



Data Collection Worksheet

Please Note: The Data Collection Worksheet (DCW) is a tool to aid integration of a PhenX protocol into a study. The PhenX DCW is not designed to be a data collection instrument. Investigators will need to decide the best way to collect data for the PhenX protocol in their study. Variables captured in the DCW, along with variable names and unique PhenX variable identifiers, are included in the PhenX Data Dictionary (DD) files.

1. 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 $<-20\text{ }^{\circ}\text{C}$ 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.

2. 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

°C. Avoid freezing.

2. Rheumatology std SL, cat # OQKZ 13: The reagent is ready to use; 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 °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 °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.

b. 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 Healthcare Diagnostics, Inc., New Castle, DE).

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

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	1:801:2560
Standard Curve Measuring Range (At initial dilution; approximate values, range is dependent upon standard value)	0.105.50 mg/dL

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

*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, TX).
5. Pipettors and disposable tips (Rainin, Emeryville, CA).
6. Gloves, disposable (Any manufacturer).

c. 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.

d. Preparation of Quality Control Materials

Two levels of control materials are purchased from BioRad (Hercules, CA), and the 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 to store vials at -20 °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 °C for up to 30 days.

3. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

a. Calibration Curve

Prepare instrument recommended standard dilutions. 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 (including zero) standard curve with a direct relationship of measured light scatter to concentration of C-reactive protein in the serum sample.

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.

4. 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 instrument and computer; the instrument will automatically initialize and prime.

b. Instrument Operation

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

2. Uncap and load reagents in the reagent racks. Load the rack in a reagent lane.

3. Select the appropriate C-reactive protein test for all specimens.

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

5. Remove specimens, controls, and reagents. Return controls and

reagents to the refrigerator.

5. 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.

6. 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.

7. 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 600 mg/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.

8. 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 NHANES III suggest that age, sex, and race or ethnicity influence the upper limit of the reference range of CRP.

9. SPECIMEN STORAGE AND HANDLING DURING TESTING

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

Protocol source: <https://www.phenxtoolkit.org/protocols/view/660601>