| YALE-NEW HAVEN HOSPITAL | TITLE: **DNase B, Siemens BNII Nephlelometer**<br><br>**Soft Code: DNSB** | | **DEPT OF LAB MEDICINE**<br>**Immunology, Flow Cytometry, and Molecular Diagnostics Laboratories Policy and Procedure Manual**<br>**DOCUMENT # IMM 194**<br>Page 1 of 9 |
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| **WRITTEN BY:**<br>Kathy Radziunas<br>Penny Smith | **EFFECTIVE DATE:**<br>January 22, 2013 | **REVISION:**<br>New | **SUPERCEDES:**<br>**IMM 43 - Immage 800 Quantitation of proteins by Nephelometry** |

## I. Intended Use

*In-vitro* diagnostic reagents for the quantitative determination of anti-streptococcal DNase B (ADNase B) in human serum by means of particle-enhanced immunonephelometry using the BN™ II System.

## II. Introduction

ADNase B antibodies react against the exoenzyme desoxyribonuclease B produced by streptococci. The detection of these antibodies provides evidence of an existing or past streptococcal infection (rheumatic fever, scarlet fever, tonsillitis, glomerulonephritis, and others). The antibody reaction against streptococcal DNase B starts later than the antibody production against streptolysin O, but it can then be detected in a greater percentage of patients[1]. An increase in the antistreptolysin concentration rarely occurs with skin infections, while a rise in the ADNase B titer can be observed.

## III. Principle of the Assay

Immunochemical reaction between streptococcal DNase B and antibodies against streptococcal DNase B bound to polystyrene particles. The presence of antibodies against streptococcal DNase B in the serum causes the added streptococcal DNase B to be bound. The result is either no agglutination or a weakened agglutination of the particles. The absence of antibodies against streptococcal DNase B in the serum results in agglutination of the polystyrene particles by streptococcal DNase B. The higher the content of anti-DNase B in the sample, the lower the intensity of the scattered light. The intensity of the scattered light produced by the agglutinates in the nephelometer is proportional to the anti-streptococcal DNase content, so that the anti-DNase B content of samples can be determined in 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. Serum 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. 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 (N Latex ADNase B REF# OWTI-11 kit containing reagents, standard and control. DO MIX KIT LOTS)

1. N Latex ADNase B reagent (2ml vial)

**Composition:**

N ADNase B Reagent consists of lyophilized polystyrene particles coated with antibodies against streptococcal DNase B from rabbits.

**Preparation:**

**Reconstitute the lyophilized contents of a vial with 2.0 mL of distilled water.** Let stand 15 minutes, Shake carefully to mix before first use.

**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, the reagent 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: Varies. Assay is calibrated daily, discard reagent if calibration or QC is unacceptable.

**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 ADNase B Suplemental Reagent

**Composition:**

N ADNase B Supplementary Reagent consists of a stabilized streptococcal DNase B solution with the addition of rabbit serum.

**Preparation:**

N ADNase B Supplementary Reagent is ready to use and requires no additional preparation.

**Storage:**

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, the reagent 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: Varies. Assay is calibrated daily, discard reagent if calibration or QC is unacceptable.

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

**B. Standards**

**Note: Calibration is required daily.**

1. N Latex ADNase B Standard serum

**Composition and Standardization:**

N Latex ADNase B Standard serum (human) consists of a mixture of human serum. The concentration of ADNase B was calibrated with reference to internal preparations of Siemens Healthcare Diagnostics. **The analytical value for the N ADNase Standard Serum is listed in the product insert for each lot number and must be entered manually into the system.**

**Preparation of the Standard**

N Latex ADNase B Standard serum 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: 4 weeks if stored tightly closed at +2 to +8 °C directly after each use.

## C. Controls

1. N ADNase B Control Serum (human)

**Composition:**

N ADNase B Control Serum (human) consists of a mixture of human serum. The concentration of ADNase B was calibrated with reference to the N ADNase B Standard Serum (human). The assigned value and confidence interval for N ADNase B Control Serum (human) are listed on the Table of Assigned Values insert sheet and must be entered manually into the system.

**Preparation of the Controls**

N ADNase B Control Serum (human) is ready to use and requires no additional preparation.

1:2 Dilution of N ADNase B Control Serum. Prepare a 1:2 dilution by adding 100uL of N diluent to 100 uL of Control Serum.

**Control Storage and Stability:**

Stability at +2 to +8 °C:

See expiry date on label.

Stability once opened:

Four weeks if stored at +2 to +8 °C securely capped immediately after each use.

### D. Consumables

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

## VI. Assay Procedure

**Note: DNASE B will only be run on first shift**

### 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 1:5 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 will be reported as less than 80 U/mL.
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 ADNase Standard are automatically prepared by the instrument using N Diluent. The standard dilutions are to be used within four hours.

2. The reference curve is valid for one day on the BN™ II System. A new reference curve must be generated for every newly reconstituted vial of N ADNase B.

### B. When to Calibrate

1. **Each day the test is to be run.**
2. If a newly reconstituted vial of N ADNase B is put into use.
3. Major instrument maintenance has been performed.

### C. How to calibrate

1. Use N ADNase 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.

## VIII. Quality Control

### A. Quality control Material

- Siemens N ADNase Control serum
- Siemens N ADNase Control serum 1:2
  Add 100uL of N diluent to 100 uL of Control Serum.

### B. Frequency

1. Both levels are to be run following calibration.

### C. Quality Control Guidelines

1. **The acceptable percent deviation from the mean 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. The manufactures mean will be used until at least 30 data points are collected. After 30 points an in-house range will be calculated.

## IX. Interpretation of Results

### A. Reporting Results

1. The instrument automatically calculates and prints the concentration of DNASE B in U/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 50,000 mg/dL, the SOFT LIS will report the result as >50,000 mg/dL.
3. If the result is lower than the AMR, the result will be reported as <80 U/mL. **Results should only be reported as <80 U/mL 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 DNase-B Antibody 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. Interpretation of DNase-B results should be based on change in titer. Acute and convalescent titers showing a two-fold or greater increase in DNase-B Antibody 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 <80 U/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: 75-3000 mg/dL
Initial Dilution: 1:5
Maximum allowable dilution: 1:100
Minimum allowable dilution: 1:5 (same as initial dilution)

Clinical Reportable Range: 80 – 50,000 U/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

<201 U/mL

## XII. Limitations

### A. Interferences

1. Interference from rheumatoid factors (up to 1500 U/mL) are suppressed by the use of N ADNase B Supplementary Reagent. Very high rheumatoid factors concentrations can interfere with the assay, however, in individual cases. These samples must be measured with a different method.
2. Turbidity and particles in the samples may interfere with the determination. Therefore, samples containing particles must be centrifuged prior to testing. Lipemic samples which cannot be clarified by centrifugation (10 minutes at approximately 15,000 x g), as well as heat-inactivated samples 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 ADNase B Standard Serum. 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 (4.3% and 5.3%). The assay was linear on both instruments within the SEa (2.8% and 1.9% Error).

**Correlation:**

Correlation was performed by comparing results of 41 patient samples tested at ARUP 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.

BNII to ARUP:

Slope 0.966 (0.927 to 1.005), Intercept 2.13 (-12.83 to 17.09), R 0.9925

BNII to BNII

Slope 1.013 (0.999 to 1.026), Intercept 0.88 (-5.12 to 6.87), R .9995

**Precision:**

**Intrarun Precision:** Intra-assay performance was evaluated on both instruments by testing the 2 levels of control 10 times on a single run. The acceptable CV limit for

intrarun precision is 10%. Both levels of control had %CV's of less than 6% on each instrument.

**Interrun Precision:** Inter-assay performance was evaluated on both instruments by testing the N ADSN control on 5 different days. The acceptable CV limit for interrun precision is 20%. Both levels had %CV's of less than 9.0% on each instrument.

**Reference Range Verification:**

The manufacturer's suggested reference range of <201 U/mL was verified by assaying 120 YNHH patient samples. The data was analyzed using EP Evaluators Reference Interval Estimation module and reviewed by the medical director.

**CAP Proficiency Results:**

CAP does not offer a proficiency survey for DNASE B. Future proficiency testing will be done by sending samples to ARUP for testing.

## XIV. References:

1. Siemens N Latex ADNase B [package insert]. Newark, DE: Siemens Healthcare Diagnostics; July 2011 Edition.

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**Signature Approval for Annual Review**
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| BRIAN SMITH | LAB DIRECTOR | | 1/18/2013 | NEW | | 1/22/2013 |
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