Length 1 340 ▼
Sub W.Length ▼

Method

CORR.
SLOPE INTER
Y = 1 X + 0

CALIBRATION
Type Spline 1 ▼ Auto Fill
Standard
1 # 4 #
2 # 5 #
3 # 6 #

NORMAL RANGE

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

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Item Name 11 IgA1

ASPIRATION
KIND ○ Single ● Double
VOLUME
SAMPLE 15
REAGENT1 VOL 190 **µL**
REAGENT2 VOL 50

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	30	31

ABSORBANCE LIMIT
LOW -3.0
HIGH 3.0

FACTOR
Blank correction *
ENDPOINT LIMIT 2.0
LINEAR CHECK (%) 0

Reaction Check
○ ON ● OFF
CHECK POINT
LOW -3
HIGH 3

DILUTION
Diluent ● 99: Dil 1 ○ 100: Dil 2
Pre Dilution Rate 10 ▼
Auto Rerun Dilution Rate High ▼
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 11 IgA1

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
Reagent2 R1	10	Reagent2 R1
Reagent2 R2	3	Reagent2 R2

Auto Rerun Condition (Absorbance)

Absorbance Range
Lower ● On ○ Off
Higher ○ On ● Off

Prozone Range ○ On ● Off

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NB. The calibrator (Standard #) values are found in the Quality Control Certificate (SIN178.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 IgA1 assay when using the standard 1/10 sample dilution is 300 – 6000 mg/L.

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 SPAPLUS Operation Manual.

9.2 Quality control testing should be performed in accordance with regulatory requirements and each laboratory's standard procedure. Should a control measurement be out of range when assayed with a store curve the assay must be calibrated. 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 (SIN178.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 value be out of range against a stored curve, it is recommended that the control should be re-assayed using the same calibration curve. If the control value is still out of range the curve should be recalibrated and the controls re-assayed. If the control values are out of range against the new calibration curve check the instrument and parameters entered before repeating the assay. If problems persist, refer to the supplier.

10.4 Diagnosis cannot be made and treatment must not be given on the basis of IgA1 measurements alone. Clinical history and other laboratory findings must be taken into account.

11 EXPECTED VALUES

The ranges provided have been obtained from a limited number of samples and are intended for guidance purposes only. Wherever possible it is strongly recommended that local ranges are generated.

Adult serum ranges

These ranges were obtained using this kit, by measuring the IgA1 concentration of sera taken from healthy adult UK blood donors. The reference interval was calculated using non-parametric statistics and represents the central 95% of the population.

	Number (n)	Mean (mg/L)	Median (mg/L)	95 Percentile Range (mg/L)
IgA1	120	1865.9	1751.79	760.81 – 3282.03

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.

IgA1 Precision summary									
	Mean	Within run		Between run		Between day		Total	
	(mg/L)	SD	CV %	SD	CV %	SD	CV %	SD	CV %
Serum 1	4813.76	78.15	1.6	63.70	1.3	172.93	3.6	200.17	4.2
Serum 2	3138.39	54.93	1.7	63.42	2.0	163.52	5.2	183.79	5.8
Serum 3	554.76	11.31	2.0	13.84	2.5	29.22	5.2	34.25	6.1

12.2 Comparison

A correlation study was performed on 112 samples (30 normal serum and 82 clinical serum) using this kit on a SPAPLUS and an alternative commercially available IgA1 assay. The study demonstrated excellent agreement with the following Passing Bablok plot:

y = 0.99x+12.59 (mg/L) (y = SPAPLUS IgA1; x = alternative assay)

correlation coefficient r = 0.980 (calculated by linear regression)

12.3 Limit of Blank and Limit of Detection

Based on *CLSI document EP17-A - Protocols for Determination of Limits of Detection and Limits Quantitation; Approved Guideline* the limit of detection represents the lowest measurable analyte level that can be distinguished from zero. This has been estimated at 15.33 mg/L (n = 60).
The limit of quantitation for this assay is defined as the lowest point of the calibration curve i.e. 34.2mg/L based upon measuring neat sample.

12.4 Linearity

A linearity study was performed based on NCCLS *Evaluation of the Linearity of Quantitative Measurement Procedures* (NCCLS 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 = 1.0178x - 69.577 (y = measured IgA1 concentration, x = theoretical concentration) over the range of 449.31-6000mg/L using the analyser's 1/10 sample dilution.

12.5 Interference

No significant assay interference by 1530 formazine turbidity units (FTU) of chyle, 200mg/L bilirubin, 4.83g/L haemoglobin has been demonstrated at the minimum sample dilution (1/1).

	Bilirubin	Hb	Chyle
Mean IgA1 (mg/L)	254.66	231.89	237.41
% interference	-3.07%	-1.73%	-5.04%

12.6 Antigen excess

No antigen excess was observed to a level of four times the top point of the assay; approximately 24g/L.

13 BIBLIOGRAPHY

1. Protein Reference Unit Handbook of Clinical Immunochemistry (1990) Ed. A. Milford Ward, Publ. PRU Publications, Sheffield, 133-134.
2. Mestecky, J. *et al* (1989). Comparative studies of the biological properties of human IgA subclasses. Protides of the Biol. Fluids 36, 179-182.
3. Conley, M.E. *et al* (1983). Serum levels of IgA1 and IgA2 in children and in patients with IgA deficiency. Mol. Immunol. 20, 977-981.