en

Immunoassay

REF **CMR0101/CMR0102/CMR0103/CMR0104/CMR0105**

50 tests*1 / 100 tests*1 / 100 tests*2 / 100 tests*5 / 50 tests*2

hs-CRP CLIA Microparticles

This assay is based on a chemiluminescent microparticle immunoassay (CLIA Microparticles) for the quantitative determination of hs-CRP (high-sensitivity C-Reactive Protein) in human serum and plasma (EDTA, heparin or sodium citrate).

Key to Graphical Symbols Used

LOT	batch code		use by
	manufacturer	Σ	contains sufficient for <n> tests
IVD	*in vitro* diagnostic medical device		temperature limitation
REF	catalogue number		consult instructions for use
EC REP	authorized representative in the European Community		

EC REP

OBELIS S.A.
Bd. Général Wahis, 53
1030 Brussels
Belgium

AUTOBIO DIAGNOSTICS CO., LTD.
No.87 Jingbei Yi Road
National Eco & Tech Development Area
Zhengzhou
China
450016

CE IVD

For any technical assistance please contact us in English at:
Email: customerservice@autobio.com.cn
Contact your local dealer for all product-related questions in your local language

Introduction

C-reactive protein (CRP) is an annular (ring-shaped), pentameric protein found in blood plasma, whose levels rise in response to inflammation. It is an acute-phase protein of hepatic origin that increases following interleukin-6 secretion by macrophages and T cells. CRP is synthesized by the liver[1] in response to factors released by macrophages and fat cells (adipocytes).[2] It was named as such for its ability to bind and precipitate the C-polysaccharide of pneumococcus.[3]

CRP is one of the acute-phase proteins, the serum or plasma levels of which rise during general, nonspecific response to a wide variety of diseases. CRP may also be found in patients with Guillain-Barre syndrome and multiple sclerosis, certain viral infections, tuberculosis, acute infectious hepatitis, many other necrotic and inflammatory diseases, burned patients and after surgical trauma.[4] CRP levels rise in circulation within 24-48 hours following acute tissue damage, reach a peak (up to 1000 times the constitutive level) and decrease with the resolution of trauma or inflammation. The elevated levels of CRP may last for several days before reaching back to normal levels.

Measurement of CRP by high sensitivity CRP assays adds to the predictive value of other cardiac markers like Myoglobin, CK-MB, cTnI and cTnT to assess the risk of cardiovascular and peripheral vascular disease. With the advent of sensitive methodologies, the use of high sensitivity CRP assays is becoming more routine to aid in the determination of inflammation due to cardiovascular trauma.

Measurement Principle

This assay is based upon the two-step sandwich method. The sample and anti-CRP coated microparticles are combined in the first incubation. After addition of enzyme linked anti-CRP, CRP present in the sample is allowed to react simultaneously with the two antibodies, resulting in the CRP being sandwiched between the microparticles and enzyme-linked antibodies. After washing, a complex is generated among the microparticles, the CRP within the sample and enzyme-linked antibodies by immunological reactions. Chemiluminescent Substrate is then added and catalyzed by this complex, resulting in a chemiluminescent reaction. The resulting chemiluminescent reaction is measured as RLUs. The RLU is proportional to the concentration of CRP in the patient sample.

Materials provided

1. Calibrators

6 vials lyophilized Calibrator A through F. The matrix is Tris buffer containing BSA (bovine serum albumin) and ADP. Contains a selection of preservatives.

Reconstitute each lyophilized calibrator with 1.0 mL of distilled water. Allow the reconstituted material to stand for at least 5 minutes. Then invert the calibrator to mix it completely.

2. Reagent pack

Reagent pack provided ready to use.

Σ	50*1	100*1	100*2	100*5	50*2
Microparticles Solution	1.2mL*1	2.3mL*1	2.3 mL*2	2.3 mL*5	1.2 mL*2
Enzyme Conjugate	5.5mL*1	11.0mL*1	11.0mL*2	11.0mL*5	5.5mL*2
Sample Diluent	5.5mL*1	11.0mL*1	11.0mL*2	11.0mL*5	5.5mL*2

- Microparticles Solution

Mouse monoclonal coated microparticles in PBS buffer. Contains a selection of preservatives.

- Enzyme Conjugate

HRP (horseradish peroxidase) labeled mouse monoclonal anti-CRP in Tris-NaCl buffer containing casein. Contains a selection of preservatives.

- Sample Diluent

Tris buffer containing casein. Contains a selection of preservatives.

Assay Analyzers on which the kit can be used

- AutoLumo A2000 Plus
- AutoLumo A2000 Plus B
- AutoLumo A1000

The chemiluminescent microparticle immunoassay (CLIA Microparticles) is intended for use on Assay Analyzers which is AutoLumo A2000 Plus, AutoLumo A2000 Plus B or AutoLumo A1000.

Materials Required but not Provided

1. Assay Analyzer
2. Reaction vessel(s) for sample and reagent reaction
3. Sample tube(s) or cup(s) for sample containing
4. Chemiluminescent Substrate
5. Diluent Universal
6. System Wash for washing the pipetting needle
7. Wash Buffer used in the washing procedure
8. Distilled or deionized water

Metrological Traceability of Calibrators

The analyte in these hs-CRP calibrators is traceable to the manufacturer's working calibrators. Traceability process is based on EN ISO 17511.The assigned values were established using representative samples from this lot of calibrator and are specific to the assay methodologies of the reagents. Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

Warnings and Precautions

1. For professional use only.
2. Follow the instruction for use carefully. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this instruction for use.
3. Refer to the material safety data sheet and product labeling for any chemical hazards that may be present in this assay.
4. Handle the potentially contaminated materials and wastes safely according to local requirement.
5. CAUTION: the calibrators contain human sourced components, which have been tested and found non-reactive for HBsAg, HIV-1 and HIV-2, HCV and Syphilis. It is recommended that all materials of human origin be considered potentially infectious. This assay contains materials of animal origin. Bovine components originate from countries where bovine spongiform encephalopathy (BSE) has not been reported.
6. Do not smoke, drink, eat or use cosmetics in the working area.
7. Wear protective clothing and disposable gloves when dealing with samples and reagents. Wash hands after operations.
8. Conduct the assay away from bad ambient conditions. e.g. ambient air containing high concentration corrosive gas such as sodium hypochlorite acid, alkaline, acetaldehyde and so on, or containing dust.
9. Do not mix or use components from kits with different batch codes.
10. When storing the calibrators, be certain the vials are securely sealed.
11. Ensure the microparticles are resuspended before loading it on the analyzer.
12. Avoid foam formation in all reagents and sample types (samples, calibrators and controls).

13. Do not substitute any reagent in this kit from other manufacturers or other lots.
14. When any damage to the protective packaging or any change of analytical performance is observed, do not use the kit.
15. Do not use reagents beyond the labeled expiry date.

Storage

1. Store the kit at 2-8 ℃. Do not freeze. Avoid strong light.
2. The expiry date is shown on the container label.
3. Refrigerate the reagent pack at 2-10 ℃ for a minimum of 2 hours prior to use.
4. Store the unsealed reagents pack upright on the analyzer or 2-10℃ for a maximum of 28 days. After 28 days, the reagent pack must be discarded. Once they are removed from the analyzer, store them at 2-10 ℃ in an upright position.
5. Seal and return the remaining calibrators at 2-8 ℃ immediately after the experiment, under which conditions the stability will be retained for 28 days.

Sample

1. Collect samples in accordance with correct medical practices.
2. Do not use heat-inactivated samples. Do not use sodium azide preservative in samples.
3. Do not use samples with obvious microbial contamination.
4. Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in samples has taken place prior to centrifugation. Some samples, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the sample is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results. Be sure that the samples are not decayed prior to use.
5. Prior to shipment, it is recommended that samples be removed from the clot or red blood cells.
6. Insufficient processing of sample or disruption of the sample during transportation may cause depressed results.
7. Avoid grossly hemolytic, lipemic or turbid samples.
8. Cap and store the samples at 18-25 ℃ for no more than 8 hours, for longer use samples should be capped and stored at 2-8 ℃ up to 48 hours. Or freeze the samples that need to be stored or transported for more than 48 hours at -20 ℃. Avoid multiple freeze-thaw cycles. Mix thawed samples thoroughly by low speed vortex or by inverting 10 times. Visually inspect the samples, if layering or stratification is observed, continue mixing until samples are visibly homogeneous. After thawing, bring to room temperature and mix well by gently shaking.
9. Centrifuge the thawed samples containing red blood cells or particulate matter, or which are hazy or cloudy in appearance prior to use to ensure consistency in the results.
10. Note that interfering levels of fibrin may be present in samples that do not have obvious or visible particulate matter.
11. If proper sample collection and preparation cannot be verified, or if samples have been disrupted due to transportation or sample handling, an additional centrifugation step is recommended. Centrifugation conditions should be sufficient to remove particulate matter.
12. For optimal results, inspect all samples for bubbles. Remove bubbles with a tip prior to analysis. Use a new tip for each sample to prevent cross contamination.

Measurement Procedure

1. Check the consumable materials
- Verify adequate volume of consumable materials is present prior to running the test.
- Refer to the Assay Analyzer's operation manual.

2. Load the kit
- Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the analyzer. Avoid foam formation in all reagents. Don't invert the open (punctured) packs. If necessary, shake gently to mix horizontally after the first loading.
- Read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Refer to the Assay Analyzer's operation manual.

3. Order tests
- Place the sample tube(s) or cup(s) on the sample rack, 10 μL of serum or plasma samples are automatically diluted 1:40 with 390 μL of Diluent Universal and mixed well (note: the calibrators have been diluted in advance and can be used directly, please avoid diluting again). But consider the sample container and 150 μL of system dead volumes, which can be refer to the appropriate Assay Analyzer manuals for the minimum sample volume required.
- Load the sample rack and input the sample information on the system software interface.
- Select "run" to start the test, the analyzer automatically operates tests. It performs the following functions:
  - Moves the sample to the set point
  - Loads a reaction vessel into the process path
  - Aspirates and transfers 100 μL of the calibrators into the reaction vessel; other reaction vessels will be added by 10 μL of diluted sample and 90 μL of Sample Diluent
  - Adds Microparticles Solution to the reaction vessel
  - Mixes, incubates and washes the reaction mixture
  - Adds Enzyme Conjugate to the reaction vessel
  - Mixes, incubates and washes the reaction mixture
  - Adds Chemiluminescent Substrate
  - Measures chemiluminescent emission to determine the quantity of CRP in the sample
  - Discards the used reaction vessel
  - Calculates the result
- Refer to the Assay Analyzer's operation manual.

4. Calibrate the curve
- Analyzer can read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Transfer the calibrators into the sample tube (s) or cup(s) and place the sample tubes on the sample rack. Conduct duplicate detection on the system.
- Load the sample rack and input calibrators' information on the system software interface.
- Select "run" to start the test and generate the calibration curve, calibration is required every 28 days.
- Once a calibration curve is accepted and stored, all subsequent samples may be tested without further calibration unless:
  - Controls are out of range after repeated measurements
  - A reagent kit and Chemiluminescent Substrate with new batch code is used
  - Beyond the expiration date of a calibration curve
  - Important parts of the analyzer are replaced or repaired

- Refer to the Assay Analyzer's operation manual.

5. Dilute the sample

Samples with a CRP value exceeding 60 mg/L may be diluted manually. Diluent Universal is used to dilute the samples. After dilution, multiply the result by the dilution factor.

- The concentration of the sample after dilution should not be less than 0.5 mg/L.

Measurement Results

The sample test results are determined automatically by the system software. The amount of CRP in the samples is determined from the measured light production by means of the stored calibration data. Refer to the Assay Analyzer's operation manual on reviewing the stored results.

Control Procedure

Controls for the various concentration ranges should be run individually when the test is in use, once per reagent kit, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Limitations of the Procedure

1. This assay is intended as an aid for the clinical diagnosis. Conduct this assay in conjunction with clinical examination, patient's medical history and other test results.
2. If the results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
3. The US Centers for Disease Control and Prevention (CDC) and the Heart Association (AHA) provide the following advice on the risk assessment of cardiovascular disease using hs-CRP:
   a) Experts does not recommend hs-CRP for screening in adult populations
   b) Hs-CRP cannot replace the traditional cardiovascular risk factor assessment, the clinical diagnosis of acute coronary syndrome cannot be only dependent on the concentration of hs-CRP
   c) For unknown reason hs-CRP concentration value continuously exceed 10mg/L, it should consider as non-cardiovascular disease
   d) Patients with infectious diseases, systemic inflammation or trauma are not subjected to risk assessment
   e) hs-CRP is an independent risk assessment indicator
   f) hs-CRP cannot be used to detect therapeutic effects
   g) Before the risk assessment, it is best to isolate the two weeks to repeat the detection of hs-CRP, the average of the two results used to assess the risk is more appropriate
4. Heterophilic antibodies and rheumatoid factors in samples may interfere with test results. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis. This kind of samples is not suitable to be tested by this assay.
5. Performance of this test has not been established with neonatal samples.
6. This assay was designed and validated for use with human serum or plasma from individual patient and donor samples. Pooled samples must not be used since the accuracy of their test results has not been validated.
7. This test measures concentrations within the range of 0.5- 60 mg/L. If CRP concentrations above the measuring range to be expected, it is recommended to dilute samples with Diluent Universal, the maximum dilution is 1:49 of this test, allowing samples to be quantitated up to approximately 3000 mg/L.

Biological Reference Interval

A study of 500 normal adult population individuals was undertaken to determine reference intervals for this assay, results are as follow:

＜5.0 mg/L (95% percentile)

The US Centers for Disease Control and Prevention (CDC) and the Heart Association (AHA) advise the following hs-CRP reference interval for the risk assessment of cardiovascular disease:

CRP (mg/L)	Risk Level
＜1.0	Low
1.0-3.0	Medium
＞3.0	High

It is recommended that each laboratory establish its own normal range which may be unique to the population it serves depending upon geographical, patient, dietary, or environmental factors.

Performance Characteristics

1. Measurement Precision

3 samples were assayed in replicate of 2, twice per day across 20 testing days. Data from this study are summarized in the following table.

Sample	n	Mean	Within-run	Total
			%CV	%CV
1	80	7.21	6.29	6.50
2	80	12.20	4.83	6.33
3	80	29.22	3.32	4.71

*Representative data; results in individual laboratories may vary from these data

2. Analytical Sensitivity

Limit of Blank＝0.01mg/L

Limit of Detection＝0.5mg/L

3. Analytical Specificity

Cross reaction: the following substances with such concentrations were tested and found no cross reactivity.

Substances	Concentration (ng/mL)
MYO	1000 ng/mL
cTnI	100 ng/mL
CK-MB	300 ng/mL
PCT	100 ng/mL

Interference: No interference with 5 g/L of hemoglobin, 0.4 g/L of Bilirubin, 30 g/L of triglyceride.

4. Measurement Accuracy by Correlation

A study was performed where samples were tested using this assay and a hs-CRP reference assay. Data were analyzed and are summarized in the following table.

Correlation Method	Number of Samples	Intercept	Slope	Correlation Coefficient
Linear Regression	280	0.6688	1.0262	0.9790

Literature References

1. Pepys MB, Hirschfield GM (2003). "C-reactive protein: a critical update". The Journal of Clinical Investigation. 111 (12): 1805–12.

2. Lau DC, Dhillon B, Yan H, Szmitko PE, Verma S (May 2005). "Adipokines: molecular links between obesity and atheroslcerosis". American Journal of Physiology. Heart and Circulatory Physiology. 288 (5): H2031–41.

3. Schultz, D.R., and Arnold P.I (1990), "Properties of four acute phase proteins: C-reactive protein, serum amyloid A protein, glycoprotein, and fibrinogen." Seminars in Arthritis and Rheumatism. 20: 129-147.

4. Hedlund, P.: Clinical and experimental studies on C-reactive protein (acute phase protein). (1961), Thesis Acta Med Scand, 128 (Suppl, 361):1-71.