Human IgA liquid reagent kit for use on SPAPLUS®

For *in vitro* diagnostic use only

Product Code: NK010.S

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FDA (USA) Information
Analyte Name: Immunoglobulins IgA
Complexity Cat.: Moderate

CE

1 INTENDED USE

This kit is intended for the quantitative *in vitro* determination of human IgA in serum, lithium heparin or EDTA plasma, using the Binding Site SPAPLUS turbidimetric analyser. Measurement of IgA aids in the diagnosis of abnormal protein metabolism and the body's lack of ability to resist infectious agents. The test results are to be used in conjunction with other clinical and laboratory findings.

2 SUMMARY AND EXPLANATION

IgA is the major immunoglobulin class of sero-mucous secretions, part of the defence system for external body surfaces. The monomeric form is composed of two alpha heavy chains and two light chains. Two subclasses of IgA have been identified in humans, IgA1 and 2. Normal serum levels of IgA vary with age. Raised IgA serum levels are associated with breast feeding, chronic infections, liver disease and myeloma. Reduced levels may be associated with certain protein losing conditions and immunodeficiency (refs 1-6).

3 PRINCIPLE

The determination of soluble antigen concentration by turbidimetric methods involves the reaction with specific antiserum 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 protein concentration in the test sample. Concentrations are automatically calculated by reference to a calibration curve stored within the instrument.

4 REAGENTS

4.1 **Human IgA Antiserum:** This is monospecific and is supplied in stabilised liquid form. It contains 0.099% sodium azide, 0.1% E-amino-n-caproic acid (EACA), 0.5% BSA and 0.01% benzamidine as preservatives.

4.2 **Calibrator and Controls:** These consist of pooled human serum and are supplied in stabilised liquid form. The concentration of IgA given on the quality control certificate has been obtained by comparison with European Reference Material ERM-DA470k. They contain 0.099% sodium azide, 0.1% EACA and 0.01% benzamidine as preservatives.

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 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 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 reagents, 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. The Human IgA Antiserum and IgA 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, lithium heparinised or EDTA plasma samples (ref 7). Samples should be obtained by venepuncture and in the case of plasma separated as soon as possible. Blood should be allowed to clot and the serum separated as soon as possible to prevent haemolysis. Samples may be stored at 2-8°C for up to 7 days, but for prolonged storage samples should be kept frozen at -20°C or below. Repeated freeze/thaw cycles should be avoided. Microbially contaminated serum samples, samples containing particulate matter and lipaemic or haemolysed serum samples should not be used.

8 METHODOLOGY

8.1 Materials provided

8.1.1 1 x 100 Tests *Human IgA Antiserum*
8.1.2 1 x *Human IgA 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 100 Tests *IgA Reaction Buffer*

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) Binding Site 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 9.

Item Name 9 IgA
DATA INFORMATION
Units g/L
Decimals 3

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 9 IgA

ASPIRATION
KIND ○ Single ● Double
VOLUME µL
SAMPLE 8
REAGENT1 VOL 170
REAGENT2 VOL 30

Third mix ● **OFF** ○ **ON**
R1 Blank ● **Water – Blank**

MONITOR
0 LEVEL SPAN 1
SPAN 3.0

DATA PROCESS
READ **ABSORBANCE LIMIT**

	START	END	
MAIN	53	54	LOW -3.0
SUB	30	31	HIGH 3.0

FACTOR **Reaction Check**
Blank correction * ○ ON ● OFF
ENDPOINT LIMIT 2.0 CHECK POINT
LINEAR CHECK (%) 0 LOW -3
HIGH 3

DILUTION
Diluent ● 99: Dil 1 ○ 100: Dil 2
Pre Dilution Rate 10 ▼
Auto Rerun Dilution Rate High 40 ▼
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 9 IgA

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	Reagent2 R1	0
Reagent2 R2	4	Reagent2 R2	0

Auto Rerun Condition (Absorbance)

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

Prozone Range ○ On ● Off

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N.B. The calibrator (Standard #) values are found in the Quality Control Certificate (SIN143.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 IgA assay is shown in the table below.

Overall dilution	Analyser dilution	Manual pre-dilution	Approximate range (g/L)
1/1	1/1	-	0.02 - 0.70
1/10	**1/10**	**-**	**0.2 - 7.0**
1/40	1/40	-	0.8 - 28.0

Where results are greater than the measuring range samples should be rerun at 1/10 with a manual offline dilution of 1/10 to give an overall dilution of 1/100.

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 (SIN143.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 Should a control measurement be out of range when assayed with a stored curve, the control should be repeated. If the repeat is out of range 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.

10.3 Diagnosis cannot be made and treatment must not be given on the basis of IgA 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.

11.1 Adult serum ranges

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

	Number (n)	Mean (g/L)	Median (g/L)	95 Percentile Range (g/L)
IgA	258	2.464	2.297	0.845-4.990

11.2 Paediatric expected ranges

Reference ranges according to ERM-DA470k (replacement for CRM470) Protein Standardisation (ref 8)

Age Group (years)	Number (n)	95 Percentile Range (g/L)
Less than 1	75	0.00-0.83
1-3	52	0.20-1.00
4-6	41	0.27-1.95
7-9	55	0.34-3.05
10-11	38	0.53-2.04
12-13	38	0.58-3.58
14-15	38	0.47-2.49
16-19	74	0.61-3.48

12 PERFORMANCE CHARACTERISTICS

12.1 Precision

A study was performed following NCCLS *Evaluation of Precision Performance of Clinical Chemistry Approved Guideline* (NCCLS Document EP5-A)**.** 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.

IgA Precision Summary									
	Mean (g/L)	Within run		Between run		Between day		Total	
		SD	CV %	SD	CV %	SD	CV %	SD	CV %
Serum 1	5.895	0.06	1.0	0.08	1.4	0.18	3.1	0.21	3.5
Serum 2	3.606	0.025	0.7	0.06	1.7	0.11	3.1	0.13	3.6
Serum 3	0.340	0.003	0.9	0.01	1.0	0.02	4.9	0.02	5.1
Serum 4	0.073	0.001	2.0	0.0004	0.5	0.003	3.8	0.003	4.3

12.2 Comparison

12.2.1 Correlation Study

A correlation study was performed on 262 samples (88 normal, 152 known elevated or suppressed IgA samples and 22 low level samples diluted to obtain results between 0.02 and 0.07g/L) using this kit on a SPAPLUS analyser and an alternative commercial IgA assay on the Modular P analyser. However, a total of 55 samples were excluded: 39 samples with results of <0.04 g/L which is the LoQ of predicate device and 16 samples with results of >25.12 g/L which was the upper limit of this assay. The study demonstrated the following Passing & Bablok fit:

y=1.00x + 0.0 (g/L) (y = Binding Site IgA; x = alternative assay)

correlation coefficient r = 0.995 (calculated by linear regression)

Serum versus plasma correlation; 30 normal serum and matched plasma samples were tested over the range of 1.0 to 6.3 g/L. The following linear regressions were obtained:

Serum versus lithium heparin plasma

y =0.9747x - 0.006 (g/L) (y =li hep plasma; x = serum) r =0.996

Serum versus EDTA plasma

y =0.9775x + 0.032 (g/L) (y =EDTA plasma; x = serum) r =0.997

12.2.2 Percent agreement

A percent agreement calculation was performed for the 262 samples used in the comparison study (section 12.2). A result of less than 0.07g/L was considered to be positive (i.e. an IgA deficient subject) and a result of greater than or equal to 0.07g/L was considered to be negative (ref 3).

		Alternative IgA kit		Total
		positive	negative	
IgA SPAPLUS kit	positive	59	3	62
	negative	3	197	200
	Total	62	200	262

Positive percent agreement = **95.16%**
Negative percent agreement = **98.5%**
Overall percent agreement = **97.71%**

12.2.3 Clinical Study

A clinical study evaluated 33 samples on this kit ; 17 IgA deficient samples (11 of which were paediatric: 3-17 yrs), 7 non-target disease samples (1 chronic lymphocytic leukaemia, 1 lambda light chain paraprotein, 1 primary biliary cirrhosis, 1 myeloma and 3 Waldenström's Macroglobulinemia) and 9 samples from normal individuals. The 17 IgA deficient clinical samples all had IgA levels below 0.07 g/L (ESID category for IgA deficiency – ref 3) and the 16 remaining samples had levels above 0.07 g/L. These results support our claim that this kit is able to identify IgA deficiency according to the ESID Guideline.

12.3 Limit of Blank and Limit of Detection

A study has been carried out according to CLSI *Protocols for Determination of Limits of Detection and Limits Quantitation; Approved Guideline* (CLSI document EP17-A).The limit of detection represents the lowest measurable analyte level that can be distinguished from zero and has been estimated at 0.003g/L (n = 60). The limit of quantitation is defined as the lowest amount of analyte that can be quantitatively determined and has been estimated as 0.02 g/L for this assay.

12.4 Linearity

Linearity

A linearity study was performed following CLSI *Evaluation of the Linearity of Quantitative Measurement Procedures: Approved Guideline* (CLSI document EP6-A). The linearity was evaluated at 1/1 1/10 and 1/40 dilutions.

A linear regression equation of *y = 0.9664x + 0.004 g/L* (y = measured IgA concentration, x = theoretical concentration) *r=0.9990* was obtained for the range of 0.02-0.60g/L at the neat (1/1) sample dilution.

A linear regression equation of *y = 0.9874x + 0.074 g/L* (y = measured IgA concentration, x = theoretical concentration) *r=0.9998* was obtained for the range of 0.32-7.64g/L at the 1/10 sample dilution.

A linear regression equation of *y = 1.007x + 0.209 g/L* (y = measured IgA concentration, x = theoretical concentration) *r=0.9996* was obtained for the range of 2.49–30.9g/L at the 1/40 sample dilution.

12.5 Interference

No significant assay interference by 1500 formazine turbidity units (FTU) of chyle, 200mg/L bilirubin, or 5g/L haemoglobin has been demonstrated at the minimum sample dilution (neat). No interference is demonstrated with rheumatoid factor (RF 546 IU/mL).

Concentration	Bilirubin	Hb	Chyle
Mean (g/L)	0.250	0.235	0.238
% interference	-1.96	-1.26	+0.14

There is no cross reactivity between IgA and IgG or IgM under normal assay conditions.

12.6 Antigen excess

No antigen excess was observed to a level of six times the top point of the assay; approximately 40.0 g/L.

13 BIBLIOGRAPHY

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