YALE-NEW HAVEN HOSPITAL	TITLE: **Haptoglobin, Siemens BNII Nephlelometer** **Soft Code: HAPT**		**DEPT OF LAB MEDICINE** **Immunology, Flow Cytometry, and Molecular diagnostcs Laboratories Policy and Procedure Manual** **DOCUMENT # IMM 190** 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 haptoglobin in human serum by means of immunonephlometry on the BNII system (Siemens).

II. Introduction

Haptoglobin binds hemoglobin which is released during erythrocyte lysis. The haptoglobin/hemoglobin complex is rapidly eliminated from the bloodstream. Increased release of hemoglobin due to intravascular hemolysis results in a reduction in the haptoglobin concentrations, and during severe hemolysis, to complete consumption of the haptoglobin. In children haptoglobin has lower physiological concentrations and therefore is not suited for hemolysis testing. Haptoglobin is an acute-phase protein which can develop very high levels during inflammatory conditions.

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. 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. Ex Vivo Hemolysis, such as may occur in blood drawing may result in a decrease in Haptoglobin concentration, therefore samples that are slightly to moderately hemolyzed will be run but the comment listed below will be added to the

result. 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 = 250 uL
Minimum Aliquot volume = 100 uL
Slightly to Moderately Hemolyzed: Add Comment Below

> "Sample hemolyzed. Ex Vivo Hemolysis, such as may occur during blood drawing, may result in a decrease in Haptoglobin concentration."

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 Haptoglobin (2ml vial) – REF# OSAV15

Composition:

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

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 haptoglobin. 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 haptoglobin 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.
4. **Add result comment to all samples slightly or moderately hemolyzed.**

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 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 Haptoglobin 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 14,000 mg/dL, the SOFT LIS will report the result as >14,000 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 <10 mg/dL. **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.

AMR: 26 – 830 mg/dL
Maximum allowable dilution: 1:400

Minimum allowable dilution: 1:5
Clinical Reportable Range: 10 – 14000 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

30-200 mg/dL (all ages)

XII. Limitations

A. Interferences

1. No interference with the determinations in serum was detected for concentrations of triglycerides up to 8.2 g/L, 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%.

The accuracy test passed, the maximum deviation for a mean recovery from 100% on both instruments was accurate within the SEa (9.5% and 7.9%). The assay was linear on both instruments within the SEa (6.2% and 2.5% Error).

Correlation:

Correlation was performed by comparing results of 42 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.

BNII to Immage 800:

Slope 0.890 (0.799 to 0.980), Intercept 14.06 (-5.99 to 34.11), R 0.9573

BNII to BNII

Slope 0.985(0.958 to 1.012), Intercept 3.4 (-2.2 to 9.0), R 0.9969

Precision:

Intrarun Precision: Intra-assay performance was evaluated 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 7% on each instrument.

Interrun Precision: Inter-assay performance was evaluated 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:

The manufactures adult reference range of 30 to 200 mg/dL was verified by testing serum from 25 healthy individuals from within the YNHH population. 96.0% of the individuals had levels within the suggested range which is within the acceptability limit of 90%. The same reference range was verified for ages <18 years by running 20 patient samples from children of various ages. 90.0% of the children had levels within the suggested range (18 of 20).

CAP Proficiency Results:

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

Hemoglobin Interference Study:

The effect of red cell hemolysis was evaluated by measuring Haptoglobin on 14 non-hemolyzed specimens. The samples were then disturbed causing moderate hemolysis and retested. The results showed a slightly negative bias (slope 0.877).

After reviewing the data, the Director of the Immunology Lab was determined that Haptoglobin results for samples with slight to moderate hemolysis will be reported with the following comment:

"Sample hemolyzed. Ex Vivo Hemolysis, such as may occur during blood drawing, may result in a decrease in Haptoglobin concentration."

XIV. References:

1. Siemens N Antisera to Human Transferrin and Haptoglobin [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
TEODORICO LEE	LAB MANAGER	Teodorico Lee	10/5/12	NEW		9/11/12
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