Laboratory Procedure Manual

Analyte:	**C-Reactive Protein**
Matrix:	**Serum**
Method:	**Nephelometry**
Method No.:	
Revised:	**January 26, 2011**
as performed by:	*University of Washington Medical Center* *Department of Laboratory Medicine* *Immunology Division*
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Important Information for Users

The University of Washington Medical Center Laboratory periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

Public Release Data Set Information

This document details the Lab Protocol for testing the items listed in the following table.

File Name	Variable Name	SAS Label
CRP_F	LBXCRP	C-reactive protein(mg/dL)

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

This method quantifies C-reactive protein (CRP) by latex-enhanced nephelometry. Particle-enhanced assays are based on the reaction between a soluble analyte and the corresponding antigen or antibody bound to polystyrene particles. For the quantification of CRP, particles consisting of a polystyrene core and a hydrophilic shell are used in order to link anti-CRP antibodies covalently. A dilute solution of test sample is mixed with latex particles coated with mouse monoclonal anti-CRP antibodies. CRP present in the test sample will form an antigen-antibody complex with the latex particles.

Light scattering, measured by a nephelometric procedure after 6 min, is proportional to the concentration of the analyte present in the sample. An automatic blank subtraction is performed. CRP concentrations are calculated by using a calibration curve. Data reduction of the signals is performed by using a storable logit-log function for the calibration curve. These assays are performed on a Siemens/Behring Nephelometer for quantitative CRP determination.

The clinical usefulness of quantitative CRP determinations has been demonstrated for various indications. In response to an inflammatory stimulus, a rise of CRP may be detected within 6 to 10 hours, and it may increase by as much as 4000-fold at the peak of the acute phases response (1-3).

Elevated values can be found among people with certain chronic inflammatory diseases, i.e. rheumatoid arthritis, juvenile chronic arthritis, ankylosing spondylitis and Crohn's disease; in diagnosis and therapy of infections, and in premature rupture of membranes or prediction of chorioamnionitis; differential diagnosis of pyelophritis versus cystitis, bacterial versus viral infections, necrotizing pancreatitis versus edematous interstitial pancreatitis; and suspected renal allograft rejection (4-7).

C-reactive protein has been of increasing interest because of the current availability of quantitative assays. CRP has been called the classical acute-phase reactant; in contrast to the erythrocyte sedimentation rate (ESR), it provides a direct measurement of a serum protein that rises and falls rapidly in response to acute inflammation and/or tissue destruction. As a result, although CRP is still a nonspecific indicator, increasing numbers of investigators advocate its quantification for early detection of bacterial infections in a wide variety of clinical settings and for following disease activity and therapy in a number of chronic diseases (e.g., rheumatoid arthritis and inflammatory bowel disease).

Recently, concentrations of CRP have been explored as risk factors for cardiovascular diseases.

2. SAFETY PRECAUTIONS

Consider all samples received for analysis potentially positive for infectious agents including HIV and the hepatitis B virus. Observe universal precautions. Wear gloves, lab coat, and safety glasses when handling all human blood products and infectious viruses. Place disposable plastic, glass, paper, and gloves that contact blood in a biohazard bag or discard pan to be autoclaved. Disinfect all work surfaces with Vesphene. Dispose of diluted specimens and any other potentially contaminated materials in a biohazard bag at the end of the analysis to be autoclaved prior to final disposal. Autoclaved or disinfect other non-disposable material at the end of the working day.

Do not pipette by mouth. Do not eat, drink or smoke in designated work areas. Wash hands thoroughly after removal of personal protective devices used in handling specimens and kit reagents.

Material safety data sheets for all reagents used in the performance of this assay are kept in the Immunology Division, University of Washington Medical Center (UWMC).

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

a. Each shipment of specimens received from the NHANES mobile unit arrives with a corresponding transmittal sheet and an electronic version of the shipping/resulting file. The file structure is determined by NHANES and is described in the National Health and Nutrition Examination Survey (NHANES) Contract Laboratory Manual.

b. After the testing is completed results from the BNII are transferred to the laboratory server system, which is backed up daily. This instrument file contains the following information for each sample, control and calibrator tested.

Patient ID
Sample ID
Date and time of calibration
Test name and number
Cuvette number
Sup reagent lot number
Reagent lot number
Dilution
Start bit value
Pre-reaction bit value
Final value with units
Date
Completion time

c. QC results are transferred to an Excel file using laboratory-developed software. This file calculates the QC statistics, plots Levey-Jennings charts, displays relevant instrument flags, tracks reagent lots and recent calibrations. QC results are reviewed prior to resulting samples.

d. Sample results are transferred to an Excel file using laboratory-developed software that enters results after matching sample identifiers from the instrument file with those provided in the NHANES shipping/resulting file. This Excel file is formatted to match the NHANES shipping/resulting file and the program uses the conventions outlined in the NHANES Contract Laboratory Manual.

e. Data entry is checked for errors.

f. The result file is transmitted electronically to NHANES WESTAT. Electronic and hard copies of the files are kept in the laboratory.

g. Technical support for this system is provided by Westat, Rockville, MD (1-301-294-2036)

4. SPECIMEN COLLECTION, STORAGE, AND HANDLING PROCEDURES; CRITERIA FOR SPECIMEN REJECTION

a. No special instructions such as fasting or special diets are required.

b. Fresh or frozen human serum, heparin and EDTA plasma samples are acceptable. Specimens should be frozen at $\leq$-20 ^{o}C if testing is not done within 24 hours of

collection.

c. Blood should be collected aseptically and the serum separated by standard laboratory techniques. Specimens may be collected by using regular or serum-separator Vacutainers. Serum or plasma should be separated from the cells within 60 minutes of collection.

d. The requested sample volume for the assay is 1.0 mL, and the minimum sample volume is 0.3 mL.

e. Specimens may be stored in glass or plastic vials, as long as the vials are tightly sealed to prevent desiccation of the sample.

f. Contamination or introduced particulate matter can lead to erroneous results. Heat inactivated specimens should not be used. Very lipemic specimens should be clarified by centrifugation (10 minutes at approximately 15,000 g) prior to testing.

g. Avoid repeated freeze/thaw cycles.

5. PROCEDURES FOR MICROSCOPIC EXAMINATIONS; CRITERIA FOR REJECTION OF INADEQUATELY PREPARED SLIDES

Not applicable for this procedure.

6. PREPARATION OF REAGENTS, CALIBRATORS (STANDARDS), CONTROLS, AND ALL OTHER MATERIALS; EQUIPMENT AND INSTRUMENTATION

a. Reagents and Standard Materials

All reagents are purchased from Siemens Healthcare Diagnostics, 1717 Deerfield Road, Deerfield, IL 60015-0778, USA

1. N Latex CardioPhase hsCRP Reagent, cat. #OQIY 21
   Each vial contains a solution of polystyrene particles coated with mouse monoclonal anti-CRP and preservatives: Gentamicin 6.25 mg/L, Amphotericin 0.625 mg/L. The reagent is ready to use and is stable until the manufacturer's expiration date. Store at 4-8 ^{o}C Avoid freezing.

2. Rheumatology std SL, cat # OQKZ 13
   The reagent is ready to use and unopened it is stable until the manufacturer's expiration date. Once opened, the standard can be used for at least two weeks provided it is stored well-closed and promptly refrigerated after use. Lot specific calibrator values are provided. Store at 4-8 ^{o}C. Avoid freezing.

3. N Supplementary Reagent/Precipitation, cat #OUMU15
   The reagent is ready to use and is stable until the manufacturer's expiration date. Store at 4-8 ^{o}C. Avoid freezing.

4. N Diluent, cat # OUMT 65
   The reagent is ready to use and is stable until the manufacturer's expiration date. Store at room temperature.

5. N Rx buffer. cat # OUMS65
   The reagent is ready to use and is stable until the manufacturer's expiration date. Store at room temperature.

c. Instrumentation

1. Siemens/Behring Nephelometer II Analyzer System (BNII) with a 3-channel, 3-valve dilutor with 2500-uL, 1000 uL and 250-uL syringes, 840 nm ± 25 wavelength analyzer and terminal equipped with a Power Macintosh 7200/75 computer (Siemens Heathcare Diagnostics, Inc. New Castle, DE).

   The instrument is fully automated. The analyzer includes a dilutor with temperature controlled (37 ^{o}C) transfer arms; reagent, sample, standards rack stations (with barcode reading); buffer compartment; dilution racks; temperature controlled (37 ^{o}C) cuvette rotor; cuvette washing device; bar code wand reader; and optical system.

   The CRP assay parameter settings for the BNII instrument are as follows:

Parameter	Setting
Protein Name	CRP
Sample Dilution*	1:100
N Supplemental reagent	5 uL
Latex reagent	40 uL
Buffer for Reagent	60 uL N Diluent
No. of Standard Points	6
Standard Dilutions	1:80-1:2560
Standard Curve Measuring Range (At initial dilution; approximate values, range is dependent upon standard value)	0.10- 5.50 mg/dL

*Automatic sample predilution with N Diluent

2. BNII dilution wells, Cat # OVIC11

3. Air Driven Ultracentrifuge, model 340400 (Beckman Instruments, Fullerton, CA).

4. Computers (Dell Computer Corporation, Round Rock, Texas).

5. Pipettors and disposable tips (Rainin, Emeryville, CA).

6. Gloves, disposable (Any manufacturer).

d. Standards/Calibration Preparation

N Rheumatology Standard SL
The standard is provided ready for use. The standard was prepared by Siemens/Behring Diagnostics and standardized against the WHO International Reference Preparation (IRP) of C-reactive protein serum, available from the National Institute of Biological Standards and Controls, London, UK. This material is an internationally recognized source of purified human C-reactive protein.

e. Preparation of Quality Control Materials

Two levels of control materials are purchased from BioRad (Hercules, CA) and third control is lab prepared. New controls are analyzed for at least 20 runs in parallel with the current control. Purchase/prepare sufficient quantity to provide QC for 2 years, and store vials at -20 ^{o}C or colder until needed. Thaw vials as needed, transfer contents to plastic tube labeled with control name, lot #, and thaw date. Store thawed controls at 2-8 ^{o}C for up to 30 days.

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

a. Calibration Curve

The standard is diluted 1:80, 1:160, 1:320, 1:640, 1:1280, and 1:2560 by the instrument. These dilutions must be used within 4 hours of preparation.

Light scattering is measured with an automatic blank subtraction. CRP concentrations are calculated by using a calibration curve. Data reduction of the signals is performed by using a storable logit-log function for the calibration curve. This method results in a linearized 6-point (including zero) standard curve with a direct relationship of measured light scatter to concentration of C-reactive protein in the serum sample. Serum results are expressed as mg/dL.

A valid standard curve for the CRP assay must be stored in the BNII memory before sample results can be quantified. Reference curves are determined monthly or whenever new lot numbers of CRP latex are placed into use.

b. Verification

1. Three levels of control are run for each test series. If, within a testing series, these controls do not conform to specifications as defined in the quality control manual, the entire series is invalidated.

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

a. Preliminaries

1. Bring all controls and patient specimens to room temperature before use. Mix any specimens or controls that have been frozen.

2. Check the levels of buffer and diluent. Verify that the dilution wells are empty.

3. Turn power on BN2 and computer, the instrument will automatically initialize and prime.

b. Instrument Operation (see operator's manual for BN II for more details).

1. Gently mix, uncap and load specimens into serum racks, with the barcode in the open slot. Make sure there are no bubbles. Load the racks in the specimen lanes.

2. Uncap and load reagents in the reagent racks, with the barcode in the open slot. Load the rack in a reagent lane. (Note: reagents do not need to be at room temperature.)

3. Select the CRP NHNS test for all specimens. Testing is done in singlicate. The instrument will automatically rerun the specimen with a higher or lower dilution if the initial result is outside the range of the standard curve.

4. The instrument automatically calculates all results. After testing is completed, results are printed and review by the technologist.

5. Remove specimens, controls, and reagents. Return controls and reagents to the refrigerator

6. Perform scheduled instrument maintenance (daily, weekly, and monthly) as outlined on the maintenance log. See the operator's manual for specific instructions.

7. Follow the on-screen instructions for shutting the instrument down.

c. Recording of Data

1 Using a lab developed program, specimen results are transferred from the instrument data file into the assay specific results table created from the send file corresponding to the specific sample box. The file format is Excel (Microsoft Corporation, Redmond WA). A copy of this file is printed out and checked for accuracy of data entry.

2. Control results are entered to the Assay Specific QC/Levy-Jennings Table using the Excel program. Compliance with the Westgard rules is evaluated. A copy of this table is printed out and checked for accuracy of data entry.

d. Replacement and Periodic Maintenance of Key Components

1. Daily Maintenance:
-Inspect tubing, syringes, and valves for bubbles or leaking during start-up.
-Clean rotor cover and dilution frame
-Replace used dilution cups
-Clean dispense probes

2. Monthly Maintenance:
-Replace cuvettes
-Clean level sensors
-Clean barcode scanner
-Clean mouse
-Decontaminate the fluid system

3. Semi-annual Maintenance
-Replace syringe

4. Periodic Maintenance to be performed by the manufacturer's service engineer.

9. REPORTABLE RANGE OF TEST RESULTS

Results are reported to the nearest hundredth (0.01). The lowest reportable CRP result is approximately 0.02 mg/dL. This will vary slightly with different calibrator lots. The assay does not have a maximum reportable limit since the instrument automatically prepares a higher dilution and retests specimens with results above the linearity of the assay to obtain reactions within the linear range for the assay.

Estimates of imprecision can be generated from long-term quality control pool results.

10. QUALITY CONTROL (QC) PROCEDURES

a. The method described in this protocol has been used for several years. The method has proven to be accurate, precise, and reliable. The instrumentation used is state-of-the-art. The primary standard used was prepared by Siemens/Behring Diagnostics and standardized against the WHO International Reference Preparation (IRP) of C-reactive protein serum, available from the National Institute of Biological Standards and Controls, UK. This material is an internationally recognized source of purified human C-reactive protein. Estimates of imprecision can be generated from long-term

quality control pool results.

b. Bench quality controls are used in this analytical method. Bench quality control specimens are tested with each analytical run (a set of consecutive assays performed without interruption) so that judgments may be made on the day of analysis. The data from these materials are then used in estimating methodological imprecision and in assessing the magnitude of any time-associated trends.

c. The bench controls are purchased in sufficient quantity to provide serum samples for all the assays for approximately 1 year. Ranges are established after 20 parallel runs with previously established controls. The quality control pools comprise two levels of concentration spanning the borderline and high ranges for C-reactive protein.

d. Bench quality controls are placed at the beginning of each analytical run. After analysis, the long-term quality control charts (Levey-Jennings) for each control material are consulted to determine if the system is in control. The Levey Jennings chart plots the quality control material observations on the y-axis and the date of the observation on the x-axis. Quality control material observations are compared with the 95% and 99% confidence limits as well as with the center line (the overall mean of the characterization runs) prior to reporting any results. The system is out of control if any of the following events occur for any one of the quality control materials:

   -The observation from a single pool falls outside the 99% confidence limits.
   -The observations from two pools fall either both above or both below the 95% confidence limits.
   -The observations from eight successive runs for one pool fall either all above or all below the center-line and the current result is above or below the 95% confidence limits.

11. REMEDIAL ACTION IF CALIBRATION OR QC SYSTEMS FAIL TO MEET ACCEPTABLE CRITERIA

If the run is declared "out of control", the system (instrument, calibration standards, etc.) is investigated to determine the root of the problem before any results are released. Consult with the supervisor for appropriate actions.

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

a. The upper reportable value is virtually unlimited. The upper limit of this assay=s default dilution is determined by the calibration material supplied by the manufacturer. Values exceeding this upper limit are repeated on dilution until values, prior to correction for dilution, fall between approximately 0.10-5.50 mg/dL

b. The lowest reportable value is approximately 0.02 mg/dL. The lower limit of this assay=s default dilution is determined by the calibration material supplied by the manufacturer. Values exceeding this lower limit are repeated on a decreased dilution until values, prior to correction for dilution, fall between approximately 0.10-5.50 mg/dL until the lower reportable limit is reached.

c. Avoid contamination of assay reagents and disposables by particulate matter, especially dust and lint. Cover all reagents immediately after use.

d. Clear serum samples are recommended for analysis. Markedly increased serum lipids can interfere with nephelometric determinations. If such interference is suspected, centrifuge samples before assaying.

e. In manufacturer's studies no interference was seen with bilirubin levels up to 600mg/L, hemoglobin levels up to 10 g/L and triglycerides up to 16 g/L.

f. Specimens from patients with human antimouse antibodies (HAMA) could react with the mouse antibody coating the polystyrene beads, leading to falsely elevated results. HAMA is more common in patients previously treated with mouse proteins.

13. REFERENCE RANGES (NORMAL VALUES)

The reference range was determined to be 0-1.0 mg/dL in normal healthy adults by in-house testing of serum from over 300 patients from March 1990 to April 1990. NOTE: while the trend of CRP response during inflammatory processes is predictable, the degree of change varies from person to person, and without baseline measurements, it can be difficult to interpret. For example, undiagnosed disease processes may be contributing to an observed acute-phase response. Pregnancy or the use of intrauterine devices or hormonal contraceptives may also raise CRP concentrations. "Normal" values should be used only as a guide by the physician and must be interpreted together with other clinical signs and symptoms. Data from NHANESIII suggests that age, sex, and race or ethnicity influence the upper limit of the reference range of CRP.

14. CRITICAL CALL RESULTS ("PANIC VALUES")

Not applicable to this procedure.

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens should be maintained at 20-25 ^{o}C during testing. After testing, the samples are stored at -70 ^{o}C or colder.

16. ALTERNATIVE METHODS FOR PERFORMING TEST OR STORING SPECIMENS IF TEST SYSTEM FAILS

There are no acceptable alternative methods of analysis. Specimens may be stored at 4-8 ^{o}C for no longer than 8 days. Otherwise, specimens should be stored -70 ^{o}C or colder until the system is returned to functionality.

17. TEST RESULT REPORTING SYSTEM; PROTOCOL FOR REPORTING CRITICAL CALLS (IF APPLICABLE)

Not applicable to this procedure.

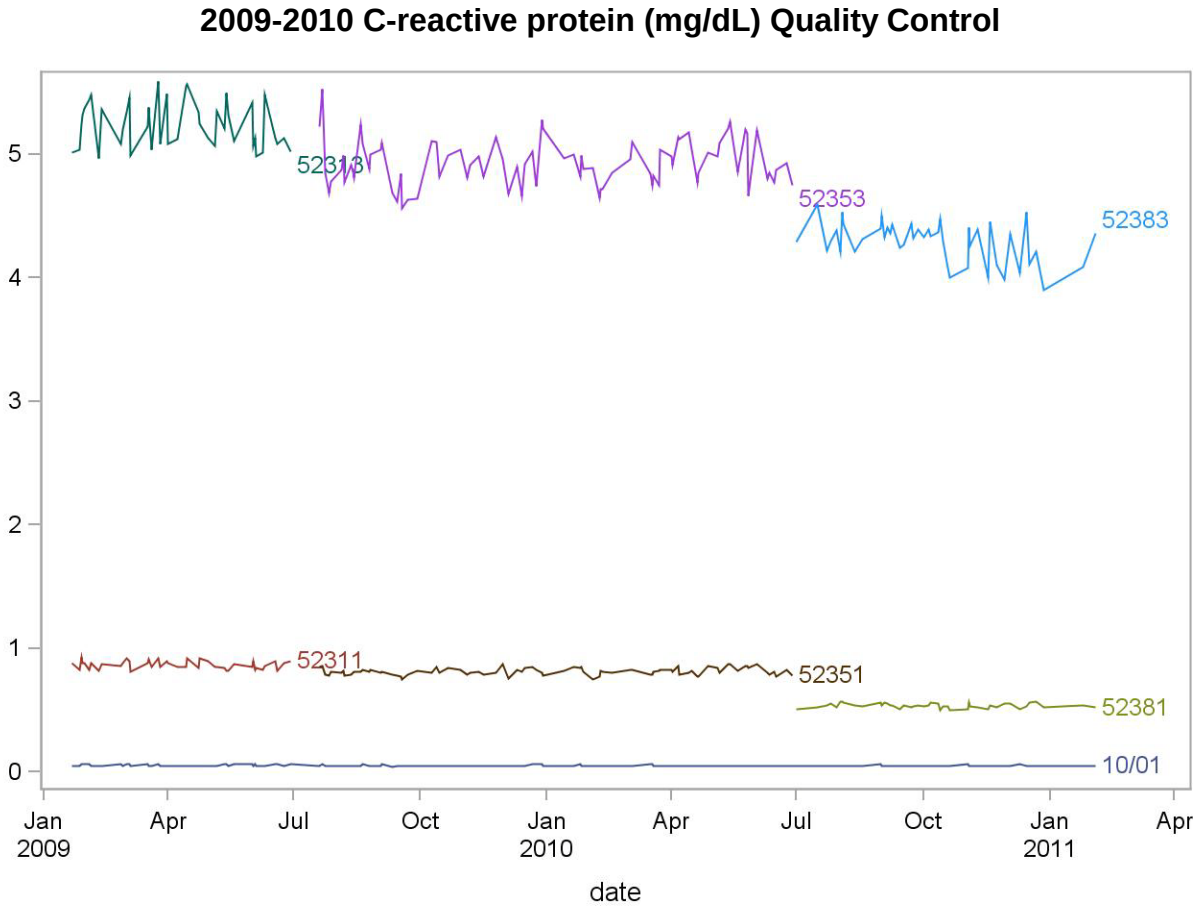
18. TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

Standard record keeping should be used for tracking specimens. Samples are inspected upon arrival and new boxes are added to an Excel worksheet (sample log) used to track boxes. This sample log is used to track the status of testing and resulting.

The residual serum is stored at $\leq$ -70 ^{o}C for 6 months after analysis, then it is returned to the NHANES Repository in Rockville, MD for long-term storage.

19. SUMMARY STATISTICS AND QC GRAPHS

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
52313	43	21JAN09	29JUN09	5.236	0.187	3.6
52311	43	21JAN09	29JUN09	0.867	0.033	3.8
10/01	171	21JAN09	03FEB11	0.052	0.004	7.5
52353	84	20JUL09	28JUN10	4.929	0.186	3.8
52351	83	20JUL09	28JUN10	0.813	0.029	3.6
52383	46	01JUL10	03FEB11	4.285	0.167	3.9
52381	46	01JUL10	03FEB11	0.535	0.019	3.6



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