Infection

AFIAS CRP

INTENDED USE

AFIAS CRP is a fluorescence immunoassay (FIA) for the quantitative determination of C-Reactive Protein (CRP) in human whole blood/serum/plasma. It is useful as an aid in management and monitoring of autoimmune diseases and infectious processes, such as rheumatoid arthritis.

For *in vitro* diagnostic use only.

INTRODUCTION

C-reactive protein (CRP) is a protein found in blood; the level of which rises in response to inflammation. CRP is first acute-phase protein to be described and is an exquisitely sensitive systemic marker of inflammation and tissue damage. The serum CRP level may rise from a normal level of < 5 mg/L to 500 mg/L during the body's general, nonspecific response to infections and other acute inflammatory conditions. Measurement of CRP concentration has been widely used as a clinical tool for monitoring the status of inflammation, effectiveness of treatment of various infections and autoimmune diseases such as rheumatoid arthritis. And high sensitive CRP (hsCRP) has been suggested that along with serum cholesterol, may be marker of diagnosis in cardiovascular diseases (CVD). And hsCRP is emerging as the strongest and most independent predictive risk factor for CVD.

PRINCIPLE

The test uses a sandwich immunodetection method.

The detector antibodies in detector bind to antigens in the sample, forming antigen-antibody complexes and migrate onto nitrocellulose matrix to be captured by the other immobilized-antibodies on a test strip.

More antigens in the sample will form more antigen-antibody complexes which lead to stronger fluorescence signal by detector antibodies, which is processed by the instrument for AFIAS tests to show CRP concentration in the sample.

COMPONENTS

AFIAS CRP consists of 'cartridges'.

- Each sealed aluminum pouch contains two cartridges.
- Each sealed cartridge packaged in an aluminum pouch has three components including a cartridge part, a detector part and detector diluent part.
- The cartridge part contains the membrane called a test strip which has mouse monoclonal anti-human CRP at the test line, human C-reactive protein at the antigen line, and streptavidin at the control line.
- The detector part has a granule containing mouse monoclonal anti-human CRP-fluorescence conjugator, mouse monoclonal anti-human CRP, Biotin-BSA fluorescence conjugator, bovine serum albumin (BSA) and sucrose as a stabilizer and sodium azide as a preservative in phosphate buffered saline (PBS).
- The detector diluent part contains antifoam and tween 20 as a detergent, sodium azide as a preservative in phosphate buffered saline (PBS).

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Follow instructions and procedures described in this 'Instructions for use'.
- Use only fresh samples and avoid direct sunlight.
- Lot numbers of all the test components (cartridge and ID chip) must match each other.
- Do not interchange the test components between different lots or use the test components after the expiration date, either of which might yield incorrect test result(s).
- Do not reuse cartridges, pipette tips, and C-tips. It should be used for testing one sample only.
- The cartridge should remain sealed in its original pouch until just before use. Do not use the cartridge, if pouch is damaged or has already been opened.
- Frozen sample should be thawed only once. For shipping, samples must be packed in accordance with local regulations. Sample with severe hemolysis and/or hyperlipidemia must not be used.
- If test components and/or sample are stored in refrigerator, then allow cartridge and sample to be at room temperature for approximately 30 minutes before use.
- The instrument for AFIAS tests may generate slight vibration during use.
- Used cartridges and pipette tips, and C-tip should be handled carefully and discarded by an appropriate method in accordance with relevant local regulations.
- The cartridge contains sodium azide (NaN_3), and they may cause certain health issues like convulsions, low blood pressure and heart rate, loss of consciousness, lung injury and respiratory failure. Avoid contact with skin, eyes, and clothing. In case of contact, rinse immediately with running water.
- Biotin interference was not observed in **AFIAS CRP** when biotin concentration in the sample was up to 3,500 ng/mL. In reference, if biotin is taken at a level of 300 mg/day, the biotin concentration in the body is about 1,200 ng/mL. However, if a patient has been taking biotin at dosage of more than 300mg a day, it is recommended to collect blood again 24 hours after discontinuation of biotin intake.
- **AFIAS CRP** will provide accurate and reliable results subject to the below conditions.
  - **AFIAS CRP** should be used only in conjunction with the instrument for AFIAS tests.
  - Have to use recommended anticoagulant.

Recommended anticoagulant
K_2 EDTA, K_3 EDTA, Sodium heparin, Lithium heparin, Sodium citrate

- **C-tip should be used when the following conditions are met.**
  - C-tip provided with the kit is recommended to obtain correct test result.
  - Whole blood should be immediately tested after collection.
  - Do not perform a test with C-tip on General Mode. It might cause an erroneous result.
  - Excess whole blood around the C-tip should be wiped off.
  - In order to avoid cross-contamination, please do not re-use C-tip for multiple samples.
  - AFIAS cartridge should be inserted and positioned in the cartridge holder prior to the blood sample collection.
  - While collecting blood, be careful not to create air bubbles in the C-tip.

LIMITATIONS OF THE TEST SYSTEM

- The test may yield false positive result(s) due to the cross-reactions and/or non-specific adhesion of certain sample components to the capture/detector antibodies.
- The test may yield false negative result(s) due to the non-responsiveness of the antigen to the antibodies which is the most common if the epitope is masked by some unknown components, so therefore not being able to be detected or captured by the antibodies. The instability or degradation of the antigen with time and/or temperature may also cause false negative result as it makes antigen unrecognizable by the antibodies.
- Other factors may interfere with the test and cause erroneous results, such as technical/procedural errors, degradation of the test components/reagents or presence of interfering substances in the test samples.
- Any clinical diagnosis based on the test result must be supported by a comprehensive judgment of the concerned physician in conjunction with clinical symptoms and other relevant test results.

STORAGE AND STABILITY

Storage condition			
Component	Storage Temperature	Shelf life	Note
Cartridge	2 - 30 °C	20 months	Unopened
		1 month	Resealed

- Return an unused cartridge to the spare cartridge zipper bag containing the desiccant pack. Reseal along entire edge of zip-seal.

MATERIALS SUPPLIED

REF SMFP-2

Components of **AFIAS CRP**

- Cartridge box :
  - Cartridge 24
  - Pipette tip (zipper bag) 24
  - C-tip (10 µL) 24
  - Spare cartridge zipper bag 1
  - ID chip 1
  - Instructions for use 1

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

Following items can be purchased separately from **AFIAS CRP**. Please contact our sales division for more information.

- Instrument for AFIAS tests
  - **AFIAS-1** REF FPRR019
  - **AFIAS-3** REF FPRR040
  - **AFIAS-6** REF FPRR020
  - **AFIAS-10** REF FPRR038
- **Boditech CRP Control** REF CFPO-100
- **Boditech CRP Calibrator** REF CFPO-112

SAMPLE COLLECTION AND PROCESSING

The sample type for **AFIAS CRP** is human whole blood/serum/plasma.

- It is recommended to test the sample within 24 hours after collection.
- The samples (serum, plasma) should be separated from the clot by centrifugation within 3 hours after the collection of whole blood.
- The samples (whole blood, serum, plasma) may be stored for a week at 2-8 °C prior to being tested. If testing will be delayed more than a week, samples should be frozen at below -20 °C.
- The samples stored frozen at below -20 °C for 3 months showed no performance difference.
- However, the whole blood sample should not be kept in a freezer in any case.
- As a repeated freeze-thaw cycle may affect the test result, do not refreeze previously frozen samples.
- Collection of whole blood sample using C-tip
  ① Hold the C-tip horizontally and touch the surface of the blood with the tip of the C-tip.
  ② Capillary action will automatically draw the blood sample to C-tip and stop.
  ③ Wipe off any excess blood around the tip.
  ④ Double-check if whole blood is filled accurately in the C-tip and AFIAS reader is ready for a test on the 'C-tip mode'.

TEST SETUP

- Check the components of the **AFIAS CRP** as described below.: Cartridges, pipette tips, C-tips, an ID chip, a spare cartridge zipper bag and an instructions for use.
- Ensure that the lot number of the cartridge matches that of the ID chip.
- If the sealed cartridge has been stored in a refrigerator, place them on a clean and flat surface at room temperature for at least 30 minutes before testing.
- Turn on the instrument for AFIAS tests.
- Empty the tip box.
- Insert the ID chip into the 'ID chip port'.

※ **Please refer to the instrument for AFIAS tests operation manual for complete information and operating instructions.**

TEST PROCEDURE

▶ **AFIAS-1, AFIAS-3, AFIAS-6,**

General mode

1) Insert the cartridge into the cartridge holder.
2) Insert a tip into the tip hole of the cartridge.
3) Select the 'General mode' in the instrument for AFIAS tests.
4) Take 100 µL of the sample (whole blood/serum/plasma/control) using a pipette and dispense it into the sample well of the cartridge.
5) Tap the 'Start' button on the screen.
6) The test result will be displayed on the screen after 3 minutes.

C-tip Mode

1) Insert a cartridge into the cartridge holder.
2) Take 10 µL of whole blood using a C-tip.
3) Insert the whole blood-filled C-tip into the tip hole of the cartridge.
4) Select the 'C-tip mode' in the instrument for AFIAS tests.
5) Tap the 'Start' button on the screen.
6) The test result will be displayed on the screen after 3 minutes.

▶ **AFIAS-10**

Normal mode

1) Insert a cartridge into the cartridge holder.

2) Insert a tip into the tip hole of the cartridge.
3) Tap the 'Load' button of the bay that holds the cartridge with the tip to read the barcode of the cartridge and please confirm the item name written on the cartridge.
4) Insert the sample tube into the tube rack.
5) Insert the tube rack into the loading part of the sampling station.
6) Tap the 'Start' button on the screen.
7) The test result will be displayed on the screen after 3 minutes.

Emergency mode (General tip)

1) The test procedure is same with the 'Normal mode 1) – 3)'.
2) Convert the 'Emergency mode' in AFIAS-10.
3) Select the tip type (General tip) on the screen.
4) Select the sample type (whole blood/serum/plasma) on the screen.
5) Take 100 µL of the sample using a pipette and dispense it into the sample well of the cartridge.
6) Tap the 'Start' button on the screen.
7) The test result will be displayed on the screen after 3 minutes.

Emergency mode (C-tip)

1) Insert a cartridge into the cartridge holder.
2) Take 10 µL of whole blood using a C-tip.
3) Insert the C-tip with sample into the tip hole of the cartridge.
4) Tap the 'Load' button of the bay that holds the cartridge with a tip to read the barcode of the cartridge and please confirm the item name written on the cartridge.
5) Convert the 'Emergency mode' in AFIAS-10.
6) Select the tip type (C-tip) on the screen.
7) Tap the 'Start' button on the screen.
8) The test result will be displayed on the screen after 3 minutes.

INTERPRETATION OF TEST RESULT

- The instrument for AFIAS tests calculates the test result automatically and displays CRP concentration of the test sample in terms of mg/L.
- Cut-off : 10 mg/L
- Working range : 0.5-200 mg/L
- Effect of Hematocrit

  The CRP Whole Blood of the instrument for AFIAS tests is calibrated to read the CRP serum concentration of a blood sample with a hematocrit of 40 %. If the actual hematocrit value deviates from 40 %, the result should be corrected by multiplying with the respective factor in the table: deviates from 40 %, the result should be corrected by multiplying with the respective factor in the table:

Hct (%)	Factor	Hct (%)	Factor
20-29	0.8	56-58	1.4
30-36	0.9	59-61	1.5
37-42	1.0	62-63	1.6
43-47	1.1	64-65	1.7
48-51	1.2	66-67	1.8
52-55	1.3	68-69	1.9

Reference range, HCT:
- Woman: 35-44 %
- Men: 39-48 %

QUALITY CONTROL

- Quality control tests are a part of the good testing practice to confirm the expected results and validity of the assay and should be performed at regular intervals.
- Quality control tests should also be performed whenever there is any question concerning the validity of the test results.
- Control materials are provided on demand with **AFIAS CRP**. For more information regarding obtaining the control materials, contact Boditech Med Inc.'s Sales Division for assistance.
  (Please refer to the instructions for use of control material.)

PERFORMANCE CHARACTERISTICS

- **Analytical sensitivity**
  - Limit of Blank (LoB) 0.19 mg/L
  - Limit of Detection (LoD) 0.32 mg/L
  - Limit of Quantitation (LoQ) 0.50 mg/L
- **Analytical Specificity**
  - **Cross-reactivity**

    Biomolecules such as below the ones in the table were added to the test sample(s) at concentrations much higher than their normal physiological levels in the blood. **AFIAS CRP** test results did not show any significant cross-reactivity with these biomolecules.

Cross reactants	Concentration
IL-6	50 µg/mL
PCT	50 µg/mL
Serum amyloid P	500 µg/mL
Serum amyloid A	500 µg/mL
Ferritin	500 µg/mL

  - **Interference**

    Interferents listed in the following table were added to the test sample at the concentration mentioned below. **AFIAS CRP** test results did not show any significant interference with these materials.

Interferents	Concentration
Ascorbic acid	350 µmol/L
Bilirubin (conjugated)	475 µmol/L
Albumin	60 g/L
Glucose	1,000 mg/dL
Triglyceride mixture (Triglyceride, total)	1,500 mg/dL
Hemoglobin	10 g/L
Biotin	3,500 ng/ml

- **Precision**
  - **Single site study**

    3 Lots of **AFIAS CRP** were tested for 20 days. Each standard material was tested 2 times per day. For each test, each material was duplicated.

    Repeatability (within-run precision)
    Within-laboratory precision (Total precision)
    Lot to lot precision
  - **Between persons**

    Three different persons tested one lot of **AFIAS CRP**, ten times at each concentration of the control standard.
  - **Between sites**

    One person tested **AFIAS CRP** at three different sites, ten times at each concentration of the control standard.
  - **Between readers**

    One person tested **AFIAS CRP** with three different readers, ten times at each concentration of the control standard.

Expected Value [mg/L]	Repeatability			Within-laboratory precision		
	Mean [mg/L]	SD	CV (%)	Mean [mg/L]	SD	CV (%)
2.0	1.97	0.16	8.0	2.02	0.17	8.5
10.0	9.96	0.63	6.3	10.01	0.61	6.1
150.0	148.76	13.95	9.4	149.33	13.79	9.2
Expected Value [mg/L]	Lot to lot precision			Between site		
	Mean [mg/L]	SD	CV (%)	Mean [mg/L]	SD	CV (%)
2.0	2.00	0.18	8.9	2.04	0.16	7.6
10.0	9.99	0.6	6.0	10.02	0.71	7.1
150.0	149.04	14.39	9.65	152.32	9.09	6.0
Expected Value [mg/L]	Between person			Between reader		
	Mean [mg/L]	SD	CV (%)	Mean [mg/L]	SD	CV (%)
2.0	2.02	0.13	6.5	2.00	0.18	9.0
10.0	10.03	0.81	8.1	9.93	0.70	7.0
150.0	155.47	11.92	7.7	152.49	11.79	7.7

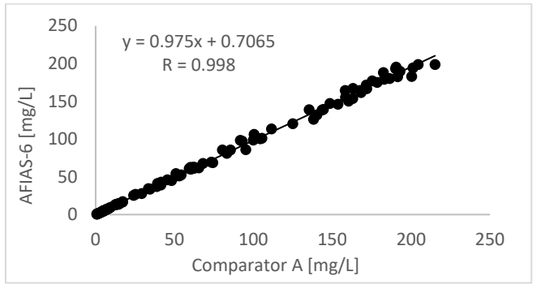
- **Accuracy**

  The accuracy was confirmed by testing 3 different lots, three times with international standard material (ERM-DA474/IFCC, human serum).

	Lo1		Lot 2		Lot 3	
	Measured Value [mg/L]	Bias (%)	Measured Value [mg/L]	Bias (%)	Measured Value [mg/L]	Bias (%)
Run 1	41.82	1.5	39.88	-3.2	42.68	3.6
Run 2	40.09	-2.7	41.42	0.5	40.52	-1.7
Run 3	42.06	2.1	41.96	1.8	40.95	-0.6

- **Comparability**

  CRP concentration of 100 clinical samples were quantified independently with **AFIAS CRP (AFIAS-6)** and **comparator A** as per prescribed test procedures. Test results were compared, and their comparability was investigated with linear regression and correlation coefficient (R). The regression equation and correlation coefficient are as follows.



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Note: Please refer to the table below to identify various symbols.

	Sufficient for <n> tests
	Read instruction for use
	Use by Date
LOT	Batch code
REF	Catalog number
	Caution
	Manufacturer
EC REP	Authorized representative of the European Community
IVD	In vitro diagnostic medical device
	Temperature limit
	Do not reuse
CE	This product fulfills the requirements of the Directive 98/79/EC on in vitro diagnostic medical devices

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