Freelite® Human Kappa Free kit for use on the SPAPLUS®

For *in vitro* diagnostic use

Product code: LK016.L.S

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Warning: The result of kappa free light chains in a given specimen determined with assays with different manufacturers can vary due to differences in assay methods and reagent specificity. The results reported by the laboratory to the physician must include the identity of the kappa free light chain assay used. Values obtained with different assay methods cannot be used interchangeably. If, in the course of serially monitoring a patient, the assay method used for determining kappa free light chain levels is changed, additional sequential testing should be carried out. Prior to changing assays, the laboratory **MUST** confirm baseline values for patients being serially monitored.

1 INTENDED USE

This kit is intended for the quantitation of kappa free light chains in serum, lithium heparin or EDTA plasma, urine and CSF on Binding Site SPAPLUS. Measurement of free light chains in serum aids in the diagnosis and monitoring of multiple myeloma, lymphocytic neoplasms, Waldenström's macroglobulinaemia, AL amyloidosis, light chain deposition disease and connective tissue diseases such as systemic lupus erythematosus (SLE) in conjunction with other laboratory and clinical findings. Measurement of free light chains in urine aids in the diagnosis and monitoring of multiple myeloma, lymphocytic neoplasms, Waldenström's macroglobulinaemia, AL amyloidosis and light chain deposition disease in conjunction with other laboratory and clinical findings.

2 SUMMARY AND EXPLANATION

Immunoglobulin molecules consist of two identical heavy chains (α, δ, ε, γ or μ) which define the immunoglobulin class and two identical light chains (κ or λ). Each light chain is covalently linked to a heavy chain and the two heavy chains are linked covalently at the hinge region. In healthy individuals, the majority of light chain in serum exists in this form, bound to heavy chain. However, low levels of free light chain (FLC) are found in serum of normal individuals due to the over-production and secretion of FLC by the plasma cells. Whilst the molecular weight of both light chains is ≈22.5kD, in serum κ free light chain (κ-FLC) exists predominantly as monomer and λ free light chain (λ-FLC) as a covalently linked dimer with a molecular weight of ≈45kD. This will lead to a differential glomerular filtration rate for κ-FLC and λ-FLC and may explain the observed ratio of κ-FLC to λ-FLC of 0.625 in serum compared to the ratio of bound κ to λ of 2.0.
FLC levels in urine are low. In a healthy kidney the tubular cells selectively reabsorb all FLC so their presence in urine is probably due to secretion into the urinary tract.
Elevated serum levels of monoclonal FLC are associated with malignant plasma cell proliferation (e.g. multiple myeloma), AL amyloidosis and light chain deposition disease. Raised serum levels of polyclonal FLC may be associated with autoimmune diseases such as SLE. The appearance of higher levels of FLC in urine may be indicative of kidney disease or malignant lymphoproliferative disease such as multiple myeloma. The monoclonal urinary FLC associated with lymphoid malignancy is called a Bence Jones protein [1-13]. CSF measurement may have a utility in identification of intrathecal synthesis of immunoglobulins [15-16].

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.

The sensitivity of turbidimetric assays can be increased by the use of particle enhancement [6]. This entails linking the antibody to a suitably sized particle that increases the relative light-scattering signal of the antigen-antibody reaction.

4 REAGENTS

4.1 **Latex reagent:** Consisting of polyclonal monospecific sheep antibody coated onto polystyrene latex. Preservative: 0.05% ProClin™, 0.1% E-amino-n-caproic acid (EACA) and 0.01% benzamidine.

4.2 **Calibrator and controls:** These consist of human sera that contain kappa free light chain. They are supplied in a stabilised liquid form and contain 0.099% sodium azide, 0.1% EACA and 0.01% benzamidine as preservatives.

4.3 **Supplementary reagent:** 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 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 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 ProClin 300 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 latex reagent, calibrator, and control 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 Kappa Free SPAPLUS Reagent* and *Human Kappa Free SPAPLUS Supplementary Reagent* may be stored, uncapped, on the analyser for up to 40 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

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. Serum, plasma and urine samples may be stored at 2-8°C for up to 21 days, but for prolonged storage they should be kept frozen at -20°C for up to 6 months[14]. CSF samples may be stored at 2-8°C for up to 7 days, for prolonged storage they should be kept frozen at -20°C. CSF samples must be centrifuged prior to testing. Repeated freeze/thaw cycles should be avoided. Microbially contaminated samples, samples containing particulate matter and lipaemic or haemolysed samples should not be used.

It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific sample stability criteria for its laboratory.[17]

8 METHODOLOGY

Note: to enable full interpretation of results, free kappa/lambda ratios should be determined; samples must therefore also be assayed using Binding Site's **Freelite** Lambda Free kit (LK018.L.S).

8.1 Materials provided

8.1.1 1 x 100 tests *Human Kappa Free SPAPLUS Reagent*
8.1.2 1 x 100 tests *Human Kappa Free SPAPLUS Supplementary Reagent*
8.1.3 1 x *Human Kappa Free SPAPLUS Calibrator Set* (6 x 1.0mL)
8.1.4 1 x 1.5mL *Human Kappa Free SPAPLUS Control*
8.1.5 1 x 1.5mL *Human Kappa Free SPAPLUS High Control*

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 Pack Code: SN080.S
8.2.5 SPAPLUS Weekly Wash Protocol and Bottles: IK050.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 1.

Item Name 1 KFree
DATA INFORMATION
Units mg/L
Decimals 2

ANALYSIS
Type End ▼

Main W.Length 1 600 ▼
Sub W.Length ▼

Method

CORR.
SLOPE INTER
Y = 1 X + 0

CALIBRATION
Type Logit 2 ▼ 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 1 KFree

DATA PROCESS

READ | **ABSORBANCE LIMIT**

	START	END		
MAIN	53	54	LOW	-3
SUB	35	36	HIGH	3

ASPIRATION
KIND ○ Single ● Double
VOLUME
SAMPLE 15
REAGENT1 VOL 145 µL
REAGENT2 VOL 80

FACTOR | **Reaction Check**
Blank correction 1 ○ 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 100 ▼
Auto Rerun Dilution Rate Low ▼

MONITOR

0 LEVEL SPAN 1
SPAN 3

PROZONE CHECK

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

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Item Name 1 KFree

Auto Rerun SW
● On ○ Off

Auto Rerun Range (Result)

	● On ○ Off Lower	● On ○ Off Higher
Serum	Cal 1 #	Cal 6 #
Urine		
Plasma		
CSF		
Dialysis		
Other		

Auto Rerun Condition (Absorbance)

Absorbance Range

Lower	● On	○ Off
Higher	● On	○ Off
Prozone Range	● On	○ Off

Bottle Size (ml)

24 Items		36 Items
Reagent1	60	Reagent1
Reagent2 R1	15.5	Reagent2 R1
Reagent2 R2	9	Reagent2 R2

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Item Name 1 KFree

Out-of-Range Table

	NEAT		Pre Dilution (*10)		Auto-rerun Dilution (*100)	
	ABOVE Cal#1	BELOW Cal#2-Cal#6	ABOVE Cal#1	BELOW Cal#2-Cal#6	ABOVE Cal#1	BELOW Cal#2-Cal#6
Serum	*	*	*	*	*	*
Urine	*	*	*	*	*	*
Plasma	*	*	*	*	*	*
CSF	#	*	*	*	*	*
Dialysis	*	*	*	*	*	*
Other	*	*	*	*	*	*

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The calibrator (Standard #), prozone check (#) and Min dOD values (#) are found in the Quality Control Certificate (SIN324.QC). Calibrator values on **Page 1** should be entered in ascending order, i.e. the lowest value first. The prozone check and Min dOD values (#) 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 Auto Fill 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.
IMPORTANT: the analyser will only update the calibrator values providing the Auto Fill button is pressed after typing the value for calibrator 6 on **Page: 1.** The assay utilises an extrapolated calibration curve for CSF samples therefore the Out-of-Range (**Page 4**) tables must be manually updated using values supplied in the Quality Control Certificate SIN324.QC) for the CSF matrix only.

8.4.2 Calibration parameters

This assay utilises an extrapolated calibration curve. To ensure accuracy, 3 blank and 3 standard-1 replicates must be programmed for each calibration curve

Calibration Parameter

CH ODR	ITEM#	Name	BLK ODR	Re CAL	BLK	STD-1	STD-2
1	1	KFree	□	□	B1 - **3**	S1 - 3	S2 - 1
□	Graph	000					

Order All Update Exit

8.4.3 The SPAPLUS can be set up to automatically calculate the serum Free Kappa / Free Lambda ratio. Refer to the SPAPLUS Reference Guide (FIN012) for details of installing calculation parameters.

8.5 Special wash procedures for urine and CSF testing

8.5.1 **Special wash procedure for urine**: Sample carryover can occur with free light chains in urine samples. To prevent this;

- **Freelite** urine tests must be batched together and run independently of other tests.
- Auto-dilutions must be turned off and repeats requested manually.
- Wash all cuvettes which have been used for urine dilutions or reactions using the weekly wash protocol. This needs to be done each time the cuvettes are used; this will allow a maximum of 30 tests in a single run (15 samples if kappa and lambda free light chains are both being tested).
- This wash protocol must be carried out after every run of urine samples and before any further testing is carried out.

8.5.2 **Special wash procedure for CSF**: CSF samples can be affected by sample carry-over from serum and urine samples. To prevent this;

- **Freelite** CSF tests must be batched together and run independently of other tests.
- Prior to running CSF samples wash all cuvettes using the weekly wash protocol.
- There is no limit to the number of CSF samples that can be run after the weekly wash protocol, providing samples are run neat.
- If any samples exceed the measuring range at neat and require running at 1/10, a manual off-line dilution must be made. It is not necessary to repeat the wash before carrying out further testing with serum or urine samples.

This wash protocol comprises the SPAPLUS weekly wash using sodium hypochlorite.
Parameters for the hypochlorite wash step are provided with the SPAPLUS Weekly Wash Protocol and Bottles (IK050.S)

8.6 Measuring range

Serum, plasma and urine samples must be assayed first at the standard 1/10 sample dilution, giving an approximate measuring range of 4.0-180mg/L. This enables a sensitivity of 0.4mg/L on neat serum and plasma samples. The upper limit of the measuring range using a sample dilution of 1/100 is 1800mg/L. For samples measuring over this limit the dilution series in the following table should be used. CSF samples must be assayed neat (1/1), and CSF must be selected as the "Specimen" type in the SPAPLUS order screen. This gives an approximate measuring range of 0.1-18.0mg/L, and a sensitivity of 0.1mg/L. Samples reporting above the measuring range at 1/1 need to be manually diluted at 1/10. Refer to the SPAPLUS Reference Guide (FIN012) for details of selecting sample dilutions.

Overall dilution	Analyser dilution	Manual pre-dilution	Approximate range (mg/L)
1/1	1/1	-	0.1 – 18.0 (CSF only)
1/10	1/1	1/10**	1 – 180 (CSF only)
1/1	1/1	-	0.4 – 18.0
1/10	1/10	-	4.0 – 180
1/100	1/100	-	40 – 1800
1/1000	1/10	1/100*	400 – 18000
1/10000	1/100	1/100*	4000 - 180000

* Make a manual dilution of 1/100 by taking 100µL of sample and add 900µL system sample diluent to achieve an initial 1/10 dilution. From this, take 100µL of this dilution and add 900µL system sample diluent to achieve a final 1/100 dilution. Use the 1/100 diluted sample for analysis. Multiply the result x 100.

** For CSF make a manual dilution of 1/10, it is suggested that a 1/10 dilution is made by taking 50µL of sample and add 450µL system sample diluent. Use the 1/10 diluted CSF sample for analysis. Multiply the result x 10.

8.7 Interpretation of results

The results of this assay should always be assessed in conjunction with the patient's medical history, clinical examinations, and other findings including previous **Freelite** results if available.

Due to the nature of monoclonal proteins, 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 8.6 is followed and the first plausible result is reported.

All immunoassays have the potential for antigen excess. In order to identify samples that are in antigen excess the SPAPLUS has the facility to monitor reaction kinetics. 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 8.6 (note: the SPAPLUS will carry out auto-dilutions up to 1/100). 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 free light chain results.

"Undetected antigen excess is a rare event but cannot be excluded. If the free light chain 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

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 Reference Guide.
Quality control testing should be performed in accordance with local 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 your local technical support organisation.
The concentrations of the controls provided are stated on the accompanying QC certificate (SIN324.QC) Sample results obtained should only be accepted if the control results are within ±20% 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 Diagnosis cannot be made and treatment must not be given on the basis of free light chain measurements alone. Clinical history and other laboratory findings must be taken into account.
10.3 This assay has not been established for use with the paediatric population.
10.4 Do not use tubes containing Fluoride Oxalate for CSF analysis as it interferes with the FLC measurement resulting in an underestimation of the reported result

11 EXPECTED VALUES

The ranges provided below 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 Serum and Plasma Reference Interval

Serum samples from 282 normal subjects aged from 20 to 90 years were assayed using Binding Site **Freelite** assays for the BN™II* [11]. The results are shown in the table below.

Adult serum	Mean conc.	Median conc.	95 Percentile range
Free kappa	8.36mg/L	7.30mg/L	3.30 - 19.40mg/L
Free lambda	13.43mg/L	12.40mg/L	5.71 - 26.30mg/L
	Mean	**Median**	**Total range**
Kappa/Lambda ratio	0.63	0.60	0.26 - 1.65

When comparing serum and plasma samples, no significant difference was observed.

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11.2 Urine Reference Interval

These ranges were obtained by measuring the free light chain concentrations of urines provided by 120 healthy adult donors on the SPAPLUS analyser. For both free kappa and free lambda measurements a number of samples ran below the measuring range of the assay.

Adult urine	Mean conc.	Median conc.	95 Percentile range
Free kappa	8.15 (mg/L)	4.93 (mg/L)	0.012 – 32.71 (mg/L)
Free lambda	0.93 (mg/L)	0.55 (mg/L)	<0.45-4.99 (mg/L)

See section 12.2 for SPAPLUS serum correlation with the BNII assay and SPAPLUS urine correlation with the Hitachi Modular P assay.

11.3 CSF Reference Interval

These ranges were obtained by measuring the light chain concentrations of CSF in 24 OCB (Oligoclonal banding) negative samples. For both free kappa and free lambda measurements a number of samples ran below the measuring range of the assay.

OCB negative CSF	Range
Free kappa	<0.1 – 1.96 mg/L
Free lambda	<0.1 – 0.39 mg/L

12 PERFORMANCE CHARACTERISTICS

12.1 Precision

Serum

A precision study was performed following CLSI guidelines *Evaluation of Precision Performance of Clinical Chemistry Approved Guideline* (EP5-A). The study was carried out over 21 working days, with two runs per day. One user assessed three different samples using three different reagent lots on three analysers. The following results were obtained for the samples tested:

Serum 1: 7.21mg/L, **Serum 2:** 35.72mg/L and **Serum 3:** 123.77mg/L.

Within-run precision

	Serum 1	Serum 2	Serum 3
SD (mg/L)	0.24	0.56	2.22
CV%	3.3	1.6	1.8

Between-run precision

	Serum 1	Serum 2	Serum 3
SD (mg/L)	0.30	0.69	2.90
CV%	4.2	1.9	2.3

Between-day precision

	Serum 1	Serum 2	Serum 3
SD (mg/L)	0.81	3.17	8.22
CV%	11.2	9.0	6.6

Total precision

	Serum 1	Serum 2	Serum 3
SD (mg/L)	0.90	3.30	8.99
CV%	12.5	9.3	7.3

Urine

A precision study was performed following CLSI guidelines *Evaluation of Precision Performance of Clinical Chemistry Approved Guideline* (EP5-A). The study was carried out over 21 working days, with two runs per day. One user assessed three different samples using one reagent lot on three analysers. The following results were obtained for the samples tested:

Urine 1: 5.82 mg/L, **Urine 2:** 44.63 mg/L and **Urine 3:** 143.65mg/L.

Within-run precision

	Urine1	Urine 2	Urine 3
SD (mg/L)	0.13	0.75	2.81
CV%	2.3	1.7	2.0

Between-run precision

	Urine1	Urine 2	Urine 3
SD (mg/L)	0.40	1.50	4.35
CV%	6.8	3.4	3.0

Between-day precision

	Urine1	Urine 2	Urine 3
SD (mg/L)	0.48	3.04	11.69
CV%	8.2	6.8	8.1

Total precision

	Urine 1	Urine 2	Urine 3
SD (mg/L)	0.63	3.47	12.78
CV%	10.9	7.8	8.9

CSF

A precision study was performed following CLSI guidelines *User Verification of Performance for Precision and Trueness; Approved Guideline- Second Edition (EP15-A2).* The study was carried out over 5 working days, with two runs per day. One user assessed three different samples using one reagent lot on one analyser. The following mean values were obtained for the samples tested:

CSF 1: 0.19mg/L, **CSF 2:** 0.45mg/L and **CSF 3:** 2.75mg/L.

Within-run precision

	CSF 1	CSF 2	CSF 3
SD (mg/L)	0.018	0.015	0.048
CV%	9.8	3.3	1.8

Between-run precision

	CSF 1	CSF 2	CSF 3
SD (mg/L)	0.019	0.018	0.188
CV%	10.1	4.0	6.8

Between-day precision

	CSF 1	CSF 2	CSF 3
SD (mg/L)	0.000	0.000	0.113
CV%	0.0	0.0	4.1

Total precision

	CSF 1	CSF 2	CSF 3
SD (mg/L)	0.026	0.024	0.225
CV%	14.1	5.2	8.2

12.2 Comparison

Serum: A correlation study was performed on 175 serum samples (119 normal, 21 from known SLE patients and 35 from known myeloma patients) using this kit on a SPAPLUS and on Binding Site **Freelite** BNII assay. The study demonstrated a good agreement giving the following Passing & Bablok comparison plot and linear regression correlation coefficient:

y = 1.0911x – 2.4285mg/L (y = SPAPLUS)

(x = Reference method)

correlation coefficient r = 0.9564

EDTA Plasma: A correlation study was performed on 55 paired serum and EDTA plasma samples using this kit on a SPAPLUS. The study demonstrated a good agreement giving the following Passing & Bablok comparison plot and linear regression correlation coefficient:

y = 0.93x + 0.19mg/L (y = EDTA Plasma)

(x = Serum)

Lithium Heparin Plasma: A correlation study was performed on 82 paired serum and Lithium Heparin plasma samples using this kit on a SPAPLUS. The study demonstrated a good agreement giving the following Passing & Bablok comparison plot and linear regression correlation coefficient:

y = 1.01x - 0.21mg/L (y = Lithium Heparin Plasma)

(x = Serum)

Urine: A correlation study was performed on 121 urine samples (29 normal and 92 clinical samples) using this kit on a SPAPLUS and on Binding Site **Freelite** Modular P assay. The study demonstrated a good agreement over the analyte concentration 0.88 – 87110 mg/L giving the following Passing & Bablok regression equation:

y = 1.04x – 0.92mg/L (y = SPAPLUS)

(x = Reference method)

Bias 2.12 mg/L at the upper limit of the reference range (32.71 mg/L).

12.3 Analytical sensitivity

Serum: Analytical sensitivity was determined by assaying ten replicates of two serum samples with concentrations equivalent to 175% and 240% of the lowest calibrator value. Two distinct sets of data were generated with CVs of 5.3% and 5.0% respectively.

Urine: An analytical sensitivity study was carried out according to CLSI guidelines EP17 Protocols for Determination of Limits of Detection and Limits Quantitation. The limit of quantitation is defined as 0.40 mg/L in urine.

12.4 Linearity

Serum: The linearity of this assay has been confirmed using a serially diluted serum sample, which gave a regression plot of $y = 1.0036x + 0.543$ *(mg/L),* $r^2 = 0.9975$ (y = measured free kappa concentration, x = theoretical concentration), over a measuring range of 2-286mg/L.

Urine: The linearity of this assay has been confirmed using a serially diluted urine sample, which gave a regression plot of $y = 0.9872x + 0.7538$ *(mg/L),* $r^2 = 0.9977$ (y = measured free kappa concentration, x = theoretical concentration), over a measuring range of 1 – 324 mg/L

12.5 Interference

Serum: Minimal assay interference by 200mg/L bilirubin (-5.0%), 3g/L haemoglobin (2.1%) and 0.1% intralipid (-9.1%) has been demonstrated using a ≤10mg/L free kappa serum at the minimum sample dilution (1/1). No significant interference (<3%) was also seen when adding purified IgG (10g/L), IgA (2g/L), IgM (1g/L) to two serum samples containing high (150mg/L) and low (20mg/L) concentrations of free kappa, apart from IgG and IgA, which showed minor interference (<20%) with the low concentration sample. Rheumatoid factor (320 IU/mL) showed minimal interference (<16%) when added to serum samples containing 20mg/L and 60mg/L free kappa.

Urine: Minimal assay interference by 40mg/L bilirubin (-4.9%), 240 mg/L haemoglobin (8.2%), 200mg/L ascorbic acid (-10.3%) and 1.25 g/L albumin (14.4%) has been demonstrated using a 32mg/L free kappa urine.

12.6 Antigen excess

Polyclonal samples: The assay was tested to a level of 330mg/L with a polyclonal serum sample. No antigen excess was observed at this level.

13 BIBLIOGRAPHY

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