YALE-NEW HAVEN HOSPITAL	TITLE: **Immnoglobulin G (IgG) Siemens BNII Nephelometer** **Soft Code: IGG**		**DEPT OF LAB MEDICINE** **Immunology, Flow Cytometry, and Molecular diagnostcs Laboratories Policy and Procedure Manual** **DOCUMENT # IMM 185** Page 1 of 9
WRITTEN BY: Kathy Radziunas Penny Smith	**EFFECTIVE DATE:** October 8, 2012	**REVISION:** New	**SUPERCEDES:** **IMM 43 - Immage 800 Quantitation of proteins by Nephlometry**

I. Intended Use

In-vitro diagnostic reagents are used for the quantitative determination of Immunoglobulin G (IgG) in human serum by means of immunonephlometry on the BNII system (Siemens). Measurements of IgG aid in the diagnosis of abnormal protein metabolism and the bodies lack of ability to resist infectious agents.

II. Introduction

Immunoglobulins are formed by plasma cells as a humoral immune response to contact of the immune system with antigens. The primary reaction after the initial contact is the formation of antibodies of the IgM class followed later by IgG and also IgA antibodies. Quantitative determination of the immunoglobulin's can provide important information on the humoral immune status.

Decreased serum immunoglobulin (Ig) concentrations occur in primary immunodeficiency conditions as well as in secondary immune insufficiencies, e.g., in advanced malignant tumors, lymphatic leukemia, multiple myeloma and Waldenstrom's disease. Increased serum immunoglobulin concentrations occur due to polyclonal or oligoclonal Ig proliferation, e.g., in hepatic diseases (hepatitis and liver cirrhosis), acute and chronic infections, autoimmune diseases as well as in the cord blood of neonates with intra-uterine and perinatal infections.

Monoclonal immunoglobulin proliferations are observed e.g. in plasmacytomas, Waldenstrom's disease and heavy-chain disease. Monoclonal immunoglobulinemia requires detailed differential diagnostic investigations in addition to the quantitative determination. Local immune reactions with the central nervous system result in elevated immunoglobulin levels, particularly IgG, in the cerebrospinal fluid.

III. Principle of the Assay

Proteins contained in human body fluids form immune complexes in an immunochemical reaction with specific antibodies. These complexes scatter a beam of light passed through the sample. The intensity of the scattered light is proportional to the concentration of the relevant protein in the sample. The result is evaluated by comparison with a standard of known concentration.

IV. Specimen Collection:

Serum:

The test should be performed on serum only (from red top tube). Separate serum by centrifugation, 3000 rpm for 15 minutes. Serum aliquots can be stored at 2-8°C for up to 8 days or at below -20°C for up to three months. Repeated freeze-thaw cycles should be avoided. Do not perform the test on grossly hemolyzed or lipemic serum. Lipemic serum should be spun at 10,000 rpm for 20 minutes to remove contaminating lipids.

Standard Aliquot volume = 250 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

1. N Antiserum to Human IgG (5ml vial) – REF# OSAS19

Composition:

N Antiserum is a liquid animal serum and is produced by immunization of rabbits with highly purified human IgG.

Preparation:

The N Antiserum is ready-for-use as supplied and requires no additional preparation.

Storage:

Stability at 2 to 8 °C: See expiration date on label.

Stability once opened: Four weeks if stored at 2 to 8 °C securely capped immediately after each use and contamination (e.g., by microorganisms) is precluded.
During storage, N Antisera can develop precipitates or turbidity which are not caused by microbial contamination and which do not affect their activity. In such cases, the antiserum should be filtered prior to use. Disposable filters with a pore size of 0.45 µm are suitable for this purpose. Do not freeze.

On-board stability: 5 days at 8 hours each day or a comparable time period.

New Reagent Lots:

All new reagent lots are verified by testing previously tested patient or CAP samples. Refer to the Immunology Policy for Pretesting of test kits and reagents (Doc# IMM 68) for procedure and acceptability limits.

Precautions:

Contains sodium azide (< 0.1 %) as a preservative. Sodium azide can react with copper or lead pipes in drain lines to form explosive compounds. Dispose of properly in accordance with local regulations.

2. N Reaction Buffer – see BNII Instrument Manual (Doc# IMM 183)
3. N Diluent – see BNII Instrument Manual (Doc# IMM 183)
4. Wash solution – see BNII Instrument Manual (Doc# IMM 183)

B. Standards

1. N Protein Standard SL – REF# OQIM 15

Composition and Standardization

N Protein Standard SL is a liquid, stabilized human serum. The protein reference preparation used for calibration of the N Antiserum to Human IgG assay is the ERM®-DA470 (known as CRM 470). The concentration of IgG contained in the standard is lot dependent.

Preparation of the Standard

N Protein Standard SL is supplied ready-for-use. Invert gently to mix. Avoid vigorous shaking and foam formation.

Standard Storage and Stability:

Stability at +2 to +8 °C: The expiration date is given on the label. Do not freeze.

Stability once opened: 14 days if stored tightly closed at +2 to +8 °C directly after each use.

C. Controls

1. Siemens BNII Protein Controls
   N/T Protein Control SL/L – REF# OQIN 19
   N/T Protein Control SL/M – REF# OQIO 19
   N/T Protein Control SL/L – REF# OQIP 19

Composition

N/T Protein Controls SL/L, M and H are liquid, stabilized human sera. The concentration of IgG is calibrated to the protein standard preparation and is lot-dependent.

Preparation of the Reagents

N/T Protein Control SL/L, M and H are supplied ready-for-use. Invert gently to mix. Avoid vigorous shaking and foam formation.

Control Storage and Stability:

Shelf life at +2 to +8 °C: The expiration date is given on the label. Do not freeze.
Stability once opened: 14 days if stored tightly closed at +2 to +8 °C after each use.

D. Consumables

1. BN™II Dilution Wells- REF# OVIC 11
2. BN™II Cuvette Segments – REF# OVIB 31

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 diluted 1:400 with N Diluent and measured. The diluted samples must be measured within four hours.
   - Results outside the analytical measuring range (AMR) will be automatically repeated at higher or lower dilutions by the instrument until a result within the AMR is obtained.
2. Short Samples
   - Samples volumes between 100uL and 250uL 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. Calibration

A. Establishment of the Reference Curve

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

2. Assigned values for Siemens standards may be scanned into the system using the barcodes found on the Table of Assigned Values sheet, which is included in each box of standards, or they may be entered manually by the operator.

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 lot of antiserum is used, a new reference curve must be generated.
3. Major instrument maintenance has been performed.

C. How to calibrate

1. Use N Protein Standard SL 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.

VIII. Quality Control

A. Quality control Material

- Siemens BNII Protein Controls
  N/T Protein Control SL/L – REF# OQIN 19
  N/T Protein Control SL/M – REF# OQIO 19
  N/T Protein Control SL/H – REF# OQIP 19

B. Frequency

1. All 3 levels of controls are to be run at the beginning of each shift or every 8 hours.
2. All 3 levels 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).
2. Total QC is set up with ranges of +/- 3 standard deviations.
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. New lots of control material are pretested until at least 30 data points are collected to determine an in-house control range of +/- 3 standard deviations.
2. If a new lot of control is put into use before 30 points are collected the manufacturer's range will be used until 30 data points are collected.

IX. Interpretation of Results

A. Reporting Results

1. The instrument automatically calculates and prints the concentration of IgG in mg/dl.
2. If the results obtained are 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 30,000 mg/dL, the SOFT LIS will report the result as >30,000 mg/dL.
3. If the results obtained are below the measuring range, the assay is automatically repeated **by the instrument using a lower dilution**. The lowest dilution the instrument will perform is 1:20. If the reported instrument value is less than 10 mg/dL, the SOFT LIS will report the result as <10 mg/dL.

B. Verification of Results

1. Results are transmitted to the SOFT LIS system and monitored via Instrument Menu. Refer to the Soft Immunology Procedure (Doc# IMM 120).
2. Results will be autoverified by SOFT unless one of the conditions below is met. Results held in instrument menu will have to be manually posted by the operator if determined that the result is acceptable.

Reason Not Autoverified	Action to be taken
Result <20 mg/dl	Check sample for presence of bubbles or fibrin before manually posting.
Delta Check Flag	Recheck result from clot before manually posting.

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.

AMR: 140 – 4600 mg/dL
Maximum allowable dilution: 1:2000
Minimum allowable dilution: 1:20
Clinical Reportable Range: 10 – 30,000 mg/dL

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

0-5 months: 106-639 mg/dL
6 months to 1 year: 124-925 mg/dL
2 – 10 years: 266-1640 mg/dL
11-17 years: 355-1887 mg/dL
≥ 18 years: 700-1600 mg/dL

XII. Limitations

A. Interferences
1. No interference with the determinations in serum was detected for concentrations of triglycerides up to 19 g/L, bilirubin at 600 mg/L, and free hemoglobin at 10 g/L.
2. No interference from commonly used drugs is known.
3. Turbidity and particles in the sample may interfere with the determination. Therefore, samples containing particles must be centrifuged prior to testing. Lipemic or turbid samples which cannot be clarified by centrifugation (10 minutes at approximately 15,000 x g) must not be used.

B. Antigen Excess
1. The immunoglobulin assays have been designed to minimize antigen excess in the initial sample dilutions. However, it cannot be completely eliminated and in rare cases very high immunoglobulin concentrations may produce falsely-low results.
2. Especially monoclonal immunoglobulins may show reactivity different from the polyclonal standard, which in isolated cases may lead to artificially decreased or non-linear results. In case of serum or plasma determinations,
3. The constellation of IgG, IgA and IgM should be assessed. In case of questionable results, the determinations should be repeated using the next higher sample dilution. For patient monitoring, consecutive immunoglobulin determinations should be performed from the same sample dilution, as far as possible.

C. Matrix Effects

1. Due to matrix effects, inter-laboratory survey samples and control samples may yield results that differ from those obtained with other methods. It may therefore be necessary to assess these results in relation to method-specific target values.

XIII. YNHH Method Validation Summary

Accuracy and Linearity:

Accuracy and linearity studies were performed by sequential dilution of the N protein standard. The studies were performed on both BNII instruments. Error limits were set as follows: Allowable Total Error (TEa): 25%, Systematic Error Budget: 50%, Allowable Systematic Error (SEa): 12.5%.

The accuracy test passed, the maximum deviation for a mean recovery from 100% on both instruments was accurate within the SEa (10.1% and 4.2%). The assay was linear on both instruments within the SEa (0.9% and 3.0% Error).

Correlation:

Correlation was performed by comparing results of 40 patient serum samples from the Beckman Immage 800 (YNHH) to results from the BNII. 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.

BNII to Immage 800:

IgG -Slope 0.965 (0.921 to 1.010), Intercept 57.9 (-7.6 to 123.3), R 0.9902

IgGC- Slope 0.879 (0.819 to 0.939), Intercept 0.06 (-0.17 to 0.29), R 0.9906

BNII to BNII

IgG -Slope 0.953 (0.916 to 0.990), Intercept 11.6 (-43.2 to 66.5), R 0.9929

IgGC- Slope 1.040 (1.018 to 1.062), Intercept 0.01 (-0.07 to 0.08), R 0.9991

Precision:

Intrarun Precision: Intra-assay performance was evaluated on both instruments by testing 3 levels of controls for IgG and 2 levels for IgGC 10 times each on a single run. The acceptable CV limit for intrarun precision is 10%. All controls on both the IgG and IgGC assays had %CV's of less than 5%.

Interrun Precision: Inter-assay performance was evaluated on both instruments by testing 3 levels of controls for IgG and 4 levels for IgGC on 5 different days. The acceptable CV limit for interrun precision is 20%. All controls on both the IgG and IgGC assays had %CV's of less than 10%.

Reference Range Verification:

The manufactures **adult** serum reference range of **700-1600 mg/dL** was verified by testing serum from 30 healthy individuals from within the YNHH population. 90.0% of the individuals tested had IgG levels within the suggested reference range. The acceptability limit of for reference range verification is 90%.

The pediatric serum references intervals below were established using two years' worth of YNHH historical data. YNHH reference ranges were created using EP Evaluator. The 0-5 month interval did not have enough data points (29) to create a YNHH reference range (103-1026 mg/dL). Instead our data was compared to the 0-5 month reference range established at Mayo Laboratories (100-334 mg/dL). After reviewing the data and excluding data points outside the bell curve, the laboratory director set the range at 106-639 mg/dL. The upper limits of the 6 months to 1 year and 11-17 year old ranges were also adjusted by excluding data points outside of the bell curve.

0-5 months: 106-639 mg/dL
6 months to 1 year: 124-925 mg/dL
2 – 10 years: 266-1640 mg/dL
11-17 years: 355-1887 mg/dL

The manufactures adult CSF reference range of <3.4 mg/dL was verified by testing CSF from 22 individuals with normal CSF total protein levels. 95.5% of the individuals tested had IgG levels within the suggested reference range. The acceptability limit of for reference range verification is 90%.

CAP Proficiency Results:

Survey S-A 2012 and ELP-A 2012 for serum and survey M-A 2012 for CSF were tested and all results were acceptable when compared to other BNII users.

XIV. References:

1. Siemens N Antisera to Human Immunoglobulins (IgG, IgA, IgM) [package insert]. Newark, DE: Siemens Healthcare Diagnostics; February 2012 Edition.

Document Author
Kathy Radziunas
Penny Smith
Sept 17, 2012

Signature Approval for Annual Review
Name: Immnoglobulin A (IgG)
Siemens BNII Nephlometer

Document #: IMM 185

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
TEODORICO LEE	LAB MANAGER	Teodorico Lee	10/5/12	NEW		10/8/12
BRIAN SMITH	LAB DIRECTOR		10/6/12	NEW		10/8/12