AFIAS IL-6

INTENDED USE

AFIAS IL-6 is a fluorescence Immunoassay (FIA) for the quantitative detection of interleukin-6 (IL-6) in human whole blood/serum/plasma. It is useful as an aid in management and monitoring of inflammatory disease.

For *in vitro* diagnostic use only.

INTRODUCTION

IL-6 (Interleukin-6) is produced by a variety of cells including T cells, B cells, fibroblasts, endothelial cells, monocytes, keratinocytes, mesangial cells, and some tumor cells. The genes for human and murine IL-6 have been cloned and sequenced. Human IL-6 has a molecular mass of 21 to 28 kDa, and is comprised of 212 amino acids that include two possible N-glycosylation sites and four cysteine residues.

IL-6 is a pleiotropic cytokine with multiple roles in the regulation of inflammation and hematopoiesis. IL-6 is produced at the site of inflammation and plays a key role in the acute phase response as defined by a variety of clinical and biological features such as the production of acute phase proteins.

IL-6 is the major regulator of the acute phase response in human hepatocytes. Due to its pleiotropic action, IL-6 has been intensively studied in many laboratories. It turned out to be an important factor in the immune and in the hematopoietic system and the major mediator in the hepatic acute phase response.

IL-6 is one of the proinflammatory cytokines and is detected in serum in the early stages of infections. Particularly in bacterial infections, IL-6 levels may be higher than CRP in early disease stages, and this may be helpful for early diagnosis. Early in infection, the CRP level may be low, but serial measurements can provide useful results and can be helpful in deciding when to discontinue antibiotic treatment. The combination of IL-6 and CRP has recently been proven to be useful in the early diagnosis of sepsis in newborns.

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-streptavidin on a test strip.

More antigens in the sample will form the more antigen-antibody complexes which lead to stronger fluorescence signal by detector antibodies, which is processed by the instrument for AFIAS tests to show IL-6 concentration in the sample.

COMPONENTS

AFIAS IL-6 consists of 'cartridges'.

- Each sealed aluminum pouch contains two cartridges.
- Each cartridge packaged in an aluminum pouch has three components including cartridge part, detector tube part and a diluent part.
- The cartridge part contains the membrane called a test strip which has streptavidin at the test line, and chicken IgY at the control line.
- The detector tube part has 2 granules containing the anti-interleukin 6 fluorescence conjugate, anti-Interleukin 6 biotin conjugate and anti-chicken IgY-fluorescence conjugate, HAMA Blocker, bovine serum albumin (BSA) as a stabilizer and sodium azide as a preservative in tris buffer and phosphate buffered saline (PBS).
- The diluent part contains sodium chloride, Tween20, TTAB and sodium azide as a preservative in the Tris buffer.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Follow instructions and procedures described in this 'Instruction 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 cartridge. A cartridge should be used for testing 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.
- Biotin interference was not observed in **AFIAS IL-6** when biotin concentration in the sample was up to 5 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 after discontinuation for 24 hours of biotin intake.
- **AFIAS IL-6** will provide accurate and reliable results subject to the below conditions.
  - **AFIAS IL-6** should be used only in conjunction with the instrument for AFIAS tests.
  - Have to use recommended anticoagulant.

Recommended anticoagulant
K_2EDTA, K_3EDTA, Lithium heparin

- 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.
  - 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
	2 - 30 °C	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-74

Components of **AFIAS IL-6**

- Cartridge Box:
  - Cartridge 24
  - Pipette tip (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 IL-6**. 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
- **C-tip (Zipper bag, 30 μL)** REF CFPO-199
- **Boditech IL-6 Control** REF CFPO-296
- **Boditech IL-6 Calibrator** REF CFPO-297

SAMPLE COLLECTION AND PROCESSING

The sample type for **AFIAS IL-6** 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 -20 °C.
- The samples (serum, plasma) stored frozen at -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) Clean the area with a pre-injection swab.
  (2) Pierce with a sterile lancet.
  (3) Wipe away the first drop of blood.
  (4) Gently massage the around the pricked fingertip for the second drop.
  (5) Hold the C-tip horizontally and touch the blood drop with the tip of the C-tip.
  (6) Capillary action will automatically draw the blood sample to C-tip and stop.
  (7) Wipe off any excess blood around the tip.
  (8) Double-check if whole blood fully 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 IL-6** as described below: cartridge, pipette tip, C-tip, an ID chip, a spare cartridge zipper bag and an instructions for use.
- 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-6, AFIAS-1, AFIAS-3**

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 100 µL of the sample (whole blood/serum/plasma/ 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 30 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/serum/plasma) on the screen.
5) Take 100 µL of the sample with 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 30 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 IL-6 concentration of the test sample in terms of pg/mL.
- Reference value: 7 pg/mL
- Working range: 2 - 2,500 pg/mL

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 **AFIAS IL-6**. For more information regarding obtaining the control materials, contact Boditech Med Inc.'s Sales Division for assistance. (Please refer to the instruction for use of control material.)

PERFORMANCE CHARACTERISTICS

- **Analytical sensitivity**

Limit of Blank (LoB)	0.5 pg/mL
Limit of Detection (LoD)	1.0 pg/mL
Limit of Quantification (LoQ)	2.0 pg/mL

- **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 IL-6** test results did not show any significant cross-reactivity with these biomolecules.

Cross-reactivity material	Conc.
Interleukin-1α	50 ng/mL
Interleukin-1β	50 ng/mL
Interleukin-2	50 ng/mL
Interleukin-3	50 ng/mL
Interleukin-4	50 ng/mL
Interleukin-8	50 ng/mL
Interferon-γ	50 ng/mL
TNF-α	50 ng/mL

- **Interference**

Interferents listed in the following table were added to the test sample at the concentration mentioned below. **AFIAS IL-6** test results did not show any significant interference with these materials.

Interference materials	Conc.
Bilirubin	342 umol/L
Cholesterol	13 mmol/L
D-Glucose	55 mmol/L
Hemoglobin	2 g/L
L-Ascorbic acid	170 umol/L
Triglyceride	37 mmol/L
EDTA	3.4 umol/L
Heparin	3,000 U/L

- **Precision**

- **Single-site study**

Repeatability (within-run precision)

within-laboratory precision (Total precision)

Lot to lot precision

3 Lots of AFIAS IL-6 were tested for 20 days. Each standard material was tested 2 times per day. For each test, each material was duplicated.

Conc. [pg/mL]	Repeatability (within-run)		within-laboratory		Lot to lot precision	
	Mean [pg/ml]	CV (%)	Mean [pg/ml]	CV (%)	Mean [pg/ml]	CV (%)
9.00	9.03	5.9	9.00	6.1	9.01	5.9
42.61	41.50	6.2	42.34	6.5	42.51	6.3
1,274	1,270.22	6.9	1,275.69	6.5	1,274.89	6.5

- **Multi-site study**

Reproducibility

1 Lot of AFIAS IL-6 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.

Conc. [pg/ml]	Reproducibility		
	Mean [pg/ml]	SD	CV (%)
9.00	8.98	0.62	6.9
42.61	42.55	2.90	6.8
1,274	1,278.58	91.40	7.1

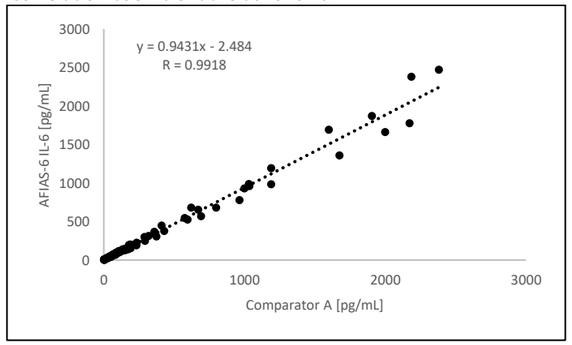
- **Accuracy**

The accuracy was confirmed by testing with 3 different lots of **AFIAS IL-6**. The tests were repeated 10 times at each concentration of the control standard.

Expected value [pg/mL]	Mean [pg/mL]			3 LOTs of AVG [pg/mL]	Recovery (%)
	Lot 1	Lot 2	Lot 3		
9.00	9.17	8.84	9.00	9.00	100.0
254.80	249.71	248.20	252.33	250.08	98.1
509.60	491.73	489.82	492.97	491.50	96.4
764.40	758.61	756.86	743.72	753.06	98.5
1019.20	1026.17	992.04	995.52	1004.58	98.6
1274.00	1288.13	1219.04	1252.84	1253.33	98.4

- **Comparability**

IL-6 concentration of 118 clinical samples were quantified independently with **AFIAS IL-6** (AFIAS-6) and Cobas e411 (Roche Diagnostics Inc. Switzerland) 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.

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

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