Human IgA CSF Kit for use on SPAPLUS®

For *in vitro* diagnostic use

Product Code: LK010.L.S

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

CE

1 INTENDED USE

Human IgA CSF Kit for use on SPAPLUS is intended for the quantitative measurement of human IgA in cerebrospinal fluid (CSF) samples using the SPAPLUS analyser. Measurement of this immunoglobulin aids in the assessment of the body's lack of ability to resist infectious disease in conjunction with other clinical and laboratory findings.

2 SUMMARY AND EXPLANATION

Serum is the predominant source for proteins present in the CSF, the levels of which are regulated by the permeability of the blood-CSF barrier and CSF flow rate. An increase in CSF protein levels can be indicative of barrier dysfunction and/or local (intrathecal) synthesis of immunoglobulin (Ig) within the central nervous system (CNS)[1]. These parameters can be evaluated by measurement of the serum and CSF concentrations of albumin, IgG, IgA and IgM.
As albumin in CSF originates exclusively from blood, the albumin CSF/serum ratio provides a measurement of barrier function. Calculation of CSF/serum ratios and comparison of the Ig ratios to the albumin CSF/serum value can differentiate between the serum-derived Ig and intrathecal Ig synthesis.
The assessment of barrier function, intrathecal synthesis and other variable CSF analytes can be useful in the diagnosis of a variety of CNS disorders. An increase of intrathecal IgA in the CSF is indicative of bacterial infections such as tuberculous meningitis.[1, 2]

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 **IgA CSF Latex Reagent:** This is a monospecific sheep antibody coated onto polystyrene latex and is supplied in stabilised liquid form. It contains 0.05% Proclin™*, 0.025% sodium azide, 0.1% E-amino-n-caproic acid (EACA) and 0.01% benzamidine as preservatives.

4.2 **Calibrator:** This consists of pooled human serum supplied in lyophilised form and contains 0.099% sodium azide, 0.1% EACA and 0.01% benzamidine as preservatives. The concentration of IgA given on the quality control certificate has been obtained by comparison with DA470k (formerly CRM470) International Reference Material.

4.3 **Controls:** These consist of pooled human serum and are supplied in liquid form. They contain 0.099% sodium azide, 0.1% EACA and 0.01% benzamidine as preservatives.

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

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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 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 IgA CSF reagent, reaction buffer, calibrators (post reconstitution), and controls may be stored for up to two months after opening providing that they are capped to avoid evaporation and kept at 2-8°C in a refrigerator. The Human IgA CSF reagent and 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

Suitable samples are human CSF tested as fresh as possible.
CSF samples may be stored at 2-8°C for up to 7 days and can be kept at -20°C for up to 6 months[3]. Samples must be centrifuged prior to testing.

8 METHODOLOGY

8.1 Materials provided

8.1.1 1 x 60 Tests *Human IgA CSF Reagent SPAPLUS*
8.1.2 1 x *Human IgA CSF SPAPLUS Calibrator set 1-6* (6 vials, the calibrator material is supplied lyophilised)
8.1.3 2 x 1.5mL *Human IgA CSF SPAPLUS High Control*
8.1.4 2 x 1.5mL *Human IgA CSF SPAPLUS Low Control*
8.1.5 1 x 60 Tests *IgA CSF 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
8.2.5 2% Alkaline wash solution (working dilution)
8.2.6 Distilled water

8.3 Calibrator Set and Reagent preparation

8.3.1 The calibrator set is supplied in lyophilised form. Each vial must be reconstituted in the volume of distilled water stated on the Quality Control Certificate (SIN214.QC), and placed on a roller mixer for 15 minutes before use.
8.3.2 Before loading the reagent, 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 31.

Item Name 31 IgA C
DATA INFORMATION
Units mg/L
Decimals 3
ANALYSIS
Type End ▼
Main W.Length 1 600 ▼
Sub W.Length ▼
Method
CORR.
SLOPE INTER
Y = 1 X + 0

CALIBRATION
Type Logit 2 ▼
Standard
1 # 4 #
2 # 5 #
3 # 6 #
AutoFill

NORMAL RANGE

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

Page : 1 Print Hard Copy Next Page Save Return

Item Name 31 IgA C
ASPIRATION
KIND ○ Single ● Double
VOLUME
SAMPLE 10
REAGENT1 VOL 165 µL
REAGENT2 VOL 60
Third mix ● **OFF** ○ **ON**
R1 Blank ● **Water – Blank**
MONITOR
0 LEVEL SPAN 1
SPAN 3.0

DATA PROCESS
READ **ABSORBANCE LIMIT**

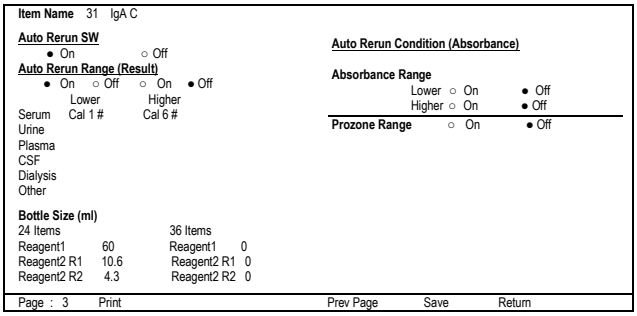
	START	END	
MAIN	53	54	LOW -3.0
SUB	35	36	HIGH 3.0

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 ▼
Auto Rerun Dilution Rate Low ▼
PROZONE CHECK

	START	END	LIMIT (%)	Min dOD	
FIRST	[ ]	[ ]			0
SECOND	[ ]	[ ]	[ ]	○ Low	● High
THIRD	[ ]	[ ]	[ ]	○ Low	● High

Page : 2 Print Hard Copy Prev Page Next Page Save Return

*Automatically calculated

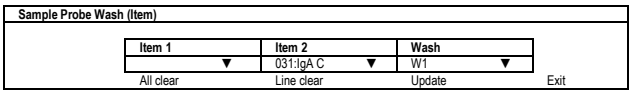


N.B. The calibrator (Standard #) values are found in the Quality Control Certificate (SIN214.QC). Calibrator values on **Page 1** should be entered in ascending order, i.e. the lowest value first. The analyser will automatically calculate and enter the correct measuring ranges on item pages 3 and 4 providing 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.

8.4.2 Sample probe wash

To protect against any potential carry-over on the sample probe a sample probe wash must be programmed.

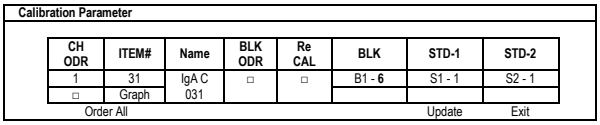
8.4.2.1 Click on **System** in the SPAPLUS software's Main Screen.
8.4.2.2 Select **Sys Para** from the drop down menu.
8.4.2.3 Select **Item** for the sample probe wash type and click **Save**. Click **OK** to save and **Exit** to return to the Main Screen.
8.4.2.4 Click on **System** in the SPAPLUS software's Main Screen.
8.4.2.5 Select **Sample Probe Wash** from the drop down menu.
8.4.2.6 Select **Item by item setting** and enter the parameters as below:



8.4.2.7 Click **Update** and **OK** to save the information and **Exit** to return to the Main Screen.

8.4.3 Calibration parameters

To protect against any potential carry-over on the sample probe from preceding assays six blank replicates must be programmed for the calibration curve.



8.4.4 Running controls and patient samples

Important: controls must be placed on the sample rack and not on the calibrator rack. If this is not followed the sample probe wash will not be activated.

8.4.4.1 Before placing the loaded sample rack onboard the analyser, fill a sample cup with 1.5mL of 2% alkaline wash solution (working dilution) and place into position W1 on the sample rack. When sample testing is complete, discard the sample cup from the W1 position.
8.4.4.2 When ordering samples select the appropriate specimen type for each sample.

Note: After running CSF samples the 'sample type' in the **Order** screen will default to CSF. Ensure that 'specimen type' is changed as appropriate when manually ordering the next serum/urine sample.

8.5 Measuring range

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

Specimen type	Analyser dilution	Approximate range (mg/L)
CSF	1/1	0.15 - 4.80
	1/10	1.50 - 48.00

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 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 (SIN214.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 IgA 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.
10.6 Carry-over may occur in conditions where IgA levels are grossly elevated e.g. with sera from multiple myeloma patients. Testing of such elevated samples should be isolated from CSF IgA testing.
10.7 Bacterial interference has not been assessed. CSF samples should be as fresh as possible to limit bacterial growth and all samples must be centrifuged prior to testing (see section 7).

11 EXPECTED VALUES

The ranges provided have been obtained from a limited number of adult samples and are intended for guidance purposes only. Wherever possible it is strongly recommended that each facility should determine its own reference intervals since values may vary depending on the individual population studied.

Reference interval for IgA in CSF: <5mg/L (after conversion to DA470k).[4]

Reference values in the true sense only exist for the CSF/serum ratio.[1,4]

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 5 working days, with two runs per day. One user assessed three different samples using one different reagent lots on one analyser.

IgA CSF Precision Summary									
	Mean (mg/L)	Within run		Between run		Between day		Total	
		SD	CV %	SD	CV %	SD	CV %	SD	CV %
Sample 1	46.164	0.19	0.4	0.50	1.1	0.63	1.4	0.82	1.8
Sample 2	5.290	0.06	1.2	0.04	0.8	0.35	6.6	0.36	6.8
Sample 3	1.986	0.10	5.0	0.12	5.9	0.00	0.0	0.15	7.8

12.2 Comparison

A correlation study was performed on 93 CSF samples (56 normal CSF and 37 clinical CSF) using this kit on a SPAPLUS and an alternative commercially available IgA CSF assay. The study demonstrated the following Passing Bablok plot:

y = 0.960x -0.05 (mg/L) (y = SPAPLUS IgA CSF; x = alternative assay)

correlation coefficient r = 0.993 (calculated by linear regression)

12.3 Limit of Detection and Limit of Quantitation

Based on CLSI document *EP17-A2 –Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline* the limit of detection represents the lowest measurable analyte level that can be distinguished from zero. This has been estimated at 0.023mg/L (n = 60).
The limit of quantitation has been calculated as 0.237mg/L (n=60) based on one lot at a 1/1 sample dilution.

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 diluted high level pool of samples using one lot of reagent on one analyser. This gave a regression plot: y = 0.993x +0.716mg/L (y = measured IgA CSF concentration, x = theoretical concentration) over the range of 1.354-49.464mg/L.

12.5 Interference

No significant assay interference by 100mg/L bilirubin, 2.5g/L haemoglobin, 200 mg/L acetaminophen and 600 mg/L aspirin has been demonstrated at the standard sample dilution (1/10).

	Bilirubin	Hb	Acetaminophen	Aspirin
Mean IgA (mg/L)	5.15	4.98	4.72	4.86
% interference	-9.08	1.92	1.87	-3.26

Bacterial interference has not been assessed (see section 10.7).

12.6 Antigen excess

No antigen excess was observed to a level of two times the top point of the assay; approximately 90mg/L.

13 BIBLIOGRAPHY

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