ichroma™ IGRA-TB 25

INTENDED USE

ichroma™ IGRA-TB is a qualitative fluorescence immunoassay (FIA) for detection of interferon gamma (IFN-γ) 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 tuberculos* 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, have been used.

ichroma™ IGRA-TB 25 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- 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 ichroma™ tests to show 'latent TB-positive' in the sample

COMPONENTS

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

- 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 antibodies conjugated with fluorescence and biotin, anti-chicken IgY-fluorescence conjugate, bovine serum albumin (BSA), sucrose, and mouse IgG as a stabilizer. All detector tubes are packed in a box.
- The detector diluent contains tween-20 as a detergent, and sodium chloride as a stabilizer in Tris-HCl, and it is pre-dispensed in a vial. The detector diluent is packed in a box.

WARNINGS 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) 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 or detector tubes. A cartridge should be used for testing one sample only. A detector tube 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 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, detector tube, detector 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 diluent and pipette tips should be handled carefully and discarded by an appropriate method in accordance with relevant local regulations.
- **ichroma™ IGRA-TB 25** will provide accurate and reliable results subject to the below conditions.
  - **ichroma™ IGRA-TB 25** should be used only in conjunction with instrument for ichroma™ tests.
  - Have to use recommended anticoagulant.

Recommended anticoagulant
Lithium Heparin

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 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 the 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	Disposable
Detector tube	2 - 30 °C	20 months	Disposable
Detector diluent	2 - 30 °C	20 months	Unopened
		20 months	Opened

- After the cartridge pouch is opened, the test should be performed immediately.

MATERIALS SUPPLIED

REF CFPC-86-1

Components of **ichroma™ IGRA-TB 25**

- Cartridge Box:
  - Cartridge 150
  - Detector tube 150
  - Detector diluent 6
  - ID Chip 1
  - Instructions for use 1

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

Following items can be purchased separately from **ichroma™ IGRA-TB 25**.

Please contact our sales division for more information.

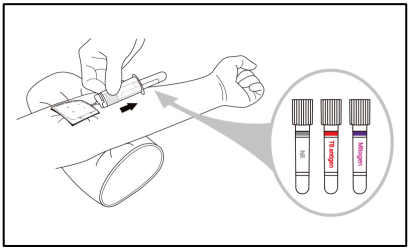
- Instrument for ichroma™ tests
  - **ichroma™ II** REF FPRR021
  - **ichroma™ III** REF FPRR037
  - **ichroma™ M2** REF FPRR031
- **Boditech IGRA-TB Control** REF CFPO-294
- **ichroma™ IGRA-TB tutbe** REF CFPO-206

SAMPLE COLLECTION AND PROCESSING

The sample type for **ichroma™ IGRA-TB 25** 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

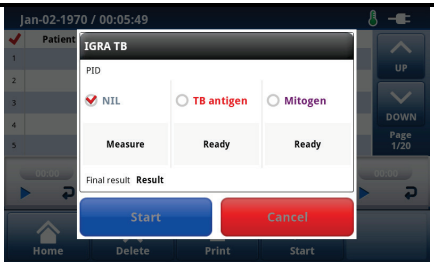
- Check the contents of **ichroma™ IGRA-TB 25**: Sealed cartridges, detector tubes, detector diluents, an ID chip and an instructions for use.
- Ensure that the lot number of the cartridge matches that of the detector tubes, the detector diluent as well as an ID Chip.
- If the sealed cartridge, the detector 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.
- Turn on the instrument for ichroma™ tests.
- Insert the ID chip into the 'ID chip port'.

※ **Please refer to the 'Instrument for ichroma™ tests Operation Manual' for complete information and operating instructions.**

TEST PROCEDURE

▶ **ichroma™ II**

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
2) Take 100 µL of detector diluent using a pipette and dispense it to the detector tube containing a granule. When the granule form is completely dissolved in the tube, it becomes detection buffer. (The detection buffer must be used immediately. Do not exceed 30 seconds.)
3) Take 50 µL of sample using a pipette to the detector tube and mix the sample thoroughly by shaking it about 10 times. (The sample mixture must be used immediately. Do not exceed 30 seconds.)
4) Take 100 µL of the sample mixture and dispense it into the sample well of the cartridge.
   * ①Nil, ②TB antigen and ③Mitogen samples are loaded sequentially.
5) Leave the cartridge at room temperature for 15 minutes.

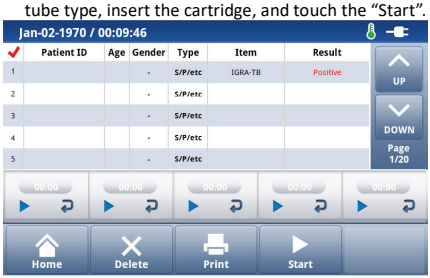


6) Insert Nil tube sample-loaded cartridge into the cartridge holder of the ichroma™ II first and touch the "Start" button. Make sure the pop-up display of IGRA-TB-specific test mode.
7) Confirm the value of Nil tube-test (IU/mL) and then insert TB antigen tube sample-loaded cartridge into the holder. Touch the "Start" on the IGRA-TB mode.

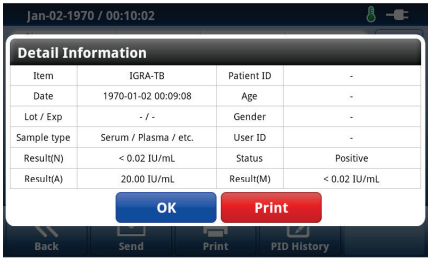
8) Confirm the value (IU/mL) and then insert Mitogen tube sample-loaded cartridge into the holder finally. Touch the "Start" on the IGRA-TB mode.
   * Eusure proper orientation and sequence of the cartridge before pushing it all the way inside the cartridge holder.

9) Make sure "Final result" on the screen and the printed result. If you want to other IGRA-TB tests, touch the "Finish" to back into Multi mode.

9-1) If you want to re-test among 3 tubes, touch the tube type, insert the cartridge, and touch the “Start”.



10) Insert new Nil tube sample-loaded cartridge into the holder and touch the “Start” to carry out new IGRA-TB test.

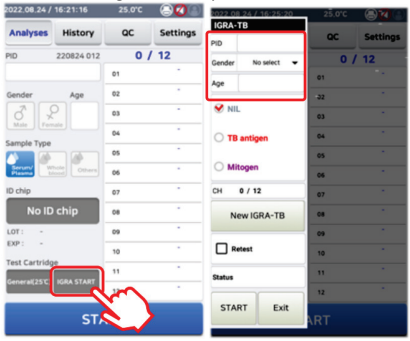


11) If you want to know detailed information of the test on the screen, double touch each result and make sure the details.

▶ **ichroma™ III**

<IGRA-TB mode>

1) Touch ‘IGRA START’ on the ‘Test Cartridge’ tab in display to start IGRA-TB mode.
2) Fill PID, gender, age in IGRA-TB mode and proceed the test according to the test procedure on below.



<Test procedure>

1) This step is same with ichroma™ II test procedure from 1 to 3.
4) Take 100 µL of Nil sample mixture using a pipette and dispense it in the sample well on the cartridge.
5) Insert Nil tube sample-loaded cartridge into the cartridge holder of ichroma™ III first and touch the “Start” button. Make sure the pop-up display of IGRA-TB specific test mode.
6) Take 100 µL of TB antigen sample mixture using a pipette and dispense it in the sample well on the cartridge with repeating of step 1 ~ 3.
7) Insert TB antigen tube sample-loaded cartridge into the cartridge holder of ichroma™ III first and touch the “Start” button. Make sure the pop-up display of IGRA-TB specific test mode.
8) Take 100 µL of Mitogen sample mixture using a pipette and dispense it in the sample well on the cartridge with repeating of step 1 ~ 3.
9) Insert Mitogen tube sample-loaded cartridge into the cartridge holder of ichroma™ III first and touch the “Start” button. Make sure the pop-up display of IGRA-TB specific test mode.
   * ①Nil, ②TB antigen, ③Mitogen samples are loaded sequentially.
10) Make sure “Final result” on the screen and the printed result. If you want to other IGRA-TB tests, touch the “New IGRA-TB” to proceed new IGRA-TB test.
10-1) If you want to re-test among 3 tubes, touch the “Retest” button to choose the test result needs re-test. And insert the cartridge and touch the “Start”.
11) Insert new Nil tube sample-loaded cartridge into the holder and touch the “Start” to the carry out new IGRA-TB test.
12) If you want to know detailed information of the test on the screen, double touch each result and make sure the details.

▶ **ichroma™ M2**

<Read Now mode>

1) Check the display "Read Now" on the ichroma™ M2 screen and set the tube type (Nil, TB antigen, Mitogen).
2) The test procedure is same with "ichroma™ II test procedure 1-5."
3) Leave the cartridge at room temperature for 15 minutes.
   ⚠ *Scan the sample-loaded cartridge immediately when the incubation time is over. If not, it will cause inaccurate test result.*
4) To scan the sample-loaded cartridge, insert it into the cartridge holder of the instrument for ichroma™ tests. Ensure proper orientation of the cartridge before pushing it all the way inside the cartridge holder. An arrow is marked on the cartridge especially for this purpose.
5) ichroma™ M2 is tested automatically after inserting.
6) The instrument for ichroma™ tests will start scanning the sample loaded cartridge immediately.
7) Read the test result on the display screen of the instrument for ichroma™ tests.

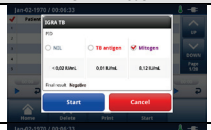
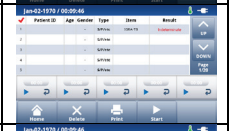
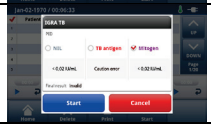
<Walk Away mode>

1) Check the display "Walk Away" on the ichroma™ M2 screen and set the tube type (Nil, TB antigen, Mitogen).
2) The test procedure is same with "ichroma™ II test procedure 1-5."
3) Insert the sample-loaded cartridge into the holder of the instrument for ichroma™ tests. Ensure proper orientation of the cartridge before pushing it all the way inside the cartridge holder. An arrow is marked on the cartridge especially for this purpose.
4) ichroma™ M2 is tested automatically after inserting.
5) The cartridge goes inside the instrument for ichroma™ tests and will automatically start scanning the sample-loaded cartridge after 15 minutes.
6) Read the test result on the display screen of the instrument for ichroma™ tests.

INTERPRETATION OF TEST RESULT

Nil (IU/mL)	TB Antigen minus Nil (IU/mL)	Mitogen minus Nil (IU/mL)	ichroma™ IGRA-TB 25 (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™ result is shown on the pop-up screen of IGRA-TB immediately.
- The results are also shown on the screen of the 'Multi-mode' as above described.

Result	Example for result	
	IGRA-TB mode	Multi-mode
Neg.		
Pos.		
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 25**. 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)	0.03 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 25** test results did not show any significant cross-reactivity with these biomolecules.

Cross-reactivity material	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 25** test results did not show any significant interference with these materials.

Interference material	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 25 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

REFERENCES

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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