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Infection

AFIAS IGRA-TB

INTENDED USE

AFIAS IGRA-TB is a qualitative fluorescence immunoassay (FIA) for detection of IFN-γ (Interferon gamma) released in response to *in vitro* stimulation by *Mycobacterium tuberculosis* specific antigen in human whole blood. It is useful as an aid in management and monitoring of Tuberculosis infection.
For *in vitro* diagnostic use only.

INTRODUCTION

Tuberculosis (TB) is a chronic disease that is infected by Mycobacterium tuberculosis. It is one of the most serious epidemics in the world, along with HIV and malaria. It is categorized into two phase, active TB and Latent TB in clinical point of view. It is crucial to detect Latent TB since about 10% of it give rise to active disease in immunocompromised patients. Diagnosis of Latent TB, however, is not easy because it is normal in the mycobacterium culture test and chest X-ray examination. To diagnose the Latent TB, the IFN-γ release assays (IGRAs), in vitro blood tests of cell-mediated immune response that measure T-cell released IFN-γ following stimulation by antigens specific to the M. tuberculosis (ESAT-6 and CFP-10), has been used. AFIAS IGRA-TB is the first lateral flow system of IGRA assays, which means that it is more simple and rapid test ever. It is useful as an aid in excluding the tuberculosis diseases.

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 more antigen-antibody complexes which lead to stronger fluorescence signal by detector antibodies, which is processed by the instrument for AFIAS tests to show Latent TB infection 'Positive', 'Negative' of the sample.

COMPONENTS

AFIAS IGRA-TB consists of 'cartridges'.

- Each sealed aluminum pouch contains two cartridges.
- Each cartridge packaged in an aluminum pouch has three components including a cartridge part, a detection buffer 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 part has two granules containing anti-IFN-γ fluorescence conjugate, anti-IFN-γ biotin conjugate, anti-chicken IgY fluorescence conjugate.
- The diluent part contains tween 20 as a detergent and sodium azide in Tris-HCl.

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 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 (plasma) 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 should be handled carefully and discarded by an appropriate method in accordance with relevant local regulations.
- The cartridge contains sodium azide (NaN3), and it may cause certain health issues like convulsions, low blood pressure and low 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 IGRA-TB** when biotin concentration in the sample was below 200 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 IGRA-TB** will provide accurate and reliable results subject to the below conditions.
  - **AFIAS IGRA-TB** should be used only in conjunction with the instrument for AFIAS tests.
  - Have to use recommended anticoagulant.

Recommended anticoagulant
Lithium heparin

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 antigens 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 antigens with time and/or temperature may also cause false negative result as it makes antigens 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-99**

Components of **AFIAS IGRA-TB**

- 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 with **AFIAS IGRA-TB**
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 IGRA-TB Control** REF **CFPO-294**
- **ichroma™ IGRA-TB tube** REF **CFPO-206**

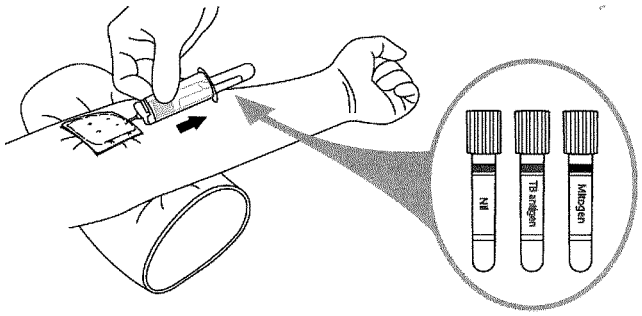
SAMPLE COLLECTION AND PROCESSING

The sample type for AFIAS IGRA-TB is Li-heparin plasma.

- For each patient collect 1 mL of blood by venipuncture directly into each of the ichroma™ IGRA-TB Tube.
  - The black line on the side of the tube indicates the range from 0.8 to 1.2 mL. If the blood level in the tube deviates off the indicated range, a new blood sample must be taken.
- Collect 1 mL of blood in the order the ichroma™ IGRA-TB Nil tube *(gray), TB antigen tube (red)*, and Mitogen tube (purple), and invert 10 times gently so that the additive and blood are well mixed.
  - Invert well to ensure that the inner wall of the tube is completely coated with blood.
  - If inverted too vigorously, hemolysis and gel division may occur, which may cause abnormal results.
- Fill in the information of the patient whose blood has been collected on the label.
- After blood collection, it must be transferred to an incubator (37 ± 1°C) immediately or within 16 hours.
  - Prior to incubation, maintain and transport the tubes at room temperature (22 ± 5°C).
  - If not incubated immediately but within 16 hours after collection, invert the tube 10 times gently again before incubation.
  - Incubate the tube by placing vertically within 16 hours of sample collection at 37 ± 1°C for 16-24 hours.

※ **If the above method is not followed, there may be errors in the results.**

- After incubation, centrifuge the blood collection tube at 2,000 ~ 3,000 RCF (g) for 15 minutes.
  - After incubation, the centrifugation should be immediately performed to obtain plasma.
- **Sample storage;**
  - The centrifuged tube can be stored for up to 1 week at 2°C to 8°C before the extraction of plasma and if harvested, should be stored at below -20°C for 1 month.
  - As a repeated freeze-thaw cycle may affect the test result, do not refreeze previously frozen samples.
  - Samples containing precipitates must be clarified by centrifugation before analysis.



Drawing into ichroma™ IGRA-TB Tube	Drawing into Lithium Heparin Tube
Collect 1 ml of blood into each of ichroma™ IGRA-TB Tubes. (Nil ▸ TB antigen ▸ Mitogen)	Collect 4 ml of blood into single Li-Heparin coated tubes and distribute it into each of ichroma™ IGRA-TB Tubes. (Nil ▸ TB antigen ▸ Mitogen)

↓

Invert tubes by 10 times.

↓

Incubate tubes at 37 ℃ ± 1 ℃ for 16-24 hours.

↓

Centrifuge tubes at 2000-3000 RCF (G) for 15 min.

TEST SETUP

- Check the components of the **AFIAS IGRA-TB** as described below. : cartridges, pipette 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'.

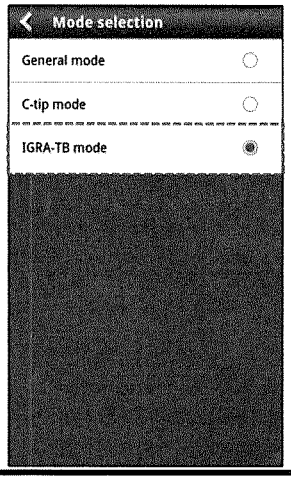
※ **Please refer to the instrument for AFIAS tests operation manual for complete information and operating instructions.**

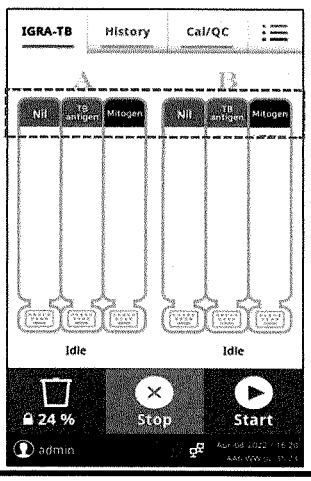
TEST PROCEDURE

▶ **AFIAS-6**

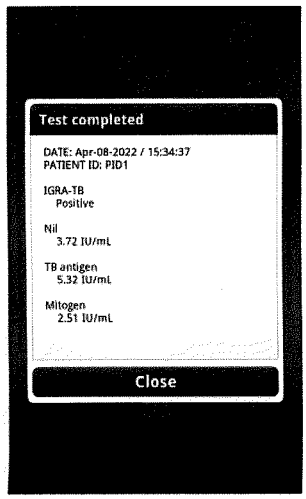
IGRA-TB Mode

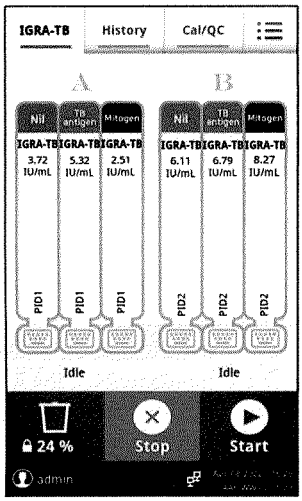
1) To select 'IGRA-TB Mode', choose either 'General Mode' or 'C-tip Mode' on the instrument when first turned on.
2) Change the mode by selecting the 'IGRA-TB Mode' in the setting menu.
   * Press the setting menu button. → Click 'Mode change' → Select the 'IGRA-TB Mode'





3) Label the 3 cartridges along with test sequence, Nil, TB antigen, and Mitogen.
   ex) N for Nil, A for TB antigen, M for Mitogen
4) Take 100 µL of sample (Li-heparin plasma/control) using a pipette and dispense it into the sample well of the cartridge.
5) Insert a cartridge into the cartridge holder.
6) Insert a tip into the tip hole of the cartridge.
7) Enter the same PID for Nil, TB antigen, and Mitogen respectively.
   * If the different PIDs were input, the results cannot be interpreted.
8) After entering the PID, select the sample type.
9) Tap the 'START' button on the screen.
10) The test results will be displayed on the screen after 15 minutes.





▶ AFIAS-3

IGRA-TB Mode

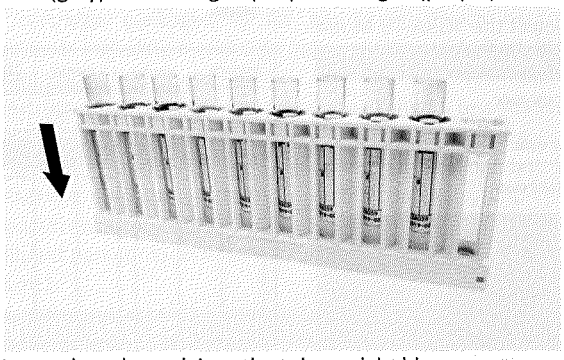
1) Select the IGRA-TB mode at the top of the display.
2) The test is conducted in the same way as AFIAS-6. (refer to 3) ~ 10) in AFIAS-6 test procedure)

▶ AFIAS-10

Normal Mode

This mode is used when using the ichroma™ IGRA-TB Tubes.

1) Attach a barcode label with patient information (ex. PID) to the tube wall of the sample obtained according to 'SAMPLE COLLECTION AND PROCESSING'.
   * The barcode labels attached to the 3 tubes (Nil, TB antigen, Mitogen tube) must be the same. If the barcodes do not match, the test cannot be started.
2) Load the processed tubes into the tube rack sequentially.
   * Nil (gray) ▶ TB antigen (red) ▶ Mitogen (purple)



3) Insert the tube rack into the tube rack holder.
4) Label the 3 cartridges along with test sequence, Nil, TB antigen, and Mitogen.
   ex) N for Nil, A for TB antigen, M for Mitogen
5) Insert the cartridges into the cartridge holder.
6) Insert a tip into the tip holder or tip hole of the cartridge.
7) 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.
8) Tap the Start " button on the screen.
9) The test results will be displayed on the screen after 15 minutes.

Emergency Mode

This mode is used when dispensing the sample into the sample well of the cartridges.

1) Label the 3 cartridges along with test sequence, Nil, TB antigen, and Mitogen.
   ex) N for Nil, A for TB antigen, M for Mitogen
3) Take 100-µL of sample (Li-heparin plasma/control) using a pipette and dispense it into the sample well of the cartridge.
4) Insert a cartridge into the cartridge holder.
5) Insert a tip into the tip holder or tip hole of the cartridge.
6) 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.
7) Convert the 'Emergency mode' and enter the same PID for Nil, TB antigen, and Mitogen respectively.
   * If the different PIDs were input, the test cannot be started.
8) Tap the 'START' button on the screen.
9) The test results will be displayed on the screen after 15 minutes.

INTERPRETATION OF TEST RESULT

- The instrument for AFIAS tests calculates the test result automatically and displays IGRA-TB concentration of the test sample in terms of IU/mL.

Nil (IU/mL)	TB Antigen minus Nil (IU/mL)	Mitogen minus Nil (IU/mL)	AFIAS IGRA-TB (IU/mL)	Report/ Interpretation
≤8.0	<0.35	≥0.5	**Negative**	M. tuberculosis infection **NOT** likely
	≥0.35 and <25% of Nil value	≥0.5		
	≥0.35 and ≥25% of Nil value	Any	**Positive**	M. tuberculosis infection likely
	<0.35	<0.5	**Indeterminate**	Results are indeterminate for TB-Antigen responsiveness
	≥0.35 and <25% of Nil value	<0.5		
>8.0	Any	Any		

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 IGRA-TB**. 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**
  - LoB (Limit of Blank) 0.02596 IU/mL
  - LoD (Limit of Detection) 0.093 IU/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 IGRA-TB** test results did not show any significant cross-reactivity with these biomolecules.

Interference material	Concentration (ng/mL)
Tumor Necrosis Factor-a	100
Tumor Necrosis Factor-b	100
Interleukin-2	100
Interleukin-4	100
Interleukin-6	100
Interleukin-10	100
Interleukin-17	100
Interleukin-23	100
Interleukin-27	100

- **Interference**

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

Interferents	Concentration
Li-Heparin	100,000 U/L
Hemoglobin	2 mg/mL
BSA	60 mg/mL
Bilirubin	0.24 mg/mL (400 μM)
Triglycerides	1.5 mg/mL
Cholesterol	7.7 mg/mL

■ Precision

- *General imprecision*

The standard materials are tested with the 3 different Lots of **AFIAS IGRA-TB** by the same operator at the same site 2 times a day (a.m./p.m.) for 20 days. For each run, the sample was tested in duplicates.

- Between person

The standard materials are tested with the 1 Lot of **AFIAS IGRA-TB** by three different operators at the same site 2 times a day (a.m./p.m.) for 5 days.

- Between sites

The standard materials are tested with the 1 Lot of **AFIAS IGRA-TB** by the same operator at three different sites 2 times a day (a.m./p.m.) for 5 days.

- Between readers

The standard materials are tested with the 1 Lot of **AFIAS IGRA-TB** by the same operator at the same sites with three different readers 2 times a day (a.m./p.m.) for 5 days.

STD no.	General imprecision		Between person	
	Positive No./ Total No.	Positive rate	Positive No./ Total No.	Positive rate
STD 1	0/240	0%	0/30	0%
STD 2	240/240	100%	30/30	100%
STD 3	240/240	100%	30/30	100%
STD no.	Between sites		Between readers	
	Positive No./ Total No.	Positive rate	Positive No./ Total No.	Positive rate
STD 1	0/30	0%	0/30	0%
STD 2	30/30	100%	30/30	100%
STD 3	30/30	100%	30/30	100%

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7. Mahomed, H., et al. "Comparison of Mantoux skin test with three generations of a whole blood IFN-γ assay for tuberculosis infection." The International Journal of Tuberculosis and Lung Disease 10.3 (2006): 310-316.
8. ECDC. 2011. Use of interferon-gamma release assays in support of TB diagnosis

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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