YALE-NEW HAVEN HOSPITAL	TITLE: **Kappa and Lambda Free Light Chains (Binding Site) on the Siemens BNII Nephelometer** **Soft Code: KALAG, UKAL**		**DEPT OF LAB MEDICINE** **Immunology, Flow Cytometry, and Molecular Diagnostics Laboratories Policy and Procedure Manual** **DOCUMENT # IMM 199** Page 1 of 13
WRITTEN BY: Penny Smith	**EFFECTIVE DATE:** December 12, 2012	**REVISION:** New	**SUPERCEDES:** IMM 64 -Kappa Lambda Freelite (Immage 800)

I. Intended Use

These kits are used for the quantitative determination of kappa and lambda free light chains in human serum and urine by means of immunonephlometry on the BNII system (Siemens).

II. Introduction

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, 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 and 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 (eg. multiple myeloma), primary amyloidosis and light chain deposition disease. Raised serum levels of polyclonal flc may be associated with autoimmune diseases such as systemic lupus erythematosus. 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

III. Principle of the Assay

Evaluating the concentration of a soluble antigen by nephelometry or 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. The antibody in the cuvette is in excess so the amount of immune complex formed is proportional to the antigen concentration. In nephelometry, the light scatter is monitored by measuring the light intensity at an angle away from incident light, whilst in turbidimetry light scatter is monitored by measuring the decrease in intensity of the incident beam of light. 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 nephelometric or turbidimetric assays can be increased by the use of particle enhancement. This entails linking the antibody to a suitably sized particle that increases the relative light scattering signal of the antigen-antibody reaction.

IV. Specimen Collection:

The test can be performed on serum or urine. Urine samples can be random or a timed collections.

Centrifuge serum (red top tube) or urine at 3000 rpm for 15 minutes. Serum or urine aliquots can be stored at 2-8°C for up to 7 days or at below -20°C for up to 3 months. Repeated freeze-thaw cycles should be avoided. Do not perform the test on grossly hemolyzed samples. Lipemic serum should be spun at 10,000 rpm for 20 minutes to remove contaminating lipids.

Standard Aliquot volume = 500 uL
Minimum Aliquot volume = 100 uL
Grossly Hemolyzed: Reject
Grossly Lipemic: Spin at 10,000 rpm for 20 min.
Stability: 7 days refrigerated, 3 months at frozen (-20°C)

V. Materials:

A. Reagents Supplied in Binging Site Kits

1. Human Kappa Free Kit for use on the BNII - REF# LK016.T
   Supplied in each Kit:
   2 x 2mL Human Kappa Free Reagent (Latex)

1 x 3mL Human Kappa Supplementary Reagent
2 x 1.0mL Human Kappa Free Standard
1 x 1.5mL Human Kappa Free Control
1 x 1.5mL Human Kappa Free High Control

2. Human Lambda Free Kit for use on the BNII - REF# LK018.T
   Supplied in each Kit:
   2 x 2mL Human Lambda Free Reagent (Latex)
   1 x 3mL Human Lambda Supplementary Reagent
   2 x 1.0mL Human Lambda Free Standard
   1 x 1.5mL Human Lambda Free Control
   1 x 1.5mL Human Lambda Free High Control

Composition:
Latex Reagent-consisting of monospecific antibody coated onto polystyrene latex. Preservative: 0.05% ProClin™, 0.1% E-amino-n-caproic acid (EACA) and 0.1% benzamidine.
Standard and Controls- these consist of human sera that contain polyclonal kappa or lambda free light chain. They are supplied in a stabilized liquid form and contain 0.099% sodium azide, 0.1% EACA and 0.01% benzamidine as preservatives.
Supplementary Reagent- containing 0.099% sodium azide as a preservative.

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.

Stability:
Unopened kits – Stored at 2-8°C until the expiry date shown on the kit box label.
After opening- The reagents, calibrator and control may be stored at 2-8°C for up to 3 months.

New Reagent Lots:

Reagents from different kit lot numbers are NOT interchangeable. The standard and control values of each new lot must be entered into the BNII (see calibration procedure for instructions). All new reagent lots must be verified by testing previously tested patient or CAP samples. The limit of acceptability is 20%. Refer to the Immunology Policy for Pretesting of test kits and reagents (Doc# IMM 68).

B. Required Reagents not supplied in Binding Site Kits

1. N Reaction Buffer – see BNII Instrument Manual (Doc# IMM 183)
2. N Diluent – see BNII Instrument Manual (Doc# IMM 183)
3. Wash solution – see BNII Instrument Manual (Doc# IMM 183)
4. BN™II Dilution Wells- REF# OVIC 11
5. BN™II Cuvette Segments – REF# OVIB 31

C. Standards

See Reagents Supplied in Binding Site Kits

D. Controls

See Reagents Supplied in Binding Site Kits

VI. Assay Procedure

A. Before Starting

1. Call a Soft pending list by Workstation. Refer to the Soft Immunology Procedure (Doc# IMM 120).
2. Allow reagents and samples to come to room temperature before testing.
3. Inspect all samples for sufficient volume (250 uL), bubbles and the presence of interfering substances such as hemolysis and lipemia.

B. Assay Protocol for the BN™ II System

1. The assay protocol is given in the Instruction Manual and software of the instrument. All steps are performed automatically by the system .Consult the BNII Instrument Manual (Doc# IMM 183) for details regarding operation of the instrument.
2. The reagents must not be used beyond the expiration date.

C. Assay of Specimens

1. Routine Samples
   - Samples are automatically with N Diluent and measured. The initial dilutions are serum 1:100 and urine 1:20. The diluted samples must be measured within four hours.
   - Results above the analytical measuring range (AMR) will be automatically diluted by the instrument until a result within the AMR is obtained.
   - Results lower than the AMR are repeated at a lower dilution. The lowest serum dilution is 1:5 and the lowest urine dilution is 1:1.

2. Short Samples
   - Samples volumes between 100 uL and 250 uL can be run in sample cups and programmed manually. Refer to the BNII Instrument Manual (Doc# IMM 183).
   - Volumes less than 100 uL cannot be tested.
   -

VII. Interpretation of Results

A. Reporting Results

1. The instrument automatically calculates and prints the concentration of Kappa and Lambda in mg/dL. The results will be held in the LIS Instrument Menu for technologist review.
2. Review each result in Instrument menu**. Before posting** check previous results by clicking on Test History. If the result does not correlate with the previous data consult a supervisor.
3. If the patient does not have any results in Test History, **and the Kappa, Lambda or Kappa/Lambda Ratio is out of the normal range**, repeat the testing at a 1:2000 dilution.

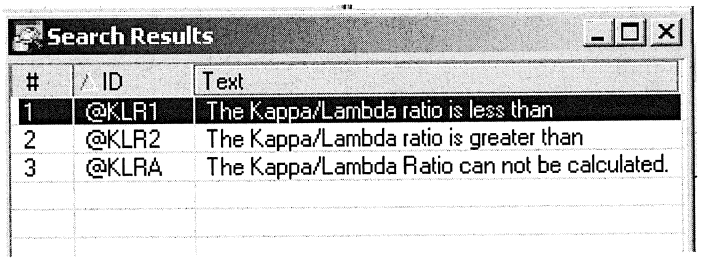
   - Go to Lab Journal
   - Open the sample by double clicking on the Order# (test)
   - Click Dilution
   - Click on 1:2000, then OK
   - Reload the sample on the BNII

   If the repeated results match the original result, post the original result. If the results do not match the original results, consult a supervisor.
4. If a result is above the measuring range, the assay is automatically repeated **by the instrument using a higher dilution**. The instrument will keep repeating on higher dilutions until a result within the AMR is obtained. If the reported instrument value exceeds 5,000 mg/dL, the SOFT LIS will report the result as >5,000 mg/dL.
5. If the result is lower than the AMR, the instrument will repeat the assay on a lower dilution. If the final result is less than 0.2 mg/dL for <u>serum</u> Kappa or Lambda, the LIS will report the result as <0.2 mg/dL. If the final result is less than 0.02 mg/dL for urine Kappa or Lambda, the LIS will report the result as <0.02 mg/dL. **Results should only be reported as less than after the sample has been evaluated for the presence of bubbles or fibrin.**

6. Calculation of Kappa/Lambda ratios will be automatically performed by the SOFT LIS after posting of results except under the following conditions listed in the chart below.

Kappa Result (serum or urine)	Lambda Result (serum or urine)	**Ratio Result**
Within the CRR	<0.2 or <0.02	> Calculated Value
<0.2 or <0.02	Within the CRR	< Calculated Value
Within the CRR	>5000	< Calculated Value
>5000	Within the CRR	> Calculated Value
Not within the CRR	Not within the CRR	The ratio cannot be calculated

- Manually calculate the ratio using the lower or upper value of the Clinical Reportable Range (0.2, 0.02 or 5000)
- Tab over to Lab Results
- Click in the Ratio field
- Click Comment, then Canned Message
- Choose the **CORRECT** comment based on the above chart.



- Free text the calculated result to the fixed comment (Do not verify).
- Do not calculate the ratio if both results are outside the CRR.
- Tab back to Instrument and Post Kappa and Lambda results.

Example:

Kappa <0.2 mg/dL; Lambda 100 mg/dL

$$\text{Ratio} = \frac{0.2}{100} = 0.002$$

Result: The Kappa/Lambda ratio is less than 0.002

VIII. Calibration

A. Establishment of the Reference Curve

1. Reference curves are generated by multi-point calibration. Serial dilutions of Human Kappa or Lambda Free Standard are automatically prepared by the instrument using N Diluent. The standard dilutions are to be used within four hours.

2. **When starting a new kit lot number update the Standard and Control values in the BNII**. The values are located on a card in each reagent kit.

Updating Standard Values

- Go to Calibration ⟶ Standard Lots
- Update the standard value, Click OK

Updating Control Values

- Go to Calibration ⟶ Control Lots
- Update the standard value, Click OK

B. When to Calibrate

1. If the controls are out of range or the Westgard rules stated in the Quality Control procedure (Doc# IMM 37) are violated.
2. If a different reagent kit lot is used, a new reference curve must be generated.
3. Major instrument maintenance has been performed.

C. How to calibrate

1. Use the Human Kappa or Lambda Free Standard to calibrate.
2. Refer to the BNII Instrument Manual (Doc# IMM 183) for instructions on programming a calibration.
3. Always run quality control after calibration.

IX. Quality Control

A. Quality control Material

For both serum and urine:

Human Kappa Free Control
Human Kappa Free High Control
Human Lambda Free Control
Human Lambda Free High Control

B. Frequency

1. Both levels of control are to be run at the beginning of each shift or every 8 hours.
2. Both levels of control are to be run following calibration.

C. Quality Control Guidelines

1. Because the BNII software lists control ranges by percent deviation, SOFT Total QC (TQC) will be used for QC monitoring. Refer to the Total QC section of the SOFT Immunology procedure (Doc# IMM 120). **The maximum allowable variation from the mean is 20%.**
2. Total QC is set up with ranges of +/- 3 standard deviations. **The maximum allowable variation from the mean is 20% so 1 SD will be equal to 0.0667% of the mean.**
3. The 10X, 2-2S and 1-3S Westgard rules will be used for QC monitoring. For more information on quality control monitoring refer to Immunology Laboratory Guidelines for Quality Control (Doc# Imm 38).

D. New lots of Quality Control

1. The quality control lot numbers are specific to each new lot. When new lots are new lots of control materials are put into use the manufactures mean +/-20% will be used.
2. An in-house mean will be established after 30 data points for each level have been collected. The range will again be set at the mean +/-20%.

X. Analytical Measuring Range (AMR)

Because the concentration of the standard varies by lot number, the AMR values listed below are approximate. Therefore, the clinical reportable range has been fixed to avoid exceeding any lot specific AMR.

Assay	Initial Dilution	Analytical Measuring Range (AMR) mg/dL	Maximum allowable dilution	Minimum allowable dilution	Clinical Reportable Range mg/dL
Serum Kappa	1:100	0.65-20.9	1:40000	1:5	0.2-5000
Serum Lambda	1:100	0.51-16.2	1:40000	1:5	0.2-5000
Urine Kappa	1:20	0.13-4.18	1:40000	1:1	0.02-5000
Urine Lambda	1:20	0.10-3.23	1:40000	1:1	0.02-5000

AMR verification does not need to be performed every 6 months because the standard curve used to calibrate contains more than 3 points.

XI. Reference Range

Serum Kappa:	0.33-1.94 mg/dL
Serum Lambda:	0.57-2.63 mg/dL
K/L Ratio:	0.26-1.65
Urine Kappa:	0.14-2.42 mg/dL
Urine Lambda:	0.02-0.67 mg/dL
K/L Ratio:	2.04-10.37

XII. Limitations

1. Nephelometric or turbidimetric assays are not suitable for measurement of highly lipemic or hemolyzed samples or samples containing high levels of circulating immune complexes (CIC's) due to the unpredictable degree of non-specific scatter these sample types may generate.
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.
3. Antigen excess:

   A small proportion of patient samples containing high concentrations of free kappa or free lambda can give a falsely low result for the "involved" light chain due to antigen excess. The amino acid composition of the light chain produced by an individual B cell clone will influence the level at which a sample may show antigen excess with the **Freelite** assay. In almost every case the concentration of the involved light chain will still be above the quoted normal range (3.30-19.40 mg/L for free kappa and 5.71-26.30 mg/L for free lambda) and/or the opposite light chain concentration will be below the quoted range and/or the free kappa/free lambda ratio will be outside the quoted range (0.26-1.65). Samples should be tested at both 1/100 and 1/2000 dilutions in order to detect antigen excess if any of the following conditions are met:

   a) If a new patient sample shows either a free light chain concentration or a free kappa/lambda ratio outside of the quoted range.
   b) Sample is from a patient that has previously demonstrated antigen excess

c) Sample result does not agree with other clinical or laboratory findings

4. Each monoclonal FLC contains unique amino acid combinations. It is therefore theoretically possible for certain monoclonal proteins to be undetectable by immunoassay leading to lower than expected measurements. In practice this occurs extremely rarely with the **Freelite** assay. Suspected samples should first be tested for antigen excess then further investigation by other laboratory methods (immunofixation and serum protein electrophoresis)

5. The nature of monocional proteins can cause a non-linear response in immunoassays, potentially leading to inconsistent results; this can be prevented by always diluting the samples in the sequence 1/100, 1/400, 1/2000, 1/8000. Omitting a dilution step will be avoided.

XIII. Validation

Accuracy and Linearity:

Accuracy and linearity studies were performed by sequential dilution of either a binding site standard or patient sample. The studies were performed on both BNII instruments. Error limits were set as follows: Allowable Total Error (TEa): 40%, Systematic Error Budget: 50%, Allowable Systematic Error (SEa): 20%. The manufactures recommended error limit is 20%.

Kappa

The accuracy test passed, the maximum deviation for a mean recovery from 100% on both instruments was accurate within the SEa (14.3% and 12.4%). The assay was linear on both instruments within the SEa (9.2% and 7.8% Error).

Lambda

The accuracy test passed, the maximum deviation for a mean recovery from 100% on both instruments was accurate within the SEa (6.6% and 10.9%). The assay was linear on both instruments within the SEa (5.8% and 4.7% Error).

Correlation:

Correlation was performed by testing a 20 sample panel (provided by Binding Site) and comparing the results to those obtained on the BNII at Binding Sites testing laboratories. In addition 20 serum samples and 20 urine samples were tested and

results compared to results from the Immage 800. Comparisons were also performed between instruments. Regression analysis was performed and the acceptability was determined by a 95% confidence interval for slope and intercept and a Correlation Coefficient (R) of greater than 0.95.

Some variability was seen at low concentrations between the Immage 800 and the BNII. The manufacturer suggests this variability is due to differences in the sensitivity of the assay on each instrument. These differences were not seen in the results from the correlation with the BNII at Binding Sites testing laboratories.

Kappa

Binding Site Sample Panel:

BNII 1 to BNII Binding Site:

Slope 1.067 (0.949 to 1.186), Intercept 0.7670 (-9.7500 to 11.2841), R 0.9748

BNII 2 to BNII Binding Site:

Slope 1.050 (0.978 to 1.121), Intercept -0.0965 (-6.4557 to 6.2627), R 0.9905

BNII 1 to BNII 2

Slope 1.015 (0.954 to 1.077), Intercept 0.9109 (-4.8484 to 6.6701), R 0.9924

Patient Serum Correlation:

BNII to Immage 800

Slope 1.232 (1.111 to 1.352), Intercept -11.0214 (-26.9656 to 4.9228), R 0.9841

BNII 1 to BNII 2

Slope 0.916 (0.895 to 0.937), Intercept 0.1914 (-3.7659 to 4.1487), R 0.9990

Patient Urine Correlation:

BNII to Immage 800

Slope 1.062 (0.947 to 1.176) Intercept -0.2228 (-0.8649 to 0.4192), R 0.9882

BNII 1 to BNII 2

Slope 1.030 (0.973 to 1.087), Intercept -0.2036 (-1.1698 to 0.7626), R 0.9938

Lambda

Binding Site Sample Panel:

BNII 1 to BNII Binding Site:

Slope 1.081 (1.055 to 1.106), Intercept 0.1027 (-1.7863 to 1.9916), R 0.9989

BNII 2 to BNII Binding Site:

Slope 1.110 (1.071 to 1.149), Intercept -1.5210 (-4.3837 to 1.3418), R 0.9975

BNII 1 to BNII 2

Slope 0.970 (0.947 to 0.992), Intercept 0.7426 (-1.1055 to 2.5908), R 0.9989

Patient Serum Correlation:

BNII to Immage 800

Slope 0.886 (0.843 to 0.929), Intercept 1.6777 (-3.5951 to 6.9505), R 0.9955

BNII 1 to BNII 2

Slope 0.982 (0.958 to 1.006), Intercept -0.484 (-1.716 to 0.747), R 0.9992

Patient Urine Correlation:

BNII to Immage 800

Slope 0.936 (0.881 to 0.991 Intercept -0.3712 (-0.5032 to -0.2391), R 0.9834

BNII 1 to BNII 2

Slope 1.051 (1.036 to 1.067), Intercept -0.0590 (-0.0907 to -0.0273), R 0.9996

Precision:

Intrarun Precision: Intra-assay performance was evaluated on both instruments by testing the low and high control 10 times each on a single run. The acceptable CV limit for intrarun precision is 10%. The Kappa controls on both instruments had %CV's of less than 7%. The Lambda controls on both instruments had %CV's of less than 3%.

Interrun Precision: Inter-assay performance was evaluated on both instruments by testing the low and high control on at least 5 different days. The acceptable CV limit for interrun precision is 20%. The Kappa controls on both instruments had %CV's less than or equal to 7%. The Lambda controls on both instruments had %CV's of less than 6%.

Reference Range Verification:

Serum:

The manufactures serum reference ranges for Kappa, Lambda and Kappa/Lambda Ratio of were verified by testing serum from 20 individuals from within the YNHH population. All were within the acceptability limit of 90%.

Kappa range: 0.33 - 1.94 mg/dL, 90% within.

Lambda range: 0.57 - 2.63 mg/dL, 95% within.

Kappa/Lambda Ratio: 0.26 – 1.65, 100% within.

Urine:

Urine samples from 20 presumably healthy individuals were used to verify the manufactures suggested reference ranges. For Lambda, 90% of the patients were within the reference range. For Kappa, only 60% were within the reference range.

The laboratory director has determined that the manufactures range will be utilized based on:

1. The manufactures range also corresponds to the range used by our reference laboratory (ARUP).

2. Although higher Kappa levels were seen in some individuals, 2 years' worth of historical data demonstrated that the elevated levels on these individuals were in the "not worry" range.

Kappa range: 0.135 – 2.419 mg/dL,

Lambda range: 0.024- 0.666 mg/dL

Kappa/Lambda Ratio: 2.04 – 10.37

CAP Proficiency Results:

Surveys SFLC-B 2012 and Q3/120 for serum and Q6/3 for urine were tested and all results were acceptable when compared to other BNII users.

XIV. References:

1. Human Kappa Free Kit for use on the Siemens BNII [package insert]. Edgbaston, Birmingham B15 1QT UK: The binding Site Group Ltd; August 2, 2011 Edition. Insert code: SIN055.

2. Human Lambda Free Kit for use on the Siemens BNII [package insert]. Edgbaston, Birmingham B15 1QT UK: The binding Site Group Ltd; August 2, 2011 Edition. Insert code: SIN056.

Quick Reference Guide: Kappa and Lambda Free Light Chains (Binding Site) on the Siemens BNII Nephelometer. Doc# IMM 199-A

Reference Ranges:

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Serum Lambda	0.57 - 2.63 mg/dL
Serum K/L Ratio	0.26 - 1.65
Urine Kappa	0.14 - 2.42 mg/dL
Urine Lambda	0.02 - 0.67 mg/dL
Urine K/L Ratio	2.04 – 10.37

Test Parameters:

Assay	Initial Dilution	Analytical Measuring Range (AMR) mg/dL	Maximum allowable dilution	Minimum allowable dilution	Clinical Reportable Range mg/dL
Serum Kappa	1:100	0.65-20.9	1:40000	1:5	0.2-5000
Serum Lambda	1:100	0.51-16.2	1:40000	1:5	0.2-5000
Urine Kappa	1:20	0.13-4.18	1:40000	1:1	0.02-5000
Urine Lambda	1:20	0.10-3.23	1:40000	1:1	0.02-5000

Ratio Calculations:

Kappa Result (serum or urine)	Lambda Result (serum or urine)	Ratio Result
Within the CRR	<0.2 or <0.02	> Calculated Value
<0.2 or <0.02	Within the CRR	< Calculated Value
Within the CRR	>5000	< Calculated Value
>5000	Within the CRR	> Calculated Value
Not within the CRR	Not within the CRR	The ratio cannot be calculated

Document Author
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December 11, 2012

Signature Approval

Name: Kappa and Lambda Free Light Chains (Binding Site) on the SiemensBNII Nephelometer

Document #: IMM 199

Name (Print)	Title	Signature	Date of Review	Revision Page and Section # (Use Procedure Review Log to document staff review)	Issue Date for Training if Applicable	Effective Date for Use
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