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# Human IgG and IgG subclass liquid reagent kits for use on the SPAPLUS<sup>®</sup>

# For in vitro diagnostic use

# Product code: NK004.S, NK006.S, NK007.S, LK008.S, LK009.S

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FDA (USA) Information Analyte name Immunoglobulins IgG subclasses Complexity Cat.: Moderate

 $\textbf{SPAPLUS}^{\otimes}$  is a registered trademark of The Binding Site Group Limited (Birmingham, UK) in certain countries.



Page no.

### INTENDED USE

These kits are intended for quantifying human IgG and IgG subclasses 1, 2, 3 and 4 in serum using the SPAPLUS analyser. Measurement of these immunoglobulins is an aid in the diagnosis of abnormal protein metabolism and the body's lack of ability to resist infectious agents in conjunction with other laboratory and clinical findings.

### 2 SUMMARY AND EXPLANATION

In normal adults, IgG constitutes approximately 75% of the total serum immunoglobulin. Within the IgG class, the usual order of concentration of the 4 subclasses is IgG1>IgG2>IgG3>IgG4, but the actual concentration of each may vary markedly between individuals. The four IgG subclasses show considerable differences in their properties, including ability to fix complement, to bind to macrophages and to pass through the placenta. Abnormal levels of one or more subclass may be associated with certain conditions, including anaphylaxis, autoimmune- and gut diseases as well as hypo- and hyper-gammaglobulinaemia (ref. 1). In particular, reduced production of IgG2 in children may be associated with recurrent infections (ref. 2). The subject has been reviewed (refs 3, 4).



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 standard curve stored within the instrument.

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

### 4 REAGENTS

- 4.1 <u>Total IgG antiserum.</u> 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) and 0.01% benzamidine.
- 4.2 <u>Human IgG1 and IgG2 antisera.</u> These are monospecific for the relevant subclass and are supplied in stabilised liquid form. Preservatives: 0.099%, sodium azide 0.1% FeCA 1mM EDTA 0.5% BSA and 0.01% herazmidine.
- sodium azide, 0.1% EACA, 1mM EDTA, 0.5% BSA and 0.01% benzamidine.
   Human IgG3 and IgG4 reagent. Consisting of polyclonal monospecific sheep antibody coated onto polystyrene latex. Preservatives: 0.05% ProClin™\*, 0.033%
- sodium azide, 0.1% EACA and 0.01% benzamidine.
   Calibrators and controls. These consist of pooled human serum and are supplied in stabilised liquid form. The concentration of IgG and IgG subclasses has been obtained by comparison with the DA470k international reference material (ref. 5, 6). Preservatives: 0.099% sodium azide, 0.1% EACA and 0.01% benzamidine.
- 4.5 <u>Supplementary reagent</u>. Contains 0.099% sodium azide as a preservative

\*ProClin<sup>™</sup> 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.

Controls from different kits may be used providing they have the same lot number; controls from different lots MUST NOT be used. All other components are NOT interchangeable. If large numbers of tests are performed, care should be taken to ensure that all components 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 antisera and latex reagents, calibrator, 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 antisera and latex reagents and Supplementary Reagent may be stored, uncapped, on the 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 8 days prior to assay, or for prolonged storage kept undiluted 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 100 tests Human IgG, IgG1 or IgG2 Antiserum SPAPLUS or 1 x 100 tests IgG3 or IgG4 Latex SPAPLUS (antiserum or latex reagent for IgG or the appropriate subclass).
- x 100 tests IgG, IgG1, IgG2, IgG3 or IgG4 Reaction Buffer SPAPLUS 8.1.2
- (supplementary reagent for IgG or the appropriate subclass). 1 × Human IgG, IgG1, IgG2, IgG3 or IgG4 SPAPLUS Calibrator 1-6 (6 × 1.0mL calibrator set for IgG or the appropriate subclass). 2 × 1.0mL Human IgG Subclass Low Control Serum (for use on the IgG and 8.1.3
- 8.1.4 IgG subclass assays)
- 2 x 1.0mL Human IgG Subclass Control Serum (for use on the IgG and IgG subclass assays) 8.1.5

#### 8.2 Materials required but not provided

- Equipment for collection and preparation of test samples e.g. sample tubes, 8.2.1
- Equipment for Concerns and a second s 8.2.2 8.2.3
- Sample Diluent (99: Dil 1). Binding Site Product Code: SN080.S. 8.2.4

#### 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

#### Test parameters 8.4.1

Assay parameters are entered into item numbers 4 – 8.

Terminame 4 rgG DATAINFORMATION Units g/L Decimals 3 ANALYSIS Type End ▼	CALIBRATION         Auto Fill           Type         Spline 1 ▼         Auto Fill           Standard         4         #           1         #         4         #           2         #         5         #           3         #         6         #
Main W.Length 1 340 ▼ Sub W.Length ▼	NORMAL RANGE
Method	MALE FEMALE LOW HIGH LOW HIGH
CORR. SLOPE INTER Y = 1 X + 0	Serum         []         []         []         []           Urine         []         []         []         []         []           Plasma         []         []         []         []         []         []           CSF         []         []         []         []         []         []         ]           Dialysis         []         []         []         []         []         ]         Other
Page: 1 Print Hard Copy	Next Page Save Return
Item Name 4 IgG ASPIRATION KIND · Single • Double VOLUME SAMPLE 6 REAGENT1 VOL 200 µL REAGENT2 VOL 60 Third mix • OFF • ON Block • Work Block	DATA PROCESS READ         ABSORBANCE LIMIT           START END SUB         START END 30         LOW -3           Biank correction         •         Reaction Check 0 ON • 0 FF           Blank correction         •         ON • 0 FF           ENDPOINT LIMIT         2         CHECK POINT LINEAR CHECK (%)         ON • 0 FF
o	Dilution Diluent ● 99: Dil 1 ∘100: Dil 2 Pre Dilution Rate Auto Rerun Dilution Rate High 40 ▼ Auto Rerun Dilution Rate Low ▼
MONITOR	PROZONE CHECK
0 LEVEL SPAN 1 SPAN 3	START END LIMIT (%) Min dOD 0           FIRST         []           SECOND         []           THIRD         []           []         ]
Page : 2 Print Hard Copy	Prev Page Next Page Save Return

\*Automatically calculated

Item Name 4 IgG <u>Auto Rerun SW</u> ● On ○ Off <u>Auto Rerun Range (Result)</u> ● On ○ Off ● On ○ Off Lower Higher Serum Cal 1 # Cal 6 # Urine Plasma		Auto Rerun Condition (Absorbance) Absorbance Range Lower • On • Off Higher • On • Off Prozone Range • On • Off				
Plasma CSF Dialysis Other						
Bottle Size (m	ıl)					
24 Items Reagent1	60 F	36 Items Reagent 1				
Reagent2 R1	21 F	Reagent2 R1				
Reagent2 R2	7 F	teagent2 R2				
Page : 3	Print		Prev Page	Save	Return	

Item Name 5 IgG1 DATA INFORMATION Units Decimals	g/L 3	CALIBRATION Type Spline 1 ▼ Auto Fill Standard
ANALYSIS Type	End V	2 # 5 # 3 # 6 #
Main W.Length 1 Sub W.Length	340 ▼ ▼	NORMAL RANGE MALE FEMALE
Method <u>CORR.</u> Y = 1 X +	INTER 0	Corv         Field         Corv         Field           Serum         []         []         []         []         []           Urine         []         []         []         []         []         []           Plasma         []         []         []         []         []         []         []         []           CSF         []
Page: 1 Print	Hard Copy	Next Page Save Return

Item Name 5 IgG1	DATA PROCESS READ ABSORBANCE LIMIT
ASPIRATION KIND ○ Single ● Double VOLUME	START END MAIN 53 54 LOW -3 SUB 30 31 HIGH 3
SAMPLE 3 REAGENT1 VOL 200 µL REAGENT2 VOL 60	FACTOR         Reaction Check           Blank correction         • ON • OFF           ENDPOINT LIMIT         2           LINEAR CHECK (%)         0           LINEAR CHECK (%)         0
Third mix ● OFF ○ ON Blank ● Water – Blank	Dilution Diluent ● 99: Dil 1 ○100: Dil 2 Pre Dilution Rate 10 ♥ Auto Rerun Dilution Rate High 40 ♥ Auto Rerun Dilution Rate Low ♥
MONITOR	PROZONE CHECK
0 LEVEL SPAN 1 SPAN 3	START END LIMIT (%) Min dOD 0           FIRST         []           SECOND         []           SECOND         []           THIRD         []           []         ]           LIMIT (%) Min dOD 0
Page: 2 Print Hard Copy	Prev Page Next Page Save Return
utomatically calculated	

Item Name 5 IgG1	
Auto Rerun SW ● On   ○ Off	Auto Rerun Condition (Absorbance)
Auto Rerun Range (Result) ● On ● Off ● On ● Off Lower Higher Serum Cal 1 # Cal 6 #	Absorbance Range         Lower         ● On         ○ Off           Higher         • On         ○ Off
Urine Plasma CSF Dialysis Other	Prozone Range o On ● Off
Bottle Size (ml)     24 ltems     36 ltems       24 ltems     36 ltems     Reagent 1       Reagent1     60     Reagent 1       Reagent2 R1     21     Reagent2 R1       Reagent2 R2     7     Reagent2 R2	
Page : 3 Print	Prev Page Save Return
Item Name 6 IgG2 DATA INFORMATION Units g/L	CALIBRATION Type Spline 1 ▼ Auto Fill
Decimals 3 ANALYSIS	Standard 1 # 4 # 2 # 5 #
Type End ▼	3 # 6 #
Main W.Length 1 380 ▼ Sub W.Length ▼	NORMAL RANGE MALE FEMALE
Method	LOW HIGH LOW HIGH
CORR. SLOPE INTER Y = 1 X + 0	Urine         []         []         []         []           Plasma         []         []         []         []         []           SF         []         []         []         []         []         []           Dialysis         []         []         []         []         []         []           Other         []         []         []         []         []         []

Page: 1 Print Hard Copy	Next Page Save Return
Item Name 6 IgG2	DATA PROCESS READ START END
KIND • Single • Double VOLUME	MAIN 53 54 LOW -3 SUB 30 31 HIGH 3
SAMPLE 18 REAGENT1 VOL 255 µL REAGENT2 VOL 25	FACTOR         Reaction Check           Blank correction         * ON         • OFF           ENDPOINT LIMIT         2         CHECK POINT           LINEAR CHECK (%) 0         LOW         -3           HIGH         3
Third mix ● OFF ○ ON Blank ● Water – Blank	DILUTION Diluent • 99: Dil 1 •100: Dil 2 Pre Dilution Rate 10 ¥ Auto Rerun Dilution Rate High 40 ¥ Auto Rerun Dilution Rate Low ¥
MONITOR	PROZONE CHECK
0 LEVEL SPAN 1 SPAN 3	START END LIMIT (%) Min dOD 0           FIRST         []           SECOND         []           []         ]           THIRD         []           []         ]
Page: 2 Print Hard Copy	Prev Page Next Page Save Return

*Automatically calculated	
Item Name 6 IgG2	
● On ○ Off	Auto Rerun Condition (Absorbance)
● On ○ Off ● On ○ Off Lower Higher	Absorbance Range Lower • On • Off Higher • On • Off
Serum Cal 1 # Cal 6 # Urine Plasma CSF Diducis	Prozone Range • On • Off
Other	
Bottle Size (ml) 24 Items 36 Items Reagent1 60 Proceed	
Reagent2 R1 26.5 Reagent2 R1 Reagent2 R2 3.5 Reagent2 R2	
Page : 3 Print	Prev Page Save Return
Item Name 7 IgG3 <u>DATA INFORMATION</u> Units g/L Decimals 3	CALIBRATION Type Logit 2 ▼ Auto Fill Standard
ANALYSIS Type End V	1 # 4 # 2 # 5 # 3 # 6 #
Main W.Length 1 600 ▼	NORMAL RANGE
Sub W.Length	MALE FEMALE LOW HIGH LOW HIGH
CORR.	Serum [ ] [ ] [ ] [ ] Urine [ ] [ ] [ ] [ ] [ ] Plazma [ ] [ ] [ ] [ ] [ ]
Y = 1   X + 0	Plasma     [     [     [     [     ]       CSF     [     ]     [     ]     [     ]       Dialysis     [     ]     [     ]     [     ]
Page: 1 Print Hard Copy	Next Page Save Return
Item Name 7 IgG3	DATA PROCESS READ ABSORBANCE LIMIT
ASPIRATION KIND • Single • Double	START END MAIN 49 50 LOW -3 SUB 35 36 HIGH 3
VOLUME SAMPLE 3 REAGENT1 VOL 170 III	FACTOR Reaction Check
REAGENT2 VOL 170 µL REAGENT2 VOL 120	Blank correction 1 ○ ON ● OFF ENDPOINT LIMIT 2 CHECK POINT LINEAR CHECK (%) 0 LOW -3 HICH 3
Third mix ● OFF ○ ON Blank ● Water – Blank	Diluttion Diluent • 99: Dil 1 • 100: Dil 2 Pre Dilution Rate = 10 ¥ Auto Rerun Dilution Rate High 40 ¥ Auto Rerun Dilution Rate Low
MONITOR	PROZONE CHECK
0 LEVEL SPAN 1 SPAN 3	START END LIMIT (%) Min dOD 0
Page 2 Print Hard Copy	SECOND [ ] [ ] [ ] ·Low • High THIRD [ ] [ ] ·Low • High Prev Page Next Page Save Potum
Item Name 7 IgG3	
Auto Rerun SW ● On ○ Off	Auto Rerun Condition (Absorbance)
Auto Rerun Range (Result) ● On ○ Off ● On ○ Off	Absorbance Range
Lower Higher Serum Cal 1 # Cal 6 # Urine Plasma CSF Dialysis	Higher • On • Off Prozone Range • On • Off
Bottle Size (ml)	
24 Items 36 Items Reagent1 60 Reagent1 Reagent2 R1 18 Reagent2 R1	
Reagent2 R2 13 Reagent2 R2	
Page: 3 Print	Prev Page Save Return
DATA INFORMATION	
Decimals 3	CALIBRATION Type Logit 2 ▼ Auto Fill Standard 1 # 4 #
Onits     g/L       Decimals     3       ANALYSIS       Type     End ▼	CALIBRATION Type         Auto Fill           Standard         4           1         #         4           2         #         5         #           3         #         6         #
grL       Decimals     3       ANALYSIS       Type       Main W.Length 1       Sub W.Length	CALIBRATION Type         Auto Fill           Standard         4           1         4           2         5           3         6           NORMAL RANGE         FEMALE           LOW         FORMAL PARTY
g/L       Decimals     3       ANALYSIS       Type     End ▼       Main WLength 1     600 ▼       Sub W.Length     ▼       Method	CALIBRATION Type         Auto Fill           Standard         Auto Fill           1 #         4 #           2 #         5 #           3 #         6 #           NORMAL RANGE MALE FEMALE LOW HIGH LOW HIGH Serum [] [] [] [] []           Serum [] [] [] [] []
g/L       Decimals     3       ANALYSIS     3       Type     End ▼       Main W.Length 1     600 ▼       Sub W.Length     ♥       Method     ▼       CORR.     SLOPE       NTER	CALIBRATION Type         Logit 2         Auto Fill           Standard         1         #         4         #           1         #         4         #         2         #         5         #           3         #         6         #         6         #         MALE         FEMALE           LOW         HIGH         LOW         HIGH         Seturn         1         1         1           Vrine         1         [         1         [         1         1         1           Plasma         1         [         1         <
grL       Decimals     3       ANALYSIS     Type       Type     End ▼       Main W.Length 1     600 ▼       Sub W.Length     ♥       Method     ♥       CORR.     SLOPE       Y =     1     X +	CALIBRATION Type Logit 2           Auto Fill         Auto Fill           1 #         4 #           2 #         5 #           3 #         6 #           MORMAL RANGE LOW HIGH LOW HIGH         FEMALE           Drine         1 [ ] [ ] [ ] [ ]           Urine         1 [ ] [ ] [ ]           Plasma         [ ] [ ] [ ] [ ]           Dialysis         [ ] [ ] [ ] [ ]
grL         grL           Decimals         3           ANALYSIS         Type           Type         End ▼           Main W.Length 1         600 ▼           Sub W.Length         ♥           Method         ♥           Y =         1           X +         0           Page :         1           Print         Hard Copy	CALIBRATION Type         Auto Fill           1         #         4           2         #         5           3         #         6           MALE         FEMALE           LOW         HIGH           LOW         HIGH           Urine         [           1         [           Plasma         [           1         [           Dialysis         [           Next Page         Save
grL         grL           Decimals         3           ANALYSIS         Type           Type         End ▼           Main W.Length 1         600 ▼           SubW.Length         ♥           Method         ▼           CORR.         NTER           Y =         1         X +         0           Page :         1         Print         Hard Copy           Item Name         8         IgG4	CALIBRATION Type         Auto Fill           Type         Logit 2         Auto Fill           1         #         4         #           2         #         5         #           3         #         6         #           NORMAL RANGE         MALE         FEMALE           LOW         HIGH         LOW         HIGH           Serum         [         [         [         ]           Urine         [         [         [         ]         [           Plasma         [         [         [         ]         [         ]           CSF         [         [         [         [         ]         ]         ]           Other         [         [         [         [         ]         ]         ]           Next Page         Save         Return         MESORBANCE LIMIT         STAPT         STAPT         END
g/L         g/L           Decimals         3           ANALYSIS         Type           Type         End ▼           Main W.Length 1         600 ▼           Sub W.Length         ♥           Method         ▼           CORR.         Y = 1           Y = 1         X + 0           Page : 1         Print           Hard Copy           Item Name 8         IgG4           ASPIRATION         ► Double           VOLUTE         ● Double	CALIBRATION Type Logit 2           Auto Fill           Standard           1 #         4 #           2 #         5 #           3 #         6 #           MORMAL RANGE LOW HIGH LOW HIGH Serum [] [] [] [] []           Uorne [] [] [] [] []           Urine [] [] [] [] []           Urine [] [] [] [] []           Users           Jamas [] [] [] [] []           Urine [] [] [] [] []           Users           Users           Users           [] [] [] [] [] []           Users           [] [] [] [] [] []           Other           [] [] [] [] [] []           Next Page           Save           READ           ABSORBANCE LIMIT           START <end< td="">           MAIN         53           Start<end< td="">           MAIN         53</end<></end<>
gL         gL           Decimals         3           ANALYSIS         Type           Type         End ▼           Main W.Length 1         600 ▼           Sub W.Length         ▼           Method         ▼           CORR.         ×           Y =         1           Y =         1           Y =         1           Y =         1           Y =         0           Page :         1           Print         Hard Copy           Item Name         8           VOLUME         SAMPLE           SAMPLE         3           REAGENTI VOL         160	CALIBRATION Type Logid 2           Auto Fill         Auto Fill           1 #         4 #           2 #         5 #           3 #         6 #           MORMAL RANGE MALE         FEMALE           LOW         HIGH           Urine         1 [ ] [ ] [ ] [ ]           Urine         1 [ ] [ ] [ ] [ ]           Urine         1 [ ] [ ] [ ] [ ]           Dialysis         1 [ ] [ ] [ ] [ ]           Next Page         Save           READ         ABSORBANCE LIMIT           START         END           MAIN         53           35         36           HIGH #         COVF
g/L         g/L           Decimals         3           ANALYSIS Type         End ▼           Main W.Length         600 ▼           Sub W.Length         ♥           Method         ▼           CORR. Y = 1         X + 0           Page : 1         Print         Hard Copy           Item Name 8         IgG4           ASPIRATION KIND         ○ Single         • Double           SAMPLE         3           REAGENT1 VOL         160         µL           REAGENT1 VOL         110	CALIBRATION Type Logit 2           Auto Fill         Auto Fill           1 #         4 #           2 #         5 #           3 #         6 #           MORMAL RANGE LOW HIGH LOW HIGH Urine [] [] [] []         FEMALE           LOW HIGH LOW HIGH Urine [] [] [] []         1           Plasma [] [] [] [] []         1           Dialysis [] [] [] [] []         1           Next Page         Save           Auto Fill         1           Next Page         Save           Atta PROCESS         READ           START         END           MAIN         53         54           LOW - 3         36           SUB         35           Text Process         Reaction Check           Blank correction         1           CHCR POINT           UNINEAR CHECK (%) 0         LUCW
Bank         Print         Hard Copy           ANALYSIS         3           Type         End ▼           Main W.Length 1         600 ▼           Sub W.Length 1         600 ▼           Method         ▼           CORR.         1           Y =         1           Y =         1           Y =         1           Y =         1           Y =         1           Y =         1           Y =         1           Y =         1           Y =         1           Y =         0           Page :         1           Print         Hard Copy           Item Name 8         IgG4           SAMPLE         3           REAGENT1 VOL         160           WL         110           Third mix<	CALIBRATION Type         Logit 2         Auto Fill           1         #         4         #           2         #         5         #           3         #         6         #           MALE         FEMALE           LOW         HIGH         LOW         HIGH           Serum         1         1         1         1           Urine         1         1         1         1         1           Plasma         1         1         1         1         1         1           Other         I         1         1         1         1         1         1           Other         I         1         1         1         1         1         1         1         1           NAIN         53         54         LOW -3         SUB         35         36         HIGH         *         *         ON< • OFF
g/L         g/L           Decimals         3           ANALYSIS Type         End ▼           Main W.Length         600 ▼           Sub W.Length         ♥           Method         ▼           CORR. Y =         1           Page :         1           Page :         1           Print         Hard Copy           Item Name         8           VOLUME         SAMPLE           SAMPLE         0           REAGENT1 VOL         160 µL           REAGENT2 VOL         110           Third mix         OFF ○           Blank         • Water - Blank	CALIBRATION Type         Logit 2         Auto Fill           1         #         4         #           2         #         5         #           3         #         6         #           MALE         FEMALE           LOW         HIGH         LOW         HIGH           Serum         [         [         [         ]           Urine         [         [         [         ]           Plasma         [         [         [         ]           Other         [         [         [         ]         [         ]           Other         [         [         [         [         ]         [         ]         [         ]         [         ]         [         ]         [         ]
g/L           Decimals         3           ANALYSIS         Type           Type         End ▼           Main W.Length 1         600 ▼           Sub W.Length         ▼           Method         ▼           CORR.         NUTER           Y =         1         X + 0           Page :         1         Print           Hard Copy         Item Name 8         IgG4           ASPIRATION         NULUME         SAMPLE           SREAGENT1 VOL         160         µL           REAGENT2 VOL         110         Third mix           Third mix         • OFF         • ON           Blank         • Water – Blank	CALIBRATION Type         Logit 2         Auto Fill           1 # # 4 # 2 # 5 # 3 # 6 #         4 # 5 # 3 # 6 #           NORMAL RANGE MALE         FEMALE FEMALE         FEMALE           LOW         HIGH         LOW         HIGH           Variable         1 [ ] [ ] [ ] [ ]         1 [ ]           Urine         1 [ ] [ ] [ ] [ ]         1 [ ]           Plasma         1 [ ] [ ] [ ] [ ]         1 [ ]           Dialysis         1 [ ] [ ] [ ] [ ]         1 [ ]           Next Page         Save         Return           Data         Start         END           START         END         ABSORBANCE LIMIT           Start         END         ABSORBANCE LIMIT           MAIN         53 54         LOW -3           SUB         35 36         HIGH #           FACTOR         1         ○N • OFF           ENDPOINT LIMIT         2         OFF           LINEAR CHECK (%)0         LOW -3         HIGH 3           Diluent         • 99: Dil 1 °100: Dil 2         Pre Dilution Rate           Pre Dilution Rate         10 <
Ones         g/L           Decimals         3           ANALYSIS         Type           Type         End ▼           Main W.Length 1         600 ▼           Sub W.Length         ♥           Method         ♥           CORR.         NTER           Y =         1         X + 0           Page :         1         Print         Hard Copy           Item Name 8         IgG4           ASPIRATION         NULUME           SAMPLE         3           REAGENT1 VOL         160 µL           REAGENT2 VOL         110           Third mix         • OFF         • ON           Blank         • Water - Blank	Auto Fill           Type Logid 2           Auto Fill           1         #         4         #           2         #         5         #           3         #         6         #           MALE         FEMALE           OW MIGH         COW MIGH           Unit of the town High           Unit of the town High           Colspan="2">Colspan="2">Colspan="2">Colspan="2">Colspan="2">Colspan="2">Colspan="2">Colspan="2">Colspan="2">Colspan="2">Colspan="2">Colspan="2">Colspan="2">Colspan="2">Colspan="2">Colspan="2">Colspan="2"           Unit of the town High         Colspan="2">Colspan="2">Colspan="2">Colspan="2"           Data process           READ         Colspan="2"           Start END         Colspan="2"           MAIN 53         Save         Return           MAIN 53         Save         Return           Dia 1         Colspan="2"           Bank correction         1            Save            Bil 1         <

Item Name 8 IgG4				
Auto Rerun SW         ○ Off           ●On         ○ Off           Auto Rerun Range (Result)         ● On         ○ Off	Auto Rerun Condition (Absorbance) Absorbance Range Lower • On • Off			
Lower Higher	Higher • On • Off			
Gerunn Carr # Car6 # Urine Plasma CSF Dialysis Other	Prozone Range • On • Off			
Bottle Size (ml)           24 Items         36 Items           Reagent1         60         Reagent1           Reagent2 R1         17         Reagent2 R1           Reagent2 R2         12         Reagent2 R2				
Basa : 2 Drint	Drev: De se Ceure Deture			

N.B. The calibrator (Standard #), prozone check (#) and Min dOD values are found in the Quality Control Certificate (SIN128.QC.1-5). Calibrator values on Page 1 should be entered in ascending order, i.e. the lowest value first. The prozone check (#), Min dOD values (#) and high absorbance limit (#) should be entered on Page 2. 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 pages 3 and 4 to ensure correct value entry.

### 8.5 Measuring range

Specificity	Approx. measu	ring range (g/L)	Approx. sensitivity (g/L)
Specificity	1/10	1/40	1/1
lgG	1.65 – 35.0	6.6-140.0	0.165
lgG1	1.50 - 36.0	6.0-144.0	0.15
lgG2	0.20 - 7.0	0.8-28.0	0.02
lgG3	0.055 -1.00	0.22-4.0	0.0055
lgG4	0.03 - 0.85	0.12-3.4	0.003

#### 8.6 Interpretation of results

The results of these assays should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings including previous IgG subclass results if available.

Some samples may exhibit non linearity when assayed at different dilutions. In order to appropriately quantify such samples it is advised that the dilution protocol described in section 8.5 is followed and the first plausible result is reported.

For the IgG4 assay the SPAPLUS has the facility to monitor reaction kinetics in order to identify samples that are in antigen excess. IgG4 samples that demonstrate unusual reaction kinetics will generate a P flag. Samples that have generated a P flag must be repeated at a higher dilution as described in section 8.5 (note: the SPAPLUS will carry out auto-dilutions up to 1/40). If upon repeat the sample gives a result that is considered implausible, the samples should be repeated at the initial dilution, reviewed and reported.

Refer to the SPAPLUS Reference Guide (FIN012) supplied with the analyser for further details of flag interpretation.

**Important Note:** No automated check will identify all cases of antigen excess and a very small percentage of samples in antigen excess may not prompt the "P" flag. It is recommended that the following statement accompany all IgG4 results.

Undetected antigen excess is a rare event but cannot be excluded. If the IgG4 results do not agree with other clinical or laboratory findings, or if the sample is from a patient that has previously demonstrated antigen excess, the result must be checked by retesting at a higher dilution. Results should always be interpreted in conjunction with other laboratory tests and clinical evidence; any anomalies should be discussed with the testing laboratory.

### 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 (SIN128.QC.1/.2/.3/.4/.5). Sample results obtained should only be accepted if the control results are within ±15% of the concentration(s) stated.
   9.4 A value for summated IgG is obtained by adding the values of the four
- subclasses together and should be within  $\pm 20\%$  of the total IgG (see 10.4).

### 10 LIMITATIONS

- 10.1 These kits are not suitable for the measurement of samples containing rheumatoid factor, paraproteins, other circulating immune complexes (CICs) or for lipaemic or haemolysed samples due to the unpredictable degree of nonspecific scatter these sample types may generate. Unexpected results should be confirmed using an alternative assay method.
- 10.2 The results obtained from measuring IgG subclasses should not be used in assessing atopy in allergic patients.
- 10.3 Diagnosis cannot be made and treatment must not be initiated on the basis of IgG subclass measurements alone. Clinical history and other laboratory findings must also be taken into account.
- 10.4 If the IgG summation (see section 9.4) is outside the acceptable range samples should be repeated with the sample manually diluted 1/2 to exclude antigen excess problems. Occasionally some samples will give poor summation to total IgG due to unusual allotype balance. Prozone warning protection ('P' flags) may be seen against IgG4 results which should be interpreted in conjunction with the IgG subclass summation results.

### 11 EXPECTED VALUES

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

#### Adult normal ranges 11.1

These ranges were obtained by measuring the subclass content of sera provided by the Birmingham Blood Transfusion Service taken from healthy adult donors and generated using Binding Site's BN™II subclass kits (NK006/7.TB, LK008/9.TB). The IgG normal range was generated on the SPAPLUS IgG assay.

Specificity	Number	Mean	Median	95 Percentile
	(n)	(g/L)	(g/L)	Range (g/L)
Total IgG	120	10.926	10.807	6.103 - 16.16
IgG1	30	6.33	6.085	3.824 - 9.286
lgG2	30	4.528	4.541	2.418 - 7.003
IgG3	30	0.7907	0.7064	0.2182 - 1.7606
lgG4	30	0.28	0.2153	0.0392 - 0.864

BN<sup>™</sup> is a trademark of Siemens Healthcare Diagnostics Inc

#### Paediatric ranges 11.2

These ranges were obtained by measuring the total IgG and subclass content of paediatric serum samples from a Birmingham Hospital using Binding Site Radial Immunodiffusion products. All concentrations are in g/L.

0-2 years	N	Mean	95%ile range
Total IgG	39	6.640	3.270 - 12.700
laG1	39	5.248	1.940 - 8.420
laG2	39	0.838	0.225 - 3.000
laG3	39	0.445	0 186 - 0 853
InG4	39	0.129	0.005 - 0.784
2-4 voare	00	0.120	0.000 0.104
Z-4 years	20	7 770	4 690 42 500
Total IgG	30	7.770	4.080 - 12.500
IgG1	36	5.495	3.150 - 9.450
IgG2	36	1.146	0.360 - 2.250
IgG3	36	0.396	0.173 – 0.676
lgG4	36	0.171	0.010 – 0.537
4-6 years			
Total IgG	49	9.280	5.320 – 13.400
lgG1	49	6.149	3.060 - 9.450
lgG2	49	1.496	0.605 - 3.450
lqG3	49	0.531	0.099 – 1.221
lgG4	49	0.329	0.018 - 1.125
6-8 vears			
Total IgG	43	8.860	4.540 - 13.600
laG1	43	5.758	2.880 - 9.180
laG2	43	1 754	0.440 - 3.750
laG3	43	0.437	0.155 - 0.853
lgG3	43	0.331	0.001 = 0.000
8-10 years		0.001	0.004 0.002
Totol laC	22	0.700	E 680 13 600
	32	9.700	5.000 - 15.000
lgG1	32	0.343	4.320 - 10.200
IgG2	32	2.120	0.720 - 4.300
IgG3	32	0.521	0.127 -0.853
1gG4	32	0.349	0.019 - 0.932
10-12 years			
Total IgG	46	10.200	5.680 - 14.900
lgG1	46	6.421	4.230 – 10.600
lgG2	46	1.980	0.760 – 3.550
lgG3	46	0.620	0.173 – 1.730
lgG4	46	0.323	0.016 – 1.150
12-14 years			
Total IgG	54	10.500	6.640 - 14.900
lgG1	54	6.581	3.420 - 11.500
laG2	54	2.486	1.000 - 4.550
lgG3	54	0.583	0.283 - 1.250
laG4	54	0.409	0.037 - 1.360
14-18 years			
Total InG	48	10 100	5 500 - 14 400
InG1	48	6.057	3 150 - 8 550
laG2	48	2 614	0.640 - 4.950
laG3	48	0.708	0.230 -1.960
1900 IaC4	49	0.700	0.110 1.570
1904	40	0.021	0.110 - 1.370

### 12 PERFORMANCE CHARACTERISTICS

#### 12.1 Precision

The precision studies were performed following CLSI Evaluation of Precision Performance of Clinical Chemistry Approved Guideline (CLSI Document EP5-A). The studies were performed over 21 working days, with two runs per day. One user assessed three different samples per assay, using three different reagent lots on three analysers.

	IgG precision summary								
	Mean Within-run			Between-run Betwe		Betwee	en-day	Total	
	(g/L)	SD	CV %	SD	CV %	SD	CV %	SD	CV %
Serum 1	2.43	0.05	2.1	0.03	1.4	0.18	7.6	0.19	8.0
Serum 2	6.00	0.09	1.5	0.07	1.1	0.29	4.8	0.31	5.1
Serum 3	33.00	0.81	2.5	0.00	0	0.61	1.91	1.02	3.1

	IgG1 precision summary								
Mean		Within-run		Between-run		Between-day		Total	
	(g/L)	SD	CV %	SD	CV %	SD	CV %	SD	CV %
Serum 1	2.55	0.05	2.1	0.08	3.2	0.22	8.7	0.24	9.5
Serum 2	3.83	0.10	2.7	0.09	2.3	0.30	7.8	0.33	8.5
Serum 3	31.36	0.70	2.2	0.46	1.5	1.65	5.3	1.85	5.9

	IgG2 precision summary								
Mean		Within-run		Between-run		Between-day		Total	
	(g/L)	SD	CV %	SD	CV %	SD	CV %	SD	CV %
Serum 1	0.245	0.01	3.6	0.02	7.3	0.01	4.9	0.02	9.6
Serum 2	2.349	0.06	2.4	0.07	3.0	0.07	3.1	0.12	4.9
Serum 3	4.145	0.03	0.8	0.15	3.5	0.07	1.6	0.16	3.9

IgG3 precision summary									
Mean		Within-run		Between-run		Between-day		Total	
	(g/L)	SD	CV %	SD	CV %	SD	CV %	SD	CV %
Serum 1	0.081	0.00	2.8	0.00	2.8	0.01	8.3	0.01	9.2
Serum 2	0.227	0.01	3.3	0.00	0.0	0.01	3.8	0.01	5.1
Serum 3	0.844	0.03	3.3	0.00	0.0	0.03	3.3	0.04	4.6

	IgG4 precision summary								
	Mean	Within	i-run	Betwee	en-run	Betwee	en-day	To	tal
	(g/L)	SD	CV %	SD	CV %	SD	CV %	SD	CV %
Serum 1	0.049	0.00	5.3	0.00	0.0	0.00	8.3	0.00	9.8
Serum 2	0.066	0.00	4.0	0.00	2.3	0.01	7.8	0.01	9.0
Serum 3	0.769	0.01	1.9	0.01	0.9	0.04	5.1	0.04	5.5

#### 12.2 Comparison

A correlation study was performed on 60 serum samples (30 normal, 30 samples with elevated levels of IgG/IgG1-4) using SPAPLUS kits and the Binding Site's IgG subclass  $BN^{TM}II$  kits for IgG1-4 and the Siemens BNII IgG kit. The study demonstrated a good agreement giving the following linear regression plot and correlation coefficient:

**IgG y = 1.0779x – 0.9028g/L** (y = IgG - SPAPLUS) correlation coefficient  $R^2$  = 0.9787

**IgG1 y = 1.0437x - 0.6121g/L**(y = IgG1 - SPAPLUS) correlation coefficient  $R^2 = 0.9850$ 

**IgG2 y = 1.059x - 0.1619g/L** (y = IgG2 - SPAPLUS) correlation coefficient  $R^2 = 0.9874$ 

IgG3 y = 1.0062x - 0.0082g/L(y = IgG3 - SPAPLUS) correlation coefficient R<sup>2</sup> = 0.9831

IgG4 y = 0.9897x - 0.0052g/L(y = IgG4 - SPAPLUS) correlation coefficient R<sup>2</sup> = 0.9959

12.3 Analytical sensitivity Analytical sensitivity was determined by assaying ten replicates of two samples with concentrations equivalent to 140% and 200% of the curve bottom point. Two distinct sets of data were generated with the following CVs:

Specificity	CV% (140%)	CV% (200%)
lgG	0.92	1.3
lgG1	1.43	1.4
lgG2	1.92	1.17
lgG3	3.33	4.69
lgG4	3.64	3.9

#### 12.4 Linearity

The linearity of these assays has been confirmed using serially diluted serum samples, giving regression equations of:

 $lgG y=1.0082x - 0.0784 (g/L), R^2 = 0.997$ 

(y = measured IgG conc., x = theoretical concentration) over the range of 0.26-30.39g/L.

IgG1 y=0.9945x – 0.2107 (g/L),  $R^2$  =0.9992 (y = measured lgG1 conc., x = theoretical concentration) over the range of 0.23-30.71g/L.

 $IgG2 y=1.0091x - 0.0046 (g/L), R^2 = 0.9996$ (y = measured IgG2 conc., x = theoretical concentration) over the range of 0.031- 4.47g/L.

IgG3 y=1.0076x - 0.001 (g/L), R<sup>2</sup> =0.9998

(y = measured IgG3 conc., x = theoretical concentration) over the range of 0.008- 0.79g/L.  $IgG4 y=0.9993x + 0.0005 (g/L), R^2 = 1.0$ 

(y = measured IgG4 conc., x = theoretical concentration) over the range of 0.005- 0.70g/L.

#### 12.5 Interference

No significant assay interference by 200mg/L bilirubin or 5g/L haemoglobin (Hb) has been demonstrated using an IgG and IgG subclass control serum. All testing was performed at the minimum sample dilution (1/1).

No significant assay interference by 4395 formazine turbidity units (FTU) of chyle has been demonstrated using a low level control serum for IgG, IgG1, IgG3 and IgG4 at the minimum sample dilution (1/1). Slight assay interference (15%) has been seen with chyle (2210 FTU) using a low level IgG2 control serum. No significant interference (<5%) was noted with chyle at 1670 FTU.

Crossificity	Concentration	Dilinuhin	ЦЬ	Chyle (FTU)			
Specificity	Concentration	Billubili	UD	4395	2210	1670	
IgG	Mean (g/L) % interference	3.783 <b>-1.74</b>	3.856 <b>+0.16</b>	0.93 <b>-2.52</b>			
lgG1	Mean (g/L) % interference	1.848 <b>-0.34</b>	1.855 <b>+0.05</b>	0.48 - <b>0.62</b>			
lgG2	Mean (g/L) % interference	0.838 <b>-0.24</b>	0.802 - <b>4.5</b>		0.226 -15.13	0.288 <b>-4.95</b>	
lgG3	Mean (g/L) % interference	0.066 <b>-4.30</b>	0.068 -1.45	0.05 <b>+1.42</b>			
lgG4	Mean (g/L) % interference	0.051 <b>-1.92</b>	0.052 0.00	0.03 <b>0.00</b>			

#### 12.6 Antigen excess

These assays were tested using polyclonal serum samples to the levels shown in the table below. No antigen excess was observed at these concentrations

Specificity	Concentration (g/L)
lgG	100.81
lgG1	53.91
lgG2	35.97
lgG3	2.90
lgG4	5.00

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