YALE-NEW HAVEN HOSPITAL	TITLE: **Albumin, Serum (and CSF Siemens BNII Nephelometer** **Soft Code: Order only as part of OLIGG (Oligoclonal Bands)**		**DEPT OF LAB MEDICINE** **Immunology, Flow Cytometry, and Molecular Diagnostics Laboratories Policy and Procedure Manual** **DOCUMENT # IMM 188** Page 1 of 10
WRITTEN BY: Kathy Radziunas Penny Smith	**EFFECTIVE DATE:** April 26, 2013	**REVISION:** New	**SUPERCEDES:** **IMM 43 - Immage 800 Quantitation of proteins by Nephelometry**

I. Intended Use

In-vitro diagnostic reagents are used for the quantitative determination of Albumin (ALB) in human serum and CSF (ALBC) by means of immunonephlometry on the BNII system (Siemens). The measurement of serum and CSF albumin, along with serum and CSF IgG, are used to calculate the CSF Index which is useful in diagnosing and monitoring patients with multiple sclerosis and other inflammatory diseases involving the brain and meninges.

II. Introduction

Albumin is the chief plasma protein in terms of quantity, normally accounting for more than half of the total protein. Albumin is formed exclusively in the liver and serves as a transport and binding protein for calcium, fatty acids, bilirubin, hormones, vitamins, trace elements and drugs. It contributes decisively towards maintaining the colloidal osmotic pressure. Diminished albumin concentrations occur in severe impairment of hepatic synthesis capacity (e.g. in hepatic cirrhosis, severe hepatitis or chronic malnutrition) as well as in marked protein loss (nephrotic syndrome, gastroenteropathy, severe burns). Defects of the glomerular filtration barrier are associated with increased urinary albumin levels. The CSF albumin concentration is a measure of the integrity of the blood/CSF barrier. The determination of the CSF/serum albumin quotient permits diagnosis of barrier dysfunction and assessment of the local synthesis of other proteins within the central nervous system.

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 can be performed on serum (from red top tube) or CSF. Separate serum by centrifugation, 3000 rpm for 15 minutes. CSF should also be spun at 3000 rpm for 15 minutes prior to testing. **Please note the presence of RBC's in CSF as Result Comment in SOFT using the canned message @1CTP.** Serum or CSF aliquots can be stored at 2-8°C for up to 7 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.

Note: Serum for CSF IgG Index calculation should be collected within 5 days of CSF collection.

Standard Aliquot volume = 500 uL
Minimum Aliquot volume = 200 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 Albumin (2ml vial) – REF# OSAL11

Composition:

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

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: **3 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.

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 Albumin assay is the ERM®-DA470 (known as CRM 470). The concentration of Albumin 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. Serum Controls

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

Composition

N/T Protein Controls SL/L, M and H are liquid, stabilized human sera. The concentration of Albumin 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.

2. **CSF Controls**
Biorad Liquicheck Spinal Fluid Control
Level 1 – REF#751 (run on a 1:2 dilution)
Level 2 – REF# 752

Composition

Biorad Liquicheck Spinal Fluid Controls are a human based control with additive constituents of human and animal origin, chemicals and preservatives.

Preparation of the Reagents

Biorad Liquicheck Spinal Fluid Controls are supplied ready-for-use. Allow controls to come to room temperature. Invert gently to mix. **For Level 1 only, prepare a 1:2 dilution with N diluent fresh daily.**

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: **30 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. Serum Albumin is run on BNII channel ALB and CSF is run on channel ALBC.
2. 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.
3. The reagents must not be used beyond the expiration date.

C. Assay of Specimens

1. Routine Samples
   - Serum samples are automatically diluted 1:400 with N Diluent and measured. The diluted samples must be measured within four hours.
   - CSF samples are automatically diluted 1:5 with N Diluent and measured. The diluted samples must be measured within four hours.
   - Serum or CSF 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 both Serum and CSF
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. Material

1. Serum
   - 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

2. CSF
   Biorad Liquicheck Spinal Fluid Control
   Level 1 – REF#751 (run on a 1:2 dilution)
   Level 2 – REF# 752

B. Frequency

1. All 3 levels of serum control and 2 levels of CSF control are to be run at the beginning of each shift or every 8 hours.
2. All 3 levels of serum control and 2 levels of CSF 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).
2. The manufactures suggested limit for deviation from the mean for both Serum and CSF is 15%. Total QC is set up with ranges of +/- 3 standard deviations with one standard deviation equal to 5% 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. 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 Albumin 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 20,000 mg/dL for Serum or 10,000 for CSF, the SOFT LIS will report the result as >20,000 mg/dL for Serum or >10, 000 mg/dL for CSF.
3. If the results obtained are below the measuring range, the assay is automatically repeated **by the instrument using a lower dilution**. For Serum, the lowest dilution the instrument will perform is 1:20. If the reported instrument value is less than 50 mg/dL, the SOFT LIS will report the result as <50 mg/dL. For CSF,

the instrument will run the assay on undiluted sample. If the reported instrument value is less than 2 mg/dL, the SOFT LIS will report the result as <2 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.

Sample Type	Reason Not Autoverified	Action to be taken
Serum	Result <50 mg/dl	Check sample for presence of bubbles or fibrin before manually posting.
CSF	Result <2 mg/dl	

C. Calculations

An IgG Index will be calculated automatically by the SOFT LIS system. The formula for calculation is listed below.

$$\text{CSF Index} = \frac{(\text{CSF IgG})(\text{Serum Albumin})}{(\text{Serum IgG})(\text{CSF Albumin})}$$

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 (CRR) has been fixed to avoid exceeding any lot specific AMR. The AMR's listed below are those at the initial dilution.

Serum:
AMR: 690 – 11000 mg/dL
Initial Dilution: 1:400
Maximum allowable dilution: 1:2000
Minimum allowable dilution: 1:20
Clinical Reportable Range: 50 – 20,000 mg/dL

CSF:
AMR: 0.3 – 10.7 mg/dL
Initial Dilution: 1:1 (undiluted)
Maximum allowable dilution: 1:400
Minimum allowable dilution: 1:1 (undiluted)
Clinical Reportable Range: 2.0 – 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

Serum: all ages 3500-5000 mg/dL
CSF: all ages $\leq$ 35.0 mg/dL
CSF Index: all ages $\leq$ 0.8

XII. Limitations

A. Interferences
1. No interference with the determinations in serum was detected for concentrations of triglycerides up to 20 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. 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 and for both the serum and CSF assays. Error limits were set as follows: Allowable Total Error (TEa): 25%, Systematic Error Budget: 50%, Allowable Systematic Error (SEa): 12.5%.

Serum (ALB) - The accuracy test passed, the maximum deviation for a mean recovery from 100% on both instruments was accurate within the SEa (3.6% and 6.1%). The assay was linear on both instruments within the SEa (1.4% and 1.1% Error).

CSF (ALBC) - The accuracy test passed, the maximum deviation for a mean recovery from 100% on both instruments was accurate within the SEa (6.0% and 7.8%). The assay was linear on both instruments within the SEa (4.6% and 3.8% Error).

Correlation:

Correlation was performed by comparing results of 40 patient serum samples and 15 patient CSF 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:

Serum (ALB) -Slope 1.077 (1.020 to 1.135), Intercept 44.1 (-166.9 to 255.1), R 0.9868

CSF (ALBC) - Slope 1.007 (0.927 to 1.087), Intercept -0.31 (-2.56 to 1.94), R 0.9913

BNII to BNII

Serum (ALB) -Slope 0.965 (0.887 to 1.043), Intercept -168.2 (-482.1 to 145.7), R 0.9694

CSF (ALBC) - Slope 0.981 (0.904 to 1.058), Intercept 0.82 (-1.35 to 2.98), R 0.9914

Precision:

Intrarun Precision: Intra-assay performance was evaluated on both instruments by testing 3 levels of controls for serum ALB and CSF ALB 10 times each on a single run. The acceptable CV limit for intrarun precision is 10%. All controls on both the serum ALB and CSF ALB assays had %CV's of less than 7.0%. Initially the serum High control had a CV of 10.5%. The intra-run precision for this level only was repeated twice with CV's of 4.0% and 5.8%.

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

Reference Range Verification:

To evaluate and determine reference ranges for Serum Albumin, CSF Albumin, CSF IgG and CSF Index a retrospective study was performed using a year's worth of YNHH data for Oligoclonal band testing. The patient data used in the study was selected based on the following: No oligoclonal bands detected in the CSF or serum, normal values for serum IgG, serum Albumin and CSF Albumin. A total of 60 samples qualified for the study.

The results of the study were then evaluated, along with the manufacturer's suggested ranges and ranges from other laboratories. The results are listed in the table below.

	Current	Siemens	Mayo	ARUP	YNHH Chem (DPP)	Historical Data	**Final Range after Data review**	Comment
CSF IgG (mg/dL)	≤4.9	≤3.4	≤8.1	≤6.0	N/A	≤5.27 (90%CI 6.04)	**≤6.0**	Used 90% CI for historical data
Serum ALB (mg/dL)	Not reported	3500-5200	3200-4800	3500-5200	3500-5000	3182-4744	**3500-5000**	Used YNHH Chem range after correlation performed
CSF ALB (mg/dL)	Not reported	≤ 35.0	≤ 27.0	≤ 35.0	N/A	≤ 35.14 (90%CI 39.82)	**≤ 35.0**	Used Siemens range which was confirmed by historical data
CSF Index	≤0.7	N/A	≤0.85	≤0.9	N/A	≤0.74 (90%CI 0.78)	**≤0.8**	Used 90% CI for historical data

CAP Proficiency Results:

Survey C-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 Albumin [package insert]. Newark, DE: Siemens Healthcare Diagnostics; February 2012 Edition.

2. **HYDRAGEL 9 CSF ISOFOCUSING** (Ref. 4355) [package insert]. Norcross, GA : Sebia-USA; 2 February 2011.

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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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