Laboratory Procedure Manual

Analyte:	**High Sensitivity C-Reactive Protein (HS-CRP)**
Matrix:	**Frozen Serum**
Method:	**Beckman UniCel® DxC 600 Synchron & Beckman UniCel® DxC 660i Synchron Access Clinical Systems (Identical Method)**
Method No.:	
Revised:	
As performed by:	*Collaborative Laboratory Services, L.L.C*
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Important Information for Users

Collaborative Laboratory Services 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	Analyte Description
HSCRP_I	LBXHSCRP	High-Sensitivity C-Reactive Protein (hs-CRP) (mg/L)

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

Blood levels of C-Reactive Protein (CRP) are known to rise rapidly from normal baseline levels of < 3 mg/L to as high as 500 mg/L as part of the body's non-specific inflammatory response to infection or injury. In more recent years, the utility of measuring CRP has expanded from its historical use as a sensitive marker of acute inflammation to include assessment of cardiac events and risk.

Measurement of C-Reactive protein (CRP) aids in evaluation of stress, trauma, infection, inflammation, surgery, and associated diseases. Cardiac CRP assays are indicated for use as an aid in the identification and stratification of individuals at risk for future cardiovascular disease. When used in conjunction with traditional clinical laboratory evaluation of acute coronary syndromes, CRP may be useful as an independent marker of prognosis for recurrent events in patients with stable coronary disease or acute coronary syndrome.

2. SAFETY PRECAUTIONS

Consider all plasma or serum specimens potentially positive for infectious agents including HIV and the hepatitis B virus. We recommend the hepatitis B vaccination series for all analysts working with whole blood and/or plasma. Observe universal precautions; wear protective gloves, laboratory coats. Place disposable plastic, glass, and paper (pipette tips, gloves, etc.) that contact plasma and any residual sample material in a biohazard bag and keep these bags in appropriate containers until disposal by maceration chlorination. Wipe down all work surfaces with Germicidal Disposable Wipe when work is finished.

Handle acids and bases with extreme care; they are caustic and toxic. Handle organic solvents only in a well-ventilated area or, as required, under a chemical fume hood.

Reagents and solvents used in this study include those listed in Section 6. Material safety data sheets (MSDSs) for these chemicals are readily accessible as hard copies in the lab.

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

A. Microsoft Excel software on a PC and our Laboratory Information Systems (L.I.S.) are used to manage the data. The test is analyzed on a Beckman Coulter UniCel® DXC 600/660i Synchron Clinical System. The DXC 600/660i is interfaced to the Laboratory Information Systems (L.I.S.) with a bi-directional interface. After tests are completed, the results will go to the L.I.S. Host Computer Interface to be verified by qualified analyst.

B. Reflex testing is set up in the L.I.S. to order a repeat of any critical result, to verify abnormal values.

C. Statistical evaluation of the runs is accomplished with Microsoft Excel software on a PC.

D. A result file is generated in the L.I.S. database. The file is opened and copied to an Excel spreadsheet for evaluation. The run numbers, and date specimens were received are entered into the Excel file. The Excel spreadsheet results file data are copied to the shipment Excel file and sent using Internet FTP transfer of files or e-mailed to Westat within 21 days of sample receipt.

E. The Excel files containing all raw data and results are backed up once a week using a CD writer or External drive for storage. Files stored on the L.I.S. network are automatically backed up nightly to tape.

F. Documentation for data system maintenance is contained in printed copies of data records, as well as in "system log" files on the local hard drives used for the archival of data.

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

A. *Interferences:*

1) No significant Interference for bilirubin within ±0.2 mg/L or ±10%

2) Lipemic specimens should be delipidated by ultra-centrifugation (90,000 x g for 10 minutes) prior to determination of CRP concentration.

B. Fasting is not required.

C. A minimum of 0.6 mL serum is needed for the Multi-Analyte Panel.

D. Sample volume for individual test is 10 µl added to 255 µL reagent.

E. Sample is run singly as part of Multi-analyte Biochemistry Panel.

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

Not applicable for this procedure

6. EQUIPMENT AND INSTRUMENTATION, MATERIALS, REAGENT PREPARATION, CALIBRATORS (STANDARDS), AND CONTROLS

A. Instrumentation: Instrumentation: Beckman Coulter UniCel® DxC 600 & DxC 660i Synchron Clinical Systems

B. Materials

1) Beckman Micro Tube (*Part #448774*)

2) S/P Plastic Transfer Pipette (*Cat. #P5214-10*)

3) S/P Brand Accutube Flange Caps (*Cat. #T1226-37*)

C. Reagent Preparation: Beckman Synchron Systems CRP Reagent (*Part #442635, 200 tests/cartridge or #476836 400 tests/cartridge*).

1) Gently invert cartridge several times to mix.

2) Check for bubbles or foam in compartments; break any bubbles.

3) CRPH reagent, when stored unopened at +2°C to +8°C, will obtain the shelf-life indicated on the cartridge label.

4) Once opened, the reagent is stable for 60 days at +2°C to +8°C unless the expiration date is exceeded.

5) Unopened reagent is stable until expiration date when stored at 2-8°C.

D. Standards Preparation: None required.

E. Control Material

1) Bio-Rad Liquid Unassayed Multiqual (Cat. #697, 699).

- Thaw bottle of control and mix very well.
- Thawed control is stable 7 days. Mix well prior to each use.

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

A. Calibrators: SYNCHRON® Systems CAL 5 Plus.

1) Refer to UniCel DXC 600/660i System *Instructions For Use (IFU)* manual for storage and stability information.

B. Calibration:

1) SYNCHRON® Systems CAL 5 Plus is stable until the expiration date printed on the calibrator bottle if stored capped in the original container at +2°C to +8°C.

2) Calibration frequency: every 30 days.

3) Required after certain parts replacement or maintenance procedures

4) Refer to UniCel DXC 600/660i System *Instructions For Use (IFU)* manual or *DXC660i and DxC600 Operating Procedure* for programming a calibration

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

A. Preliminaries

1) Enter test in L.I.S. as a part of a panel according to procedure listed in this document (*See Attachment A*).

B. Sample Preparation

1) Procedure for labeling Micro tube (CX tube) and transferring serum (See Attachment B).

C. Operation

1) Refer to Operation Procedures for programming controls/patients and loading sectors/racks in the Beckman Coulter Synchron UniCel DXC 600/660i System *Instructions For Use (IFU)* manual or *DXC660i and DxC600 Operating Procedure. (See Attachment C* for specific procedure for NHANES samples).

D. Recording of Data

1) Operator will review and verify results in the L.I.S.

2) The L.I.S. reorders tests to verify any critical results. These results are stored in the L.I.S. along with the original results. Original values are used when repeat results match the original within 3 CV.

3) Project supervisor will export data from the L.I.S. into an Excel file. The data is copied in into another Excel file for further evaluation.

4) An Excel spreadsheet printout of the results for each container ID is made and comments noted.

5) Project supervisor reviews the results. If problems noted with results or QC, Project Supervisor investigates and discusses issues if necessary with Laboratory Director. Repeat samples if necessary.

6) Daily log sheets are completed and any problems or issues noted.

E. Replacement and Periodic Maintenance of Key Components (*See Attachment D for* DXC660i Maintenance Schedule).

F. Calculations

Synchron Systems perform all calculations internally to produce the final reported result. The system will calculate the final result for sample dilutions made by the operator when the dilution factor is entered into the system during sample programming.

9. REPORTABLE RANGE OF RESULTS

A. Analytical Range:

1) 0.20 – 80.00 mg/L, or up to 380.00 mg/L with ORDAC enabled.

2) Samples which are out of ORDAC (Overrange Detection and Correction) range high should be reanalyzed after doing a manual dilution of the sample with saline. The dilution factor must be entered into the sample information. If the dilution factor is not entered into the system, the printout must be multiplied by the dilution factor to obtain the final answer.

3) Limits of detection (LOD) are established by Beckman-Coulter and linearity data verifies the reportable range. Detection of results below the reportable range is not relevant and formal limit of detection study is unnecessary.

4) Sensitivity is defined as the lowest measurable concentration which can be distinguished from zero with 95% confidence. Sensitivity for the C-reactive protein is 0.11 mg/L.

5) 0 is not a reportable value.

10. QUALITY CONTROL (QC) PROCEDURES

A. Blind QC Specimens are included in the samples received from NHANES.

B. Controls are assayed in early A.M. and if a new reagent pack is loaded, controls are assayed again. One level is assayed in middle of the day and both control levels are assayed after running NHANES samples.

C. BioRad Liquid Unassayed Multiqual Controls Levels 1 and 3 are assayed for CDC-NHANES runs to allow long term control use. Multiqual controls are analyzed at beginning and end of runs with CDC-NHANES samples.

D. Acceptable Answer:

1) Controls must be within ±2 S.D.

2) Refer to Quality Control Flow Chart for action decisions guidelines (*See Attachment F*).

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

Remedial action for out of control conditions includes examination of the pipetting and detection equipment and examination of reagent materials. The QC parameters are compared to the patient means to look for confirmatory or disconfirmatory evidence. When the 2 2s and/or 1 3s rules are violated, samples are repeated following corrective maintenance or reagent changes.

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

A. Lipemic specimens should be delipidated by ultra-centrifugation (90,000 x g for 10 minutes) prior to determination of hs CRP concentration.

B. Dust particles or other particulate matter (i.e. debris and bacteria) in the reaction solution may result in extraneous light-scattering signals, resulting in variable sample analysis.

C. For assays employing mouse antibodies, the possibility exists for interference by human anti-mouse antibodies (HAMA) in the sample. Human anti-mouse antibodies may be present in samples from patients who have received immunotherapy or diagnostic procedures utilizing monoclonal antibodies or in individuals who have been regularly exposed to animals. Additionally, other heterophile antibodies, such as human anti-goat antibodies may be present in patient samples. Interpretation of results should be done in the context of the overall clinical presentation of the patient, including symptoms, clinical history, data from additional tests and other appropriate information.

D. Refer to References for other interferences caused by drugs, disease and preanalytical variables.

13. REFERENCE RANGES (NORMAL VALUES)

High-Sensitivity C-Reactive Protein (hs CRP)

Serum or Plasma	Reference Range
HS-CRP	< 7.48 mg/L

Reference Range values were established from wellness participants with an age mix similar to our patients. These data were analyzed using non-parametric techniques described by Reed (Clin Chem 1971;17:275) and Herrara (J Lab Clin Med 1958;52:34-42) which are summarized in recent editions of Tietz' textbook. Descriptions appear in Clin Chem 1988; 34:1447 and Clinics in Laboratory Medicine June 1993; 13:481.

Pediatric Reference Range Guidelines for Synchron Systems- Multicenter study using data from Montreal, Quebec, Miami, FL and Denver, CO. Beckman 1995

14. CRITICAL CALL RESULTS ("PANIC VALUES")

There are no critical call back values for HS C-Reactive Protein (CRP).

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens arrive frozen. Specimens are kept frozen until ready to transfer to Micro tubes. Capped Micro tubes are kept refrigerated until ready to put on instrument.

Specimen vials are returned to container and refrigerated after transfer of aliquot and double checking of pour off tubes. Specimen vial container is placed in -70°C Freezer after testing is complete. Micro tubes are refrigerated, and then frozen after analysis.

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

Samples will remain in refrigerator until instrument is back in operation.

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

The collaborating agency with access to patient identifiers or the responsible medical officer receives an Excel file with all results for a specimen with any critical values. These files with critical values are sent in advance of results that are not abnormal, unless all results are ready to send at the same time. The earliest reporting of results would be the day after arrival of specimens. More frequently two to three days after receiving specimens.

Test results that are not abnormal are reported to the collaborating agency at a frequency and by a method determined by the study coordinator. Generally, data from this analysis are compiled with results from other analyses and sent to the responsible person at the collaborating agency as an Excel file, either through Internet FTP transfer of files or electronic mail or other electronic means.

All data are reported electronically to Westat within 21 days of receipt of specimens.

Internet FTP transfer of files is available and is preferred for data transfer.

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

In general, when specimens are received, the specimen ID number, and a name identifying the container ID and slot number is entered into the Laboratory Information System (L.I.S.) database. New barcodes are printed and the specimens stored in a refrigerator. Samples are aliquoted to a Micro tube with the new barcodes. The specimen ID is read off of the tube by a barcode reader. Tracked in the database are the date and time of entry into the L.I.S., date and time analysis completed, and who certified the results.

Microsoft Excel spreadsheets are used to keep records and track specimens with the data taken from the Laboratory Information System. Logs are kept including information of when samples arrive, are processed and tested, when frozen after testing, and when returned to NHANES for long term storage.

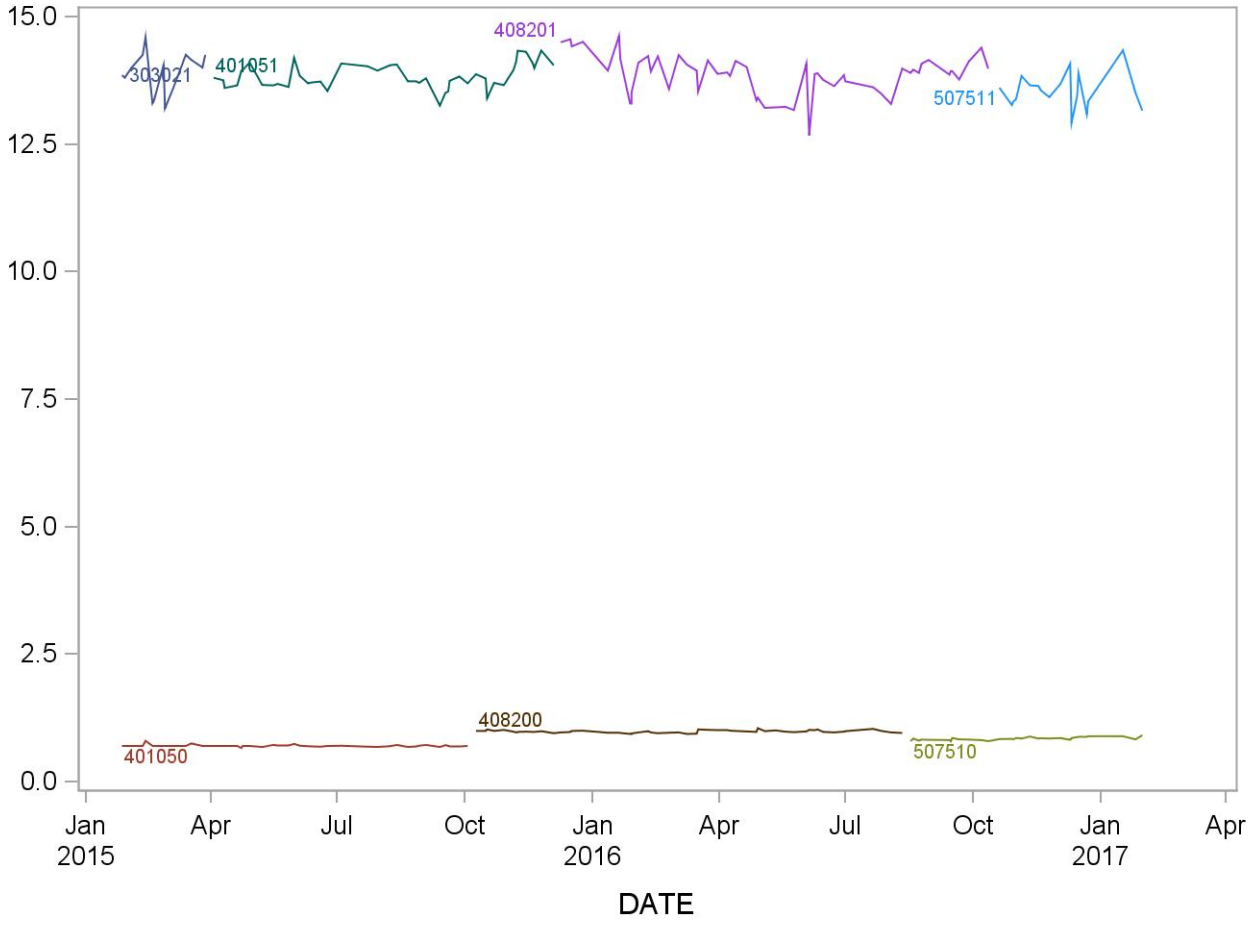
The Project supervisor is responsible for keeping a logbook containing the ID numbers of specimens prepared incorrectly, those with labeling problems, and those with abnormal results, together with information about these discrepancies. It is recommended that records, including related QA/QC data, be maintained for 10 years after completion of the NHANES study.

19. SUMMARY STATISTICS AND QC GRAPHS

See following pages

2015-2016 Summary Statistics and QC Chart for C-Reactive Protein (mg/dL)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
303021	14	27JAN15	28MAR15	13.90	0.41	3.0
401050	46	27JAN15	03OCT15	0.70	0.02	3.0
401051	45	03APR15	04DEC15	13.83	0.24	1.8
408200	56	09OCT15	11AUG16	0.98	0.03	2.7
408201	55	09DEC15	12OCT16	13.85	0.40	2.9
507510	32	17AUG16	31JAN17	0.84	0.03	3.6
507511	20	20OCT16	31JAN17	13.53	0.33	2.5



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