YALE-NEW HAVEN HOSPITAL	TITLE: **Antistreptolysin O (ASL), Siemens BNII Nephelometer** **Soft Code: ASO**		**DEPT OF LAB MEDICINE** **Immunology, Flow Cytometry, and Molecular diagnostcs Laboratories Policy and Procedure Manual** **DOCUMENT # IMM 193** Page 1 of 9
WRITTEN BY: Kathy Radziunas Penny Smith	**EFFECTIVE DATE:** January 22, 2013	**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 antistreptolysin O in human serum by means of immunonephlometry on the BNII system (Siemens). Measurements of antistreptolysin O aid in the diagnosis of disease caused by bacteria belonging to the genus *Streptococcus* and provides epidemiological information on these diseases. Pathogenic streptococci are associated with infections, such as impetigo, urinary tract infections, rheumatic fever, and kidney disease.

II. Introduction

Immunochemical determination of specific antibodies to streptococcal metabolites provides valuable information regarding a past streptococcal infection (rheumatic fever, scarlet fever, tonsillitis, glomerulonephritis, etc.). Although rheumatic fever has become more rare in some regions, the increase in mild or sub-clinical cases requires more detailed evaluations by means of serological methods. Among the assays for antibodies to various streptococcal exoenzymes, antistreptolysin O testing should be given prominence as ASL is a sensitive parameter which has been found to be elevated in 80 to 85 % of the cases. This antibody response is first noted in the second or third week after an acute episode and peaks at about four to five weeks.

III. Principle of the Assay

Polystyrene particles coated with streptolysin O are aggregated when mixed with samples containing human antistreptolysin O. These aggregates scatter a beam of light passed through the sample. The intensity of the scattered light is proportional to the concentration of the respective 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 (from red top tube) or fluid (tube without any anticoagulants). Centrifugation either specimen at 3000 rpm for 15 minutes. 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 specimens. 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

1. N Latex ASL (3.5 mL) –REF OPBU 05

Composition:

N Latex ASL Reagent consists of polystyrene particles coated with streptolysin O.

Preparation:

The N Latex ASL reagent can be used without additional preparation but must be mixed carefully before use, avoid vigorous shaking and foaming.

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. Do not freeze.

On-board stability: 5 days at 8 hours each day or a comparable time period. May vary, depending on the BN II System used and laboratory conditions.

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 Diluent – see BNII Instrument Manual (Doc# IMM 183)
3. Wash solution – see BNII Instrument Manual (Doc# IMM 183)

B. Standards

1. N Rheumatology Standard SL – REF OQKZ13

Composition and Standardization

N Rheumatology Standard SL consists of a mixture of human sera with elevated concentrations of RF, ASL and CRP. The concentration of ASL was calibrated against the reference preparation, 1st International Standard for ASL.The concentration of ASL 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 Rheumatology Control SL/1 – REF OQDB13
   N/T Rheumatology Control SL/2 – REF OQDC13

Composition

N/T Rheumatology Control SL1 and SL/2 consist of a mixture of human sera with elevated concentrations of RF, ASL and CRP with the additive pyrrolidone (approx. 50 g/L).

Preparation of the Reagents

N/T Rheumatology Controls SL are supplied ready-for-use.

Control Storage and Stability:

- Shelf life at +2 to +8 °C:
  The expiry 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.

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 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 1:100 dilution.
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 reagent is used, a new reference curve must be generated.
3. Major instrument maintenance has been performed.

C. How to calibrate

1. Use N Rheumatology 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 Rheumatology Controls
  N N/T Rheumatology Control SL/1 – REF OQDB13
  N/T Rheumatology Control SL/2 – REF OQDC13

B. Frequency

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

C. Quality Control Guidelines

1. **The acceptable percent deviation from the mean for ASL is 20%.**
2. SOFT Total QC (TQC) will be used for QC monitoring. Refer to the Total QC section of the SOFT Immunology procedure (Doc# IMM 120).

3. Total QC is set up with ranges of +/- 3 standard deviations with 1 standard deviation equal to 6.67%.
4. 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 ASL in UI/mL
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 32,000 mg/dL, the SOFT LIS will report the result as >32,000 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 after as <18 mg/dL. **Results should only be reported as <18mg/dL after the sample has been evaluated for the presence of bubbles or fibrin.**
4. **The following comment will be included on each patient report:**

"Normal reference ranges for ASLO titers are highly dependent on the season of the year, age, and geographical location of the patient. Children, especially those in the 4-17 years old range, may have higher values, even up to 650 IU/mL. Interpretation of ASLO results should be based on change in titer. Acute and convalescent titers showing a two-fold or greater increase in ASLO is supportive of recent streptococcal infection."

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 <18 UI/mL	Check sample for presence of bubbles or fibrin 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: 50-1600 UI/mL
Maximum allowable dilution: 1:8000
Minimum allowable dilution: 1:100
Clinical Reportable Range: 18-32000 UI/mL

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

<214 UI/mL

XII. Limitations

A. Interferences

1. No interference with the determinations in serum was detected for concentrations of 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.

XIII. YNHH Method Validation Summary

Accuracy and Linearity:

Accuracy and linearity studies were performed by sequential dilution of the N Rheumatology 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%.

The accuracy test passed, the maximum deviation for a mean recovery from 100% on both instruments was accurate within the SEa (5.1% and 4.6%). The assay was linear on both instruments within the SEa (4.3% and 2.5% Error).

Correlation:

Correlation was performed by comparing results of 41 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.

The BNII showed a positive bias (slope of 1.751) when compared to the Immage 800, which is reflected in the differences in suggested reference ranges by the respective manufacturers and the CAP results for each instrument. The slope of the comparison between CAP means from the Immage and BNII for survey samples from 2010 to 2012 was 1.449.

BNII to Immage 800:

Slope 1.751 (1.668 to 1.835), Intercept 0.32 (-25.58 to 26.23), R 0.9924

BNII to BNII

Slope 0.989(0.984to .995), Intercept 1.12 (-1.92 to 4.16), R .9999

Precision:

Intrarun Precision: Intra-assay performance was evaluated on both instruments by testing 2 levels of control 20 times each on a single run. The acceptable CV limit for

intrarun precision is 10%. Both levels of control had %CV's of less than 2% on each instrument.

Interrun Precision: Inter-assay performance was evaluated on both instruments by testing 2 levels of control on 5 different days. The acceptable CV limit for interrun precision is 20%. Both levels had %CV's of less than 2% on each instrument.

Reference Range Verification:

The manufactures adult reference range of <214 IU/mL (80^{th} percentile) was verified by testing serum from 31 healthy individuals from within the YNHH population. 80.6% of the individuals had levels less than 214 IU/mL.

CAP Proficiency Results:

Proficiency samples from the S-A 2011 and S-C 2011 were tested and all results were acceptable when compared to other BNII users.

XIV References:

1. Siemens N Latex ASL [package insert]. Newark, DE: Siemens Healthcare Diagnostics; January 2010 Edition.

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January 22, 2013

Signature Approval for Annual Review
Name: Antistreptolysin O (ASL)
Siemens BNII Nephlometer

Document #: IMM 193

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	1/22/13	NEW		1/22/13
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