TECHNICAL PROCEDURE MANUAL
CHRISTUS Spohn Hospital Corpus Christi Shoreline STAT Lab
C-Reactive Protein (CRP) Vario Using Abbott Architect ci4100
Proc. #: HSL0360.01

Intended Use
The MULTIGENT CRP Vario assay [CRPVa] is intended for the quantitative immunoturbidimetric determination of C-reactive protein in human serum or plasma with variable assay ranges [CRP16, CRP32, CRP48] using the ARCHITECT *c* Systems.

Clinical Significance
C-reactive protein (CRP) is an acute phase protein whose concentration rises non-specifically in response to inflammation. CRP is seen to increase as a result of the inflammatory process, most notably in response to pneumococcal (bacterial) infection, histolytic disease, and a variety of other disease states. Intraindividual variation is a major limitation of the assay when the assay is used for directing therapies. Intraindividual variations of the CRP levels are from 30% to 60%. Serial measurement may be required to estimate true mean of CRP depending on the intended use in any specific individual. CRP is used as a marker or general diagnostic indicator of infections and inflammation, in addition to serving as a monitor of patient response to pharmacological therapy and surgery.

Principle

MULTIGENT CRP Vario is a latex immunoassay developed to accurately and reproducibly measure blood CRP levels in serum and plasma. When an antigen-antibody reaction occurs between CRP in a sample and anti-CRP antibody, which has been adsorbed to latex particles, agglutination results. This agglutination is detected as an absorbance change (572 nm), with the rate of change being proportional to the quantity of CRP in the sample. Three different methods (High Sensitivity [CRP16], Standard [CRP32], and Wide Range [CRP48]) are available to cover a wide analytical measurement range.

Methodology: Turbidimetric/Immunoturbidimetric.

Specimen Collection and Handling

Suitable Specimens
• **Serum:** Use serum collected by standard venipuncture techniques into plastic tubes with or without gel barriers. Ensure complete clot formation has taken place prior to centrifugation. Centrifuge according to tube manufacturer's instructions to ensure proper separation of serum from blood cells.
Some specimens, especially those from patients receiving anticoagulant or thrombolytic therapy, may take longer to complete their clotting processes. Fibrin clots may subsequently form in these sera and the clots could cause erroneous test results.
• **Plasma:** Use plasma collected by standard venipuncture techniques into plastic tubes. Acceptable anticoagulants are lithium heparin (with or without gel barrier), sodium heparin, and EDTA. Ensure centrifugation is adequate to remove platelets. Centrifuge according to tube manufacturer's instructions to ensure proper separation of plasma from blood cells.

NOTE: Glass tubes were not tested

Specimen Storage

Temperature	Maximum Storage
20 to 25˚C	15 days
2 to 8˚C	2 months
-20˚C	3 years

NOTE: Stored specimens must be inspected for particulates. If present, mix and centrifuge the specimen to remove particulates prior to testing.

Materials and Equipment Required

TEST INSTRUMENT: Abbott ARCHITECT System

MATERIALS PROVIDED

6K26-30 MULTIGENT CRP Vario Kit
6K26-41 MULTIGENT CRP Vario Kit

MATERIALS REQUIRED BUT NOT PROVIDED

- 6K26-10 MULTIGENT CRP Calibrator Set
- 6K26-14 MULTIGENT CRP Calibrator HS
- 6K26-12 MULTIGENT CRP Calibrator WR
- 6K26-21 MULTIGENT CRP Control HS
- Biorad Immunology Control Level 1 -594
  Biorad Immunology Control Level 3-596
- Saline (0.85% to 0.90% NaCl) for specimens that require dilution

Reagent Handling and Storage:

***CAUTION*:**

1. For in vitro diagnostic use.
2. Do not use components beyond the expiration date.
3. Do not mix materials from different kit lot numbers.

CAUTION: This product requires the handling of human specimens. It is recommended that all human sourced materials be considered potentially infectious and be handled in accordance with the OSHA Standard on Bloodborne Pathogens. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.

• The following warning and precaution apply to R1 and R2:
Contains sodium azide.
EUH032 Contact with acids liberates very toxic gas.
These materials and their containers must be disposed of in a safe way.

Reagent Handling
R1 Ready for Use.
R2 Ready for Use.
• Remove air bubbles, if present in the reagent cartridge, with a new applicator stick. Alternatively, allow the reagent to sit at the appropriate storage temperature to allow the bubbles to dissipate.
To minimize volume depletion, do not use a transfer pipette to remove the bubbles.
CAUTION: Reagent bubbles may interfere with proper detection of reagent level in the cartridge, causing insufficient reagent aspiration that could impact results.

Reagent Storage
• Reagent stability is 60 days if the reagent is uncapped and onboard.
• Unopened reagents are stable until the expiration date when stored at 2 to 8°C.

Reagent Preparation:
MULTIGENT CRP Vario is supplied as a two-reagent kit which contains: **R1 & R2**

Reactive Ingredients		Concentration
R1	Glycine buffer (pH 7.0)	1.28%
R2	Anti-CRP polyclonal antibodies (rabbit) adsorbed on latex particles	0.2%

Nonreactive Ingredients:
R1 contains bovine albumin (≤ 1%) and sodium azide (< 0.1%).
R2 contains bovine albumin (≤ 0.1%) and sodium azide (< 0.1%).

Calibrator:
• 6K26-10 MULTIGENT CRP Calibrator Set
• 6K26-14 MULTIGENT CRP Calibrator HS
• 6K26-12 MULTIGENT CRP Calibrator WR

Calibration
NOTE: The MULTIGENT CRP Vario assay must be calibrated using the individual levels listed in the ASSAY PARAMETERS. Refer to the parameters for the High Sensitivity [CRP16], Standard [CRP32], and Wide Range [CRP48] methods and the MULTIGENT CRP Calibrator package insert specific for the method used in your laboratory.

Frequency:
Calibration is stable for 15 days for any one lot. Recalibration is required with each new reagent lot number.

A new calibration is required:

1. If quality control results do not meet acceptance criteria defined by your laboratory, patient values may be suspect. Follow the established quality control procedures for your laboratory. Recalibration may be necessary.
2. Review quality control results and acceptance criteria following a change of reagent or calibrator lot.

Calibrator Required:

- 6K26-10 MULTIGENT CRP Calibrator Set
- 6K26-14 MULTIGENT CRP Calibrator HS
- 6K26-12 MULTIGENT CRP Calibrator WR.

Reagents:

6K26-10 MULTIGENT CRP Calibrator Set contains the following calibration levels, prepared by diluting CRP with human serum and stabilized by adding sodium azide (< 0.1%).
The values assigned and the color of the caps corresponding to each level are indicated in the table below.

CONT	Short Name	Cap Color	CONC mg/dL	CONC mg/L	QTY
CAL	CRP05	white	0.50	5.0	1 x 2 mL
CAL	CRP10	light yellow	1.00	10.0	1 x 2 mL
CAL	CRP20	light green	2.00	20.0	1 x 2 mL
CAL	CRP40	light blue	4.00	40.0	1 x 2 mL
CAL	CRP80	pink	8.00	80.0	1 x 2 mL
CAL	CRP160	magenta	16.00	160.0	1 x 2 mL
CAL	CRP320	brown	32.00	320.0	1 x 2 mL

6K26-12 MULTIGENT CRP Calibrator WR contains the following calibration level, prepared by diluting CRP with human serum and stabilized by adding sodium azide (< 0.1%). The value assigned and the color of the cap are indicated in the table below.

CONT	Short Name	Cap Color	CONC mg/dL	CONC mg/L	QTY
CAL	CRPWR	green	48.00	480.0	1 x 2 mL

6K26-14 MULTIGENT CRP Calibrator HS contains the following calibration level, prepared by diluting CRP with human serum and stabilized by adding sodium azide (< 0.1%).
The value assigned and the color of the cap are indicated in the table below.

CONT	Short Name	Cap Color	CONC mg/dL	CONC mg/L	QTY
CAL	CRPHS	yellow	0.25	2.50	1 x 2 mL

Calibrator Preparation:
Calibrator Sets require no preparation prior to use.

Calibration Procedure:
1. Enter the calibrator values provided in the following table for the Standard method. For the High Sensitivity and Wide Range methods, refer to the tables below.
2. Mix bottles several times by gentle inversion to ensure homogeneity of the solution.
3. Open the bottles, place an appropriate amount of each calibrator in a separate sample cup, and place in the assigned positions.
4. Cap bottles tightly and return to refrigerated storage immediately after use. Always return each cap to its original bottle.
5. Perform calibration as indicated in *Section 6* of the **ARCHITECT System Operations Manual**.

Calibrators required for CRP Vario Standard Method [CRP32]

CAL	Short Name	mg/dL	mg/L
Blank	water	0.00	0.0
Cal 1	CRP05	0.50	5.0
Cal 2	CRP10	1.00	10.0
Cal 3	CRP20	2.00	20.0
Cal 4	CRP40	4.00	40.0
Cal 5	CRP160	16.00	160.0
Cal 6	CRP320	32.00	320.0

Troubleshooting and Overall Acceptance Criteria Failure

See ARCHITECT Operations Manual for further calibration troubleshooting.

Quality Control:

Controls are tested according to each location/site Quality Control Procedure.

• If quality control results do not meet the acceptance criteria defined by your laboratory, patient values may be suspect. Follow the established quality control procedures for your laboratory.
Recalibration may be necessary.
• Review quality control results and acceptance criteria following a change of reagent or calibrator lot.

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Procedure
For a detailed description of how to run an assay, refer to *Section 5* of the **ARCHITECT System Operations Manual**.

Calculations
Refer to *Appendix C* of the **ARCHITECT System Operations Manual** for information on results calculations.

Reporting Results
The result unit for the CRP Vario assay can be reported in mg/dL or mg/L. This will be reported in mg/L.

Specific Performance Characteristics
Reference Ranges
It is recommended that each laboratory determine its own reference range based upon its particular locale and population characteristics.

Serum/Plasma (Abbott Package Insert)

Range (mg/dL)	Range (mg/L)
≤ 0.5	≤ 5

CRP is an acute phase protein whose concentration rises non-specifically in response to inflammation. CRP values should not be interpreted without a complete clinical evaluation. Follow-up testing of patients with elevated values is recommended in order to help rule out a recent response to undetected infection or tissue injury

Serum/Plasma (this facility)

Critical Values *(Refer to Critical Value Policy)*

Performance Characteristics
Reportable Range
The reportable range for MULTIGENT CRP Vario is:
- Standard Method 0.02 to 32.00 mg/dL (0.2 to 320 mg/L)

Method was tested for prozone up to a CRP concentration of 100 mg/dL (1,000 mg/L). No prozone effect was observed within the linear range of the assay. At 100 mg/dL (1,000 mg/L) the observed result was correctly flagged as above the linearity of the assay.

Limit of Quantitation (LOQ)
The LOQ is the analyte concentration at which the CV = 20%. The limit of quantification for MULTIGENT CRP Vario is:
- Standard and Wide Range Methods 0.02 mg/dL (0.2 mg/L)

Dilution:
Serum and Plasma: Specimens with CRP values exceeding the linearity are flagged and may be diluted by following the Automated Dilution Protocol.

Automated Dilution Protocol

If using the Automated Dilution Protocol, the system performs a dilution of the specimen and automatically corrects the concentration by multiplying the result by the appropriate dilution factor. The dilution for each method is listed below.

Method	Dilution
Standard	**1:5**

Precision:

The precision of the MULTIGENT CRP Vario assay is ≤ 6% Total CV

CRP High Sensitivity Method

		Level 1	Level 2	Level 3	Level 4
N		40	40	40	40
Mean (mg/L)		0.462	4.92	11.35	45.88
Within Run	SD	0.019	0.035	0.148	0.298
	%CV	4.00	0.72	1.30	0.65
Between Run	SD	0.011	0.024	0.109	0.120
	%CV	2.38	0.49	0.96	0.26
Total	SD	0.022	0.043	0.183	0.389
	%CV	4.66	0.87	1.62	0.85

NOTE: %CV was calculated prior to rounding Mean and SD.

CRP Standard Method

		Level 1	Level 2	Level 3	Level 4
N		40	40	40	40
Mean (mg/L)		5.10	18.30	73.30	319.40
Within Run	SD	0.10	0.11	0.37	2.08
	%CV	1.97	0.59	0.50	0.65
Between Run	SD	0.04	0.12	0.14	1.12
	%CV	0.78	0.65	0.19	0.35
Total	SD	0.11	0.19	0.40	2.70
	%CV	2.15	1.04	0.54	0.85

NOTE: %CV was calculated prior to rounding Mean and SD.

CRP Wide Range Method

		Level 1	Level 2	Level 3	Level 4
N		40	40	40	40
Mean (mg/L)		5.20	18.40	90.20	268.00
Within Run	SD	0.06	0.18	0.69	3.32
	%CV	1.16	0.99	0.77	1.24
Between Run	SD	0.05	0.08	0.76	3.35
	%CV	0.98	0.44	0.84	1.25
Total	SD	0.1	0.22	1.25	4.92
	%CV	1.91	1.17	1.39	1.83

Liitations of Procedure

The following are limitations on the use of the High Sensitivity CRP per CDC/AHA recommendations.

- Screening the entire adult population is not recommended.
- CRP is not a substitute for traditional cardiovascular risk factors.

- Acute coronary syndrome management should not depend on CRP measurements.
- Patients with persistently unexplained CRP levels above 1.0 mg/dL (10 mg/L) should be evaluated for noncardiovascular etiologies.
- Secondary prevention measures should not depend on CRP.
- Serial measurements of CRP should not be used to monitor treatment.
- The average of two CRP results, repeated optimally two weeks apart, should be used on metabolically stable patients. In very rare cases gammopathy, particularly of the monoclonal IgM type (e.g., Waldenstrom macroglobulinemia), may cause unreliable results.

Interfering Substances

Interference studies were conducted using an acceptance criteria of+/- 5% or 0.05 mg/dL deviation, whichever is greater, from the target value. No interference was observed at the concentrations below.

Interfering Substance	Interferent Concentration	
Bilirubin, conjugated	66 mg/dL	(1129 µmol/L)
Bilirubin, unconjugated	66 mg/dL	(1129 µmol/L)
Hemoglobin	500 mg/dL	(5 g/L)
Intralipid	1,500 mg/dL	(15 g/L)
Rheumatoid factor	550 IU/mL	(550 kU/L)

References:

1. ABBOTT ARCHITECT CRP Vario package insert
   Abbott Laboratories
   Diagnostics Division
   Abbott Park, IL 60064
   Aug 2015 306731/R04
2. ABBOTT ARCHITECT CRP Vario Calibrators package insert
   Abbott Laboratories
   Diagnostics Division
   Abbott Park, IL 60064
3. Abbott ARCHITECT Operator's Guide

Alternative Method

Send to sister facility if unable to perform at this site.

Effective date of this Procedure: 7/25/2018

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