YALE-NEW HAVEN HOSPITAL	TITLE: **Complement Factors C3 and C4, Siemens BNII Nephelometer** **Soft Code: C3, C4**		**DEPT OF LAB MEDICINE** **Immunology, Flow Cytometry, and Molecular diagnostcs Laboratories Policy and Procedure Manual** **DOCUMENT # IMM 187** Page 1 of 10
WRITTEN BY: Kathy Radziunas Penny Smith	**EFFECTIVE DATE:** November 27, 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 complement factors (C3/C3c and C4/C4c) in human serum by means of immunonephlometry on the BNII system (Siemens).

II. Introduction

The complement system is an integral part of the antigen-nonspecific immune defense. It can be activated via two reaction pathways, the classical pathway which is triggered primarily by cellbound immune complexes, and the alternative pathway which is activated primarily by foreign bodies such as microorganisms. The complement component C3 is a key protein in both reaction pathways whereas C4 belongs to the classical pathway of complement activation. Complement activation is associated with consumption of component C3 or C4 so that a reduction in their concentrations can allow diagnostic conclusions to be reached. Diminished serum concentrations of C3 and C4 are observed primarily in active systemic lupus erythematosus (SLE), in forms of membrane proliferative glomerulonephritis and in immune complex diseases (serum sickness). In the case of SLE the serum concentrations of the complement factors reflect the activity of the disease. Diminished C3 values occur in acute glomerulonephritis and in membrane proliferative glomerulonephritis whereas isolated diminished levels of C4 can occur in hereditary angloedema (HAE) and in cases of autoimmune hemolytic anemia. Both complement components react as acute-phase proteins and may therefore show elevated serum concentrations in patients with inflammatory diseases. Hereditary deficiency states of both complement factors have been reported.

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:

The test should be performed on serum only (from red top tube). Separate serum by centrifugation, 3000 rpm for 15 minutes before refrigeration and within 12 hours. Serum aliquots can be stored at **2-8°C or -20°C for up to 3 days**. 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: 3 days (2-8 °C), 3 days (-20°C)

Samples refrigerated before centrifugation, add the canned message @COLD

"This sample was received after refrigeration. This could result in an incorrect low quantification."

V. Materials:

A. Reagents

1. N Antiserum to Human C3c (2ml vial/ 40 tests) – REF# OSAP09
   N Antiserum to Human C4c (2ml vial/ 40 tests) – REF# OSAO09

Composition:

N Antiserum is a liquid animal serum and is produced by immunization of rabbits with highly purified human complement factors (C3c or C4).

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 Antiserum is liquid animal serum and is produced by immunization of rabbits with highly purified human C3c and C4. The antibody titer (T) is determined by radial immunodiffusion and printed on the vial label. The titer indicates the quantity of antigen in mg which will be precipitated in an agarose gel by 1 mL of the corresponding antiserum.

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 complement factors are calibrated to the protein standard preparation and are 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, 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 1:20 with N Diluent and measured. 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 automatically rerun using a 1:5 dilution.

2. Short Samples
   - Samples volumes between 100 uL and 500 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. 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 C3c and C4 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 40,000 mg/dL for C3c or 10,000 mg/dL for C4, the SOFT LIS will report the C3 result as >40,000 mg/dL and the C4 result as >10,000 mg/dL.
3. If the result is lower than the AMR, the assay is automatically repeated **by the instrument using a lower dilution**. If that result is lower than the measurable range, it is reported as <10 mg/dL for both C3c and C4. **Results should only be reported as <10 mg/dL after the sample has been evaluated for the presence of bubbles or fibrin.**

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 <10 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.

C3
AMR: 20 – 600 mg/dL
Maximum allowable dilution: 1:2000
Minimum allowable dilution: 1:5
Clinical Reportable Range: 10 – 40000 mg/dL

C4
AMR: 6 – 190 mg/dL
Maximum allowable dilution: 1:2000
Minimum allowable dilution: 1:5
Clinical Reportable Range: 10 – 10000 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

C3: > 1 year 81-145 mg/dL

C4: > 1 year 16-39 mg/dL

Reference ranges have not been established for children less than 1 year old.

XII. Limitations

A. Interferences

1. No interference with the determinations in serum was detected for concentrations of triglycerides up to 5.7 g/L (C3) or 2.4 g/L (C4), bilirubin at 0.6 g/L, and free hemoglobin up to 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. 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.

C. Intended Use

1. Siemens has validated use of these reagents on various analyzers to optimize product performance and meet product specifications. User defined modifications are not

supported by Siemens as they may affect performance of the system and assay results. It is the responsibility of the user to validate modifications to these instructions or use of the reagents on analyzers other than those included in Siemens Application Sheets or these instructions for use.

2. Results of this test should always be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

XIII. YNHH Method Validation Summary

Accuracy and Linearity:

Accuracy and linearity studies were performed by sequential dilution of the N Protein Standard SL. 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%.

C3- The accuracy test passed, the maximum deviation for a mean recovery from 100% on both instruments was accurate within the SEa (4.7% and 3.8%). The assay was linear on both instruments within the SEa (1.7% and 0.7% Error).

C4- The accuracy test passed, the maximum deviation for a mean recovery from 100% on both instruments was accurate within the SEa (9.7% and 7.5%). The assay was linear on both instruments within the SEa (5.3% and 2.5% Error).

Correlation:

Correlation was performed by comparing results of 40 patient samples tested on the Beckman Immage 800 (YNHH) to results tested on the BNII and comparing results obtained on each 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.

C3- BNII to Immage 800: Slope 1.207 (1.089 to 1.325), Intercept 8.3 (-5.4 to 22.0), R 0.9554

C4- BNII to Immage 800: Slope 1.383 (1.249 to 1.517), Intercept -4.0 (-7.1 to -1.0), R 0.9559

C3- BNII to BNII: Slope 0.900 (0.865 to 0.935), Intercept 7.3 (2.1 to 12.5), R 0.9929

C4- BNII to BNII: Slope 1.008 (0.966 to 1.050), Intercept 0.2 (-1.0 to 1.4), R 0.9919

Precision:

Intrarun Precision: Intra-assay performance was evaluated for both C3 and C4 on both instruments by testing the low, medium and high control 10 times each on a single run. The acceptable CV limit for intrarun precision is 10%. All 3 levels of control had %CV's of less than 6% on each instrument.

Interrun Precision: Inter-assay performance was evaluated for both C3 and C4 on both instruments by testing the low, medium and high control 5 different days. The acceptable CV limit for interrun precision is 20%. All 3 levels had %CV's of less than 10% on each instrument.

Reference Range Verification:

References ranges for both C3 and C4 were established using five years' worth of YNHH historical data. The data was analyzed using EP Evaluator and reviewed by the medical director who then determined the ranges below. There were not enough data points to establish a reference range for children less than 1 year old.

C3: > 1 year 81-145 mg/dL

C4: > 1 year 16-39 mg/dL

CAP Proficiency Results:

Proficiency samples from surveys S2-A 2012 were tested and all results were acceptable when compared to other BNII users.

Complement Stability Studies:

Stability studies were performed to evaluate

1. The time period in which collected samples could remain unspun at room temperature and refrigerated.
2. The storage stability of serum samples at 2-8°C and -20°C.

See addendum A, C3 and C4 Stability Study (Doc# 187-A) for results.

XIV. References:

1. Siemens N Antisera to Human Complement Factors (C3c, C4) [package insert]. Newark, DE: Siemens Healthcare Diagnostics; June 2010 Edition.

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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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