Infection

AFIAS MxA/CRP

INTENDED USE

AFIAS MxA/CRP is a fluorescence immunoassay (FIA) for the quantitative determination of Myxovirus resistance protein A (MxA) and C-Reactive Protein (CRP) in human whole blood. This test aids in identification of viral and/or bacterial infection in patients with clinical symptoms caused by infection.

For *in vitro* diagnostic use only

INTRODUCTION

Viral and bacterial respiratory infections represent a major source of morbidity, mortality, and healthcare costs. Approximately 80% of all antimicrobials are prescribed in primary care, and up to 80% of these are for respiratory tract indications[8]. But sensitive and specific diagnostic tools to aid in the diagnosis of ARIs (particularly in differentiating bacterial and viral infections) in primary and urgent healthcare settings have been lacking[9].

Mx proteins are large GTPases and belong to a group of IFN-induced GTPases involved in the control of intracellular pathogens[5]. In humans, two Mx homologs (MxA and MxB, also called MX1 and MX2, respectively) mediate antiviral activity against a broad range of viruses include Covid-196. Elevated levels of MxA protein could be an indicator of endogenous interferon production mediated by an unknown viral activation and so, the MxA protein levels could be used as a general marker of viral infection[7].

CRP is one of the cytokine-induced acute-phase proteins[1] whose blood levels rise during a general, unspecific response to infections and non-infectious inflammatory processes[2]. CRP tests provide information for the diagnosis, therapy, and monitoring of inflammatory diseases.[10] During infectious or inflammatory disease states, CRP levels rise rapidly within the first 6 to 8 hours and peak at levels of up to 350–400 mg/L after 48 hours. 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.

MxA/CRP test will aid in the differentiation of viral and bacterial acute febrile respiratory infections.

PRINCIPLE

The test uses a sandwich immunodetection method.

The detector antibodies in buffer 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 MxA and CRP concentration in the sample.

COMPONENTS

AFIAS MxA/CRP consists of a 'cartridges'.

- Each sealed aluminum pouch contains two cartridges.
- Each cartridge packaged in an aluminum pouch has three components including a cartridge part, a detector part and a diluent part.
- The cartridge part contains the membrane called a test strip which has anti-MxA, and anti-CRP at the test line, CRP Ag at the antigen line, and chicken IgY at the control line.
- The detector part has a granule containing the anti-MxA-fluorescence complex, anti- CRP-fluorescence conjugate, anti-chicken IgY-fluorescence conjugate, and sodium azide as a preservative in phosphate buffered saline (PBS).
- The diluent part contains 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. A cartridge should be used for processing of one sample only.
- The cartridge should remain sealed in its original pouch until just before use. Do not use a cartridge, if the 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, C-tips and pipette tips 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.
- No biotin interference was observed in **AFIAS MxA/CRP** when biotin concentration in the sample was below 3,500 ng/mL. If a patient has been taking biotin at dosage of more than 0.03 mg a day. It is recommended to test again 24 hours after discontinuation of biotin intake.
- **AFIAS MxA/CRP** will provide accurate and reliable results subject to the below conditions.
  - **AFIAS MxA/CRP** should be used only in conjunction with 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.

※ The following cases may affect the measurement results.

- Receiving interferon therapy (e.g. MS, HIV, HBV, HCV) in the last 30 days.
- Immunocompromised state (e.g. HIV) or taking immunosuppressive or chemotherapeutic medications in the last 30 days (e.g. oral steroids, Methotrexate, Cyclosporine, Antimetabolite chemotherapy, interferon therapy).
- Taking antibiotics or antiviral therapy in the last 14 days.
- Received a live viral immunization in the last 14 days.
- Significant trauma or burns (> 5% total body surface area or full thickness (3rd°)) in the last 30 days.
- Major surgery (requiring intravenous anesthesia and/or respiratory assistance) in the last 30 days- - History of a myocardial infarction or stroke in the last 30 days.
- Taking high biotin-containing drugs within 24 hours.

STORAGE AND STABILITY

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

- 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-102

Components of **AFIAS MxA/CRP**

- Cartridge box:
  - Cartridge 24
  - Pipette tip (zipper bag) 24
  - C-tip (10 µL, zipper bag) 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 MxA/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 MxA Calibrator** REF CFPC-353
- **Boditech MxA Control** REF CFPC-352
- **Boditech CRP Calibrator** REF CFPO-112
- **Boditech CRP Control** REF CFPO-100
- **Boditech MxA/CRP Control** REF CFPO-382
- **Boditech MxA/CRP Calibrator** REF CFPO-383

SAMPLE COLLECTION AND PROCESSING

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

- It is recommended to test the sample within 1 hour after collection.
- The samples (whole blood) may be stored for 12 hours at 2-8°C prior to being tested.
- However, the whole blood sample should not be kept in a freezer in any case.
- 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 MxA/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 each instrument for AFIAS tests operation manual for complete information and operating instructions.

TEST PROCEDURE

▶ **AFIAS-1, AFIAS-3, AFIAS-6**

General mode

1) Insert a 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 150 µL of the sample (whole blood/control) using pipette and dispense it into the sample well of the cartridge.
5) Tap the 'Start' button on the screen.

6) The test results will be displayed on the screen after 12 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 12 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 12 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) on the screen.
5) Take 150 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 12 minutes.

Emergency mode – C-tip

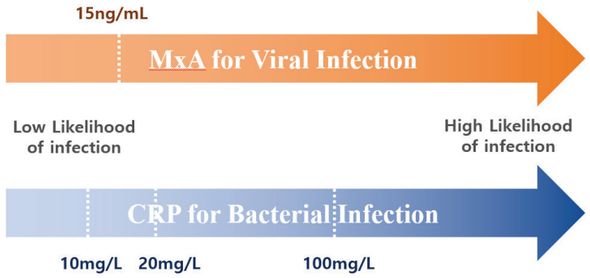
1) Insert a cartridge into the cartridge holder.
2) Take 10 µL of whole blood using a C-tip.
3) Insert the C-tip with sample into the tip hole of the cartridge.
4) Tap the 'Load' button of the bay that holds the cartridge with a tip to read the barcode of the cartridge and please confirm the item name 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 12 minutes.

INTERPRETATION OF TEST RESULT

- The instrument for AFIAS tests calculates the test result automatically and displays MxA, CRP concentration of the test sample in terms of ng/mL. and mg/L, respectively.

Unit	MxA [ng/mL]	CRP [mg/L]
Clinical Cut-off	15.0	10.0
measuring range	10.0 – 300.0	1.0-200.0

MxA [ng/mL]	CRP [mg/L]	Interpretation
MxA ≥ 15.00	CRP < 10.00	viral infection
	10.00 ≤ CRP < 20.00	viral infection and low likelihood of bacterial infection
	20.00 ≤ CRP < 100.00	viral infection and bacterial infection likely
	CRP ≥ 100.00	viral infection and bacterial infection very likely
MxA < 15.00	10.00 ≤ CRP < 20.00	likelihood of bacterial infection
	20.00 ≤ CRP < 100.00	bacterial infection likely
	CRP ≥ 100.00	bacterial infection very likely
	CRP < 10.00	non-infection



- This product is intended for screening tool only. A negative result does not preclude a viral or bacterial infection. 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.
- NICE(National Institute for Health and Care Excellence) recommend CRP test for antibiotics prescription as follows.
  Pneumonia in adults: diagnosis and management (CG191)
  [Presentation with lower respiratory tract infection[10]]
  For people presenting with symptoms of lower respiratory tract infection in primary care, consider a point care C-reactive protein test if after clinical assessment a diagnosis of pneumonia has not been made and it is not clear whether antibiotics should be prescribed. Use the results of the C-reactive protein test to guide antibiotic prescribing in people without a clinical diagnosis of pneumonia as follows:
  - Do not routinely offer antibiotic therapy if the C-reactive protein concentration is less than 20 mg/litre.
  - Consider a delayed antibiotic prescription (a prescription for use at a later date if symptoms worsen) if the C-reactive protein concentration is between 20 mg/litre and 100 mg/litre.
  - Offer antibiotic therapy if the C-reactive protein concentration is greater than 100 mg/litre.

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 MxA/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**

	MxA [ng/mL]	CRP [mg/L]
LOB	4.50	0.30
LOD	5.70	0.40
LOQ	10.00	1.00

- **Analytical specificity**

- **Cross-reactivity**

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

Cross reactants	Concentration
MxB	100 ng/mL
Human IRGM (Immunity-related GTPase family M protein)	100 ng/mL
Human GBP1 (guanylate-binding protein 1)	100 ng/mL
Human GBP2 (guanylate-binding protein 2)	100 ng/mL
Human Gvin1 (Interferon-induced very large GTPase 1)	100 ng/mL
Human Interferon-γ-Inducible Protein-10	100 ng/mL
Interferon α	100 pg/mL
Interferon β 1a	100 pg/mL
Interferon λ	100 pg/mL
TNF-α	100 pg/mL
IL-10	100 pg/mL
IL-6	100 pg/mL
IL-2	100 pg/mL
PCT	50 ng/mL

- **Interference**

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

Cross reactants	Concentration
Ascorbic acid	350 µmol/L
Bilirubin (conjugated)	475 µmol/L
Albumin	60 g/L
Glucose	1,000 mg/dL
Triglycerides mixture	1,500 mg/dL
Hemoglobin	10 g/L
K_2-EDTA	5.45mg/mL
K_3-EDTA	5.45mg/mL
Na-Heparin	10.41mg/mL
Li-Heparin	10.44mg/mL
Sodium Citrate	32 mg/mL

- **Precision**

- **Single-site study]**

Repeatability (within-run precision)
within-laboratory precision (Total precision)
Lot to lot precision

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

CRP Conc. [mg/L]	Repeatability (within-run)		within-laboratory		Lot to lot precision	
	Mean [mg/L]	CV (%)	Mean [mg/L]	CV (%)	Mean [mg/L]	CV (%)
10	10.15	5.7	10.05	6.4	10.00	6.4
40	39.90	6.5	40.13	6.4	39.75	6.3
150	151.24	6.3	150.63	6.2	149.78	6.3
MxA Conc. [ng/mL]	Repeatability (within-run)		within-laboratory		Lot to lot precision	
	Mean [ng/mL]	CV (%)	Mean [ng/mL]	CV (%)	Mean [ng/mL]	CV (%)
20	20.24	5.7	20.22	5.8	19.99	6.2
50	48.56	6.1	49.68	6.6	50.39	6.6
100	99.26	6.5	99.99	6.2	99.87	6.3

- **Multi-site study**

Reproducibility

1 Lot of **AFIAS MxA/CRP** was tested for 5 days in 3 different sites (1 person per 1 site, 1 instrument per 1 site). Each standard material was tested 1 time per and 5 replicates per day.

Multi-site study (Reproducibility)			
CRP Conc. [mg/L]	Mean [mg/L]	SD	CV (%)
10	9.95	0.57	5.7
40	39.54	2.56	6.5
150	151.54	10.41	6.9
MxA Conc. [ng/mL]	Mean [ng/mL]	SD	CV (%)
20	20.42	1.26	6.2
50	49.53	3.46	7.0
100	100.88	6.70	6.6

- **Accuracy**

The accuracy was confirmed by testing with 3 different lots of **AFIAS MxA/CRP**. Each MxA sample was tested in 10 replicates, and recovery (%) calculated. Each CRP sample was tested in 10 replicates, and bias (%) was calculated.

Expected value CRP [mg/L]	Mean [mg/L]			3 LOTs of AVG [mg/L]	Bias (%)
	Lot 1	Lot 2	Lot 3		
41.20	38.84	40.47	41.60	40.30	-2.2%
Expected value MxA [ng/mL]	Mean [ng/mL]			3 LOTs of AVG [ng/mL]	Recovery (%)
	Lot 1	Lot 2	Lot 3		
20.00	20.10	19.39	19.05	19.51	97.6
100.00	97.09	99.92	95.24	97.42	97.4

- **Comparability**

AFIAS MxA/CRP has demonstrated the following clinical performance results.
The results were evaluated as 39 samples infected with bacteria, 25 samples infected with the virus.

Clinical Diagnosis	Sensitivity (95% CI)	Specificity (95% CI)
Viral infection	88.0 (75.3 - 100.0)	94.9 (87.9 – 100.0)
Bacterial infection	87.2 (76.7 - 97.7)	88.0 (75.3 – 100.0)

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

Σ	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

For technical assistance, please contact:

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