YALE-NEW HAVEN HOSPITAL	TITLE: **Rheumatoid Factor (RF), Siemens BNII Nephelometer Serum or Fluid** **Soft Code: RF, RFF (fluid)**		**DEPT OF LAB MEDICINE** **Immunology, Flow Cytometry, and Molecular diagnostcs Laboratories Policy and Procedure Manual** **DOCUMENT # IMM 191** Page 1 of 9
WRITTEN BY: Kathy Radziunas Penny Smith	**EFFECTIVE DATE:** November 7, 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 rheumatoid factors (RF) in human serum or fluid by means of immunonephlometry on the BNII system (Siemens) as an aid in the diagnosis of rheumatoid arthritis.

II. Introduction

RF's are autoantibodies against the Fc region of human IgG which has been altered in its tertiary structure. These autoantibodies also react with animal IgG. RF belongs predominantly to the IgM class, but they also occur in all of the other immunoglobulin classes.

The detection of RF is one of the criteria of the American Rheumatism Association (ARA) for the diagnosis of rheumatoid arthritis (RA), since 70 - 90 % of patients with RA exhibit rheumatoid factors. RF plays an important role in the differential diagnosis between RA and other rheumatic diseases. Moreover, they permit prognostic statements with regard to RA. High RF concentrations are often associated with a more severe course of disease. There are, however, also seronegative types of RA without detectable RF, and RF can occur in connection with other rheumatic and non-rheumatic diseases such as hepatitis, endocarditis and parasitic or viral infections and other autoimmune diseases. With increasing age there is also an increase in the ratio of RF positive findings without corresponding signs of disease[1]. Therefore, the detection of RF alone cannot serve as diagnosis, but must be interpreted in conjunction with further clinical findings.

III. Principle of the Assay

Polystyrene particles coated with an immunocomplex consisting of human immunoglobulin and anti-human IgG from sheep are aggregated when mixed with samples containing RF. 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). Centrifuge 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 RF Kit – REF# OPCE 03 contains:
   3 X 2 mL N RF Reagent
   3 X 2.4 mL N RF Supplement

Composition:

N RF Reagent consists of a suspension of polystyrene particles coated with an immunocomplex of human-γ-globulin/anti-human-γ-globulin from sheep, i. e., a combination of human and animal γ-globulin.

N RF Supplement consists of an aqueous solution of polyethylene glycol (max. 70 g/L) containing detergent.

Preparation:

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

The N RF Supplement is ready for use.

Storage:

Stability at 2 to 8 °C: See expiration date on label.

Stability once opened: **One week** 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: 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 RF was calibrated against the reference preparation: 1st British Standard 64/002 for RF.

The concentration of RF 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).

The concentration of RF is calibrated to the protein standard preparation and is lot-dependent.

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

Note: Due to the instability of the reagent, RF's will be calibrated every Monday and Thursday. Samples may be run on Monday's and Tuesdays and Thursdays and Fridays as long as the controls are acceptable. Do not run samples on Wednesday. Once open, the reagent is only stable for one week.

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. Fluids are to be assayed in the same manner as serum samples.

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 are reported as <15.9 IU/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 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. Every Monday and Thursday.
2. If the controls are out of range or the Westgard rules stated in the Quality Control procedure (Doc# IMM 37) are violated.
3. If a different lot of reagent is used, a new reference curve must be generated.
4. 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. 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 Rheumatoid Factor 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 60,000 mg/dL, the SOFT LIS will report the result as >60,000 mg/dL.
3. If the result is lower than the AMR, it is reported after as <15.9 mg/dL. **Results should only be reported as <15.9 mg/dL after the sample has been evaluated for the presence of bubbles or fibrin.**
4. The following comment will be added automatically to all fluid RF results: "A reference interval has not been established for body fluid specimens. This test is FDA cleared, but is not labeled for use with body fluid specimens."

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 <15.9 UI/mL	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: 10-640 UI/mL (1:20)
Maximum allowable dilution: 1:2000
Clinical Reportable Range: 15.9-60000 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

Serum: <15.9 UI/mL for serum

Fluids: Not Established

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 (12.1% and 9.5%). The assay was linear on both instruments within the SEa (3.6% and 5.6% 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.

The R value for the BNII to Immage 800 correlation was below 0.95. This was attributed to a noted variability on CAP proficiency samples between the two instruments. To compare the accuracy of our BNII results vs other BNII users, 13 CAP samples were tested and regression analysis was performed using the mean CAP BNII results. The R value for this comparison was greater than 0.95 (see below).

BNII to Immage 800:

Slope 1.367 (1.205 to 1.529), Intercept -87.6 (-205.4 to 30.2), R 0.9302

BNII to CAP BNII Results

Slope 0.873 (0.832 to 0.914), Intercept 3.44 (-3.73 to 10.61), R 0.9978

BNII to BNII

Slope 1.003(0.9997 to 1.009), Intercept -3.0 (-9.0 to 3.0), R 0.9998

Precision:

Intrarun Precision: Intra-assay performance was evaluated on both instruments by testing the 2 levels of controls 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 5% on each instrument.

Interrun Precision: Inter-assay performance was evaluated on both instruments by testing 2 levels of controls on 7 different days. The acceptable CV limit for interrun precision is 20%. Both levels had %CV's of less than 10% on each instrument.

Reference Range Verification:

The manufactures adult reference range of <15.9 IU/mL was verified by testing serum from 25 healthy individuals from within the YNHH population. 95.8% of the individuals tested had RF levels below <15.9 IU/mL which is within the acceptability limit of 90%.

CAP Proficiency Results:

Proficiency samples from the S-A 2012, S-C 2011 and S-A 2011 were tested and all results were acceptable when compared to other BNII users.

References:

1. Siemens N Latex RF Kit [package insert]. Newark, DE: Siemens Healthcare Diagnostics; April 2008 Edition.

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