ichroma™ IGRA-TB

INTENDED USE

ichroma™ 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 and is one of the most serious epidemics in the world, along with HIV and malaria. It is categorized into two phases, 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), have been used.

ichroma™ 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 ichroma™ tests to show latent TB infection 'Positive', 'Negative' in the sample.

COMPONENTS

ichroma™ IGRA-TB consists of 'cartridges', 'detector tubes', and 'detector diluents'.

- The cartridge contains the membrane called a test strip which has streptavidin at the test line, and chicken IgY at the control line. All cartridges are individually sealed in an aluminum foil pouch containing a desiccant, and they are further packaged in a box.
- The detector tube has two granules containing paired anti-human IFN-γ antiboides conjugated with fluorescence and biotin, anti-chicken IgY-fluorescence conjugate. All detector tubes are packed in a box.

The detector diluent contains tween 20 as a detergent and sodium azide in Tris-HCl. The diluent is dispensed in a vial. The detector diluent is packed in a box.

WARNING AND PRECAUTIONS

- For in vitro diagnostic use only.
- Follow the 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, detector tube, detector diluent and ID chip) except for lot number of IGRA-TB tube must match each other.
- The lot number of the IGRA-TB tube is managed separately.
- 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, detector tubes and detector diluent. A cartridge should be used for testing one sample only. A detector tube should be used for processing of one sample only.
- Do not use tube if any tube shows sign of damage prior to use.
- The cartridge should remain sealed in its original pouch until just before use. Do not use 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 severehemolysis and/or hyperlipidemia must not be used.
- If test components and/or sample are stored in refrigerator, then allow cartridge, detection tube, diluent and sample to be at room temperature for approximately 30 minutes before use.
- The instrument for ichroma™ tests may generate slight vibration during use.
- Used cartridges, detector tubes, detector diluents and pipette tips should be handled carefully and discarded by an appropriate method in accordance with relevant local regulations.
- No Biotin interference was observed in **ichroma™ 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.
- ichroma™ IGRA-TB will provide accurate and reliable results subject to the below conditions.
  - ichroma™ IGRA-TB should be used only in conjunction with the instrument for ichroma™ tests.
  - Have to use recommended anticoagulant.

Recommended anticoagulant
Lithium Heparin

STORAGE AND STABILITY

Storage condition			
Components	**Storage temperature**	**Shelf life**	**Note**
Cartridge	2-30℃	20 months	Disposable
Detector tube	2-30℃	20 months	Disposable
Detector diluent	2-30℃	20 months	Unopened
	2-30℃	20 months	Opened

- After the cartridge pouch is opened, the test should be performed immediately.
- Return an unused cartridge to the spare cartridge zipper bag containing the desiccant pack. Reseal along entire edge of zip-seal.

LIMITATION 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 the 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.

MATERIALS SUPPLIED

REF CFPC-86

Components of ichroma™ IGRA-TB for ichroma™-50

- **Cartridge box:**
  - Cartridge 300
  - ID Chip 1
  - Instruction for use 1
  - Spare cartridge zipper bag 1
- **Buffer Box:**
  - Detector tube 300
  - Detector diluent 12

MATERIALS SUPPLIED

Following items can be purchased separately with ichroma™ IGRA-TB test.

Please contact our sales division for more information.

- **Instrument for ichroma™ tests**
  - ichroma™-50 REF FPRR022 (automatic analysis)
  - ichroma™-50 PLUS REF FPRR036
- **Boditech IGRA-TB Control** REF CFPO-294
- **ichroma™ IGRA-TB tube** REF CFPO-206

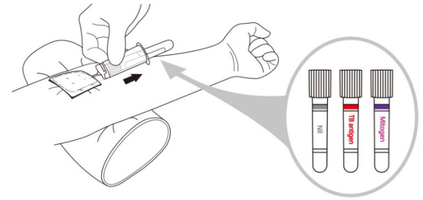
SAMPLE COLLECTION AND PROCESSING

The sample type for ichroma™ IGRA-TB is Lithium 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 of 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.

↓

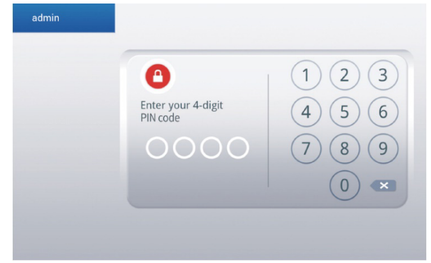
Incubate tubes at 37 ℃ ± 1 ℃ for 16-24 hours.

↓

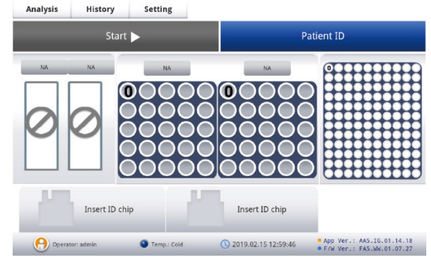
Centrifuge tubes at 2000-3000 RCF (G) for 15 min.

TEST SETUP

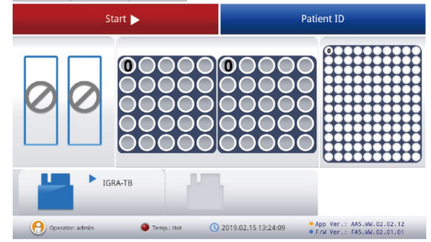
- Check the contents of ichroma™ IGRA-TB: Sealed cartridges, detector tubes, detector diluents, ichroma™ IGRA-TB tubes, an ID chip and an instructions for use.
- Ensure that the lot number of the cartridge matches that of matches that of the detector tube, the detector diluent as well as an ID chip.
- If the sealed cartridge, the detector tube and the detector diluent have been stored in a refrigerator, place them on a clean and flat surface at room temperature for at least 30 minutes before testing. Manipulate the ichroma™ as follows:



1. Turn on the Instrument for ichroma™-50 and enter the 4-digit PIN code.

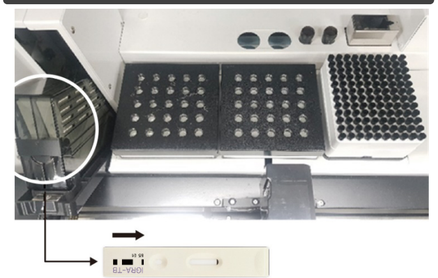


2. Make sure the initial screen



3. Insert the ID chip into the ID chip port of the ichroma™-50.

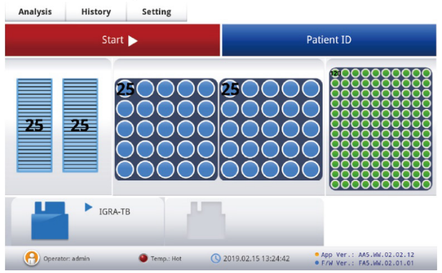
TEST PROCEDURE



1. Load the cartridges, Detector tubes, Diluent, tips and ID chip in the instrument.

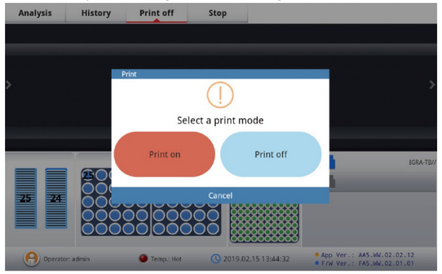
   * Confirm the orientation of the cartridges in the cartridge magazine.

* For ichroma™-50 Plus, the cartridge is packaged with the cartridge inserted into the magazine.

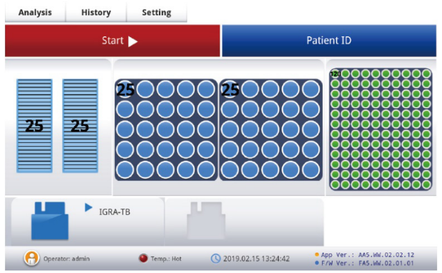


2. Adjust the number of test cartridges, tubes and tips on the touch screen. And make sure the activation of IGRA-TB ID chip.

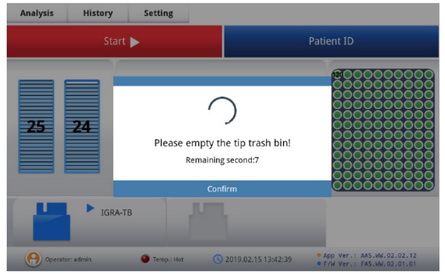
3. Load the processed tubes into the tube rack sequentially.
   * Nil (Gray) ▶ TB antigen (Red) ▶ Mitogen (Purple) per a patient



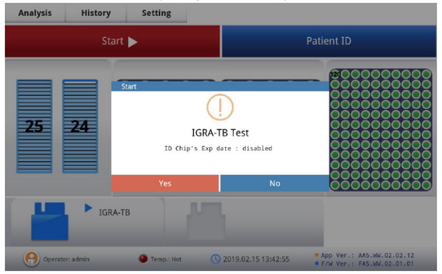
4. Make a choice to activate the print mode. If the print mode is on, the results of 3 tubes per a specimen are printed out when 3rd (Mitogen tube) test is finished.



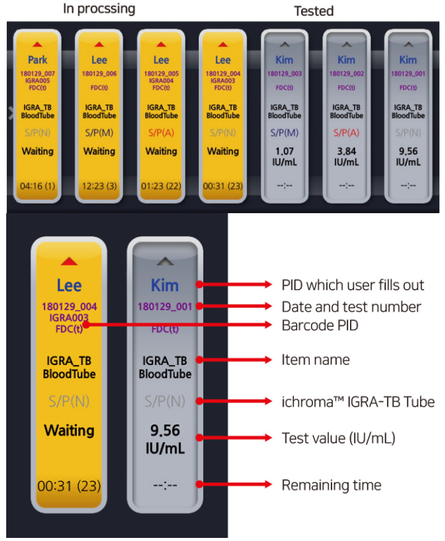
5. Press the "Start" button on the screen.



6. Clean out the remaining tips and cartridges used in the trash bin.
   * Make sure the tip ejecting area is empty.



7. Confirm the agreement between test item and a program on the instrument. If correct, Press "Yes" on the screen.
8. Make sure the running stage in progress. The contents of each test is described as below:

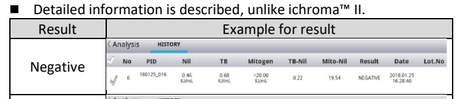
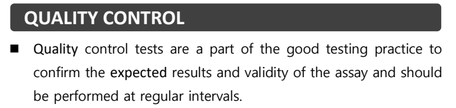


9. Press the "History" to make sure the test results. Each result can be popped up when press the result for 3 to 5 seconds.

INTERPRETATION OF TEST RESULT

Nil (IU/mL)	TB Antigen minus Nil (IU/mL)	Mitogen minus Nil (IU/mL)	ichroma™ 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		

■ Display of the ichroma™-50 is shown on the "History" section
■ Detailed information is described, unlike ichroma™ II.

Result	Example for result
Negative	
Positive	
Indeterminate	
Invalid	

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 **ichroma™ IGRA-TB**. 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.)

INTERPRETATION OF TEST RESULT

■ **Analytical sensitivity**
- Limit of Blank (LoB) 0.03 IU/mL
- Limit of Detection (LoD) 0.05 IU/mL

■ **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. **ichroma™ IGRA-TB** test results did not show any significant cross-reactivity with these biomolecules.

Cross-reactants	Conc.
Tumor Necrosis Factor-α (TNF-α)	100 ng/mL
Tumor Necrosis Factor-β (TNF- β)	100 ng/mL
Interleukin-2 (IL-2)	100 ng/mL
Interleukin-4 (IL-4)	100 ng/mL
Interleukin-6 (IL-6)	100 ng/mL
Interleukin-10 (IL-10)	100 ng/mL
Interleukin-17 (IL-17)	100 ng/mL
Interleukin-23 (IL-23)	100 ng/mL
Interleukin-27 (IL-27)	100 ng/mL

- Interference

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

Interferents	Conc.
Li-Heparin	100,000 U/L
Bilirubin	400 μM
Hemoglobin	2 mg/mL
Triglycerides	1.5 mg/mL
Cholesterol	7.7 mg/mL
BSA	60 mg/mL

■ **Precision**

Single-site study

ichroma™ IGRA-TB were tested with the 3 different Lots by the same person at the same site in duplicate 2 times a day for 20 days.

Between person

Three different people test the standard materials 2 times for 5 days.

Between site

At three different sites each, the standard materials are tested 2 times for 5 days.

Between reader

Three different readers test the standard materials 2 times for 5 days.

Single-site study				
Standard material	Single-site study		Between person	
	Positive No. / Total No.	Positive rate	Positive No. / Total No.	Positive rate
Neg.	0 / 240	0%	0 / 30	0%
Low Pos.	240 / 240	100%	30 / 30	100%
Mid Pos.	240 / 240	100%	30 / 30	100%
Standard material	Between site		Between reader	
	Positive No. / Total No.	Positive rate	Positive No. / Total No.	Positive rate
Neg.	0 / 30	0%	0 / 30	0%
Low Pos.	30 / 30	100%	30 / 30	100%
Mid Pos.	30 / 30	100%	30 / 30	100%

■ **Clinical performance**

		Comparator A		Total
		Pos.	Neg.	
ichroma™ IGRA-TB	Pos.	102	6	108
	Neg.	23	232	255
Total		125	238	363
PPA (%)		81.6% (95% C.I 73.9 ~ 87.4%)		
NPA (%)		97.5% (95% C.I 94.6 ~ 98.8%)		
OPA (%)		92.0% (95% C.I 88.8 ~ 94.4%)		
* Cohen's kappa (κ)		0.817 (95% C.I 0.754 ~ 0.881) [very good agreement]		

*<Analysis of kappa statistics>
- 0.000 ~ 0.200: poor agreement
- 0.201 ~ 0.400: fair agreement
- 0.401 ~ 0.600: moderate agreement
- 0.601 ~ 0.800: good agreement
- 0.801 ~ 1.000: very good agreement

REFERENCE

1. 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.
2. 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

For technical assistance, please contact:
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CE IVD