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ichroma™ IGRA-TB 25

INTENDED USE

ichroma™ IGRA-TB 25 is a qualitative fluorescence Immunoassay (FIA) for detection of interferon gamma (INF-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 tuberculosis and 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, has 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; Dried detector and captor antibodies in cartridge binds to IFN-γ in sample, forming antigen-antibody complexes, and migrates onto nitrocellulose matrix to be captured by immobilized streptavidin on test strip.
The more IFN-γ in sample forms the more antigen-antibody complex and leads to stronger intensity of fluorescence signal on detector antibody which is processed by instrument for ichroma™ tests to show 'latent TB-positive' in sample.

COMPONENTS

ichroma™ IGRA-TB 25 consists of 'Cartridges', 'Detector tubes', 'Detector Diluent' and an 'ID chip'.

- The cartridge contains a test strip, the membrane which has streptavidin at the test line, while chicken IgY at the control line.
- The cartridges are individually sealed in an aluminum foil pouch containing a desiccant. 25 sealed pouches are packed in a box which also contains an ID chip.
- The Detector is in the form of a dried granulated ball. It contains paired anti-human IFN-γ antibodies conjugated with fluorescence and biotin, anti-chicken IgY-fluorescence conjugate, bovine serum albumin (BSA) as a stabilizer and sodium azide in phosphate buffered saline (PBS) as a preservative.
- The Detector is pre-dispensed in a tube and sealed. 25 Detector tubes are packed in **ichroma™ IGRA-TB 25** Buffer Box.
- The Detector Diluent contains a detergent and bovine serum albumin (BSA) as a stabilizer and sodium azide as a preservative. The diluent is dispensed in a shaded vial.

WARNING 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, ID chip, Detector tube and Detector Diluent) should agree.
- Do not interchange test components between different lots or use test components after the expiration date, either of which might yield incorrect test result(s).
- Do not reuse cartridges, Detector tube or Detector Diluent. A Detector tube should be used for processing of one sample only. 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 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.
- Allow cartridge, Detector 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 Detector tubes, Detector Diluent, pipette tips and cartridges should be handled carefully and discarded by an appropriate method in accordance with relevant local regulations.
- An exposure to larger quantities of sodium azide may cause certain health issues like convulsions, low blood pressure and heart rate, loss of consciousness, lung injury and respiratory failure.
- **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 sample.

Recommended anticoagulant
Lithium Heparin

STORAGE AND STABILITY

Storage condition			
Component	Storage Temperature	Shelf life	Note
Cartridge	4-30 ℃	20 months	Disposable
Detector tubes	4-30 ℃	20 months	
Detector diluent	4-30 ℃	20 months	Unopened
	4-30 ℃	20 months	Opened

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

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. The non-responsiveness of the antibodies to the antigens is most common where the epitope is masked by some unknown components, so as not to be detected or captured by the antibodies. The instability or degradation of the antigen with time and/or temperature may cause the false negative 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 including clinical symptoms and other relevant test results.

MATERIALS SUPPLIED

REF CFPC-86-1
Components of **ichroma™ IGRA-TB 25 for ichroma™ II**

- **Cartridge Box:**
  - Cartridges 25
  - ID Chip 1
  - Instruction for Use 1
- **Buffer Box**
  - Detector tube 25
  - Detector Diluent 1

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

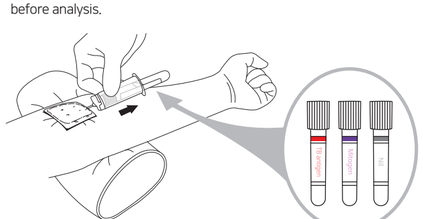
Following items can be purchased separately from **ichroma™ IGRA-TB test**. Please contact our sales division for more information.

- Instrument for ichroma™ tests
  - **ichroma™ II** REF FPRR021 (manual analysis)
- **ichroma™ IGRA-TB tube** REF CFPO-206 (**exclusive use**)

SAMPLE COLLECTION AND PROCESSING

The sample type for **ichroma™ IGRA-TB 25** is Li-heparin plasma.

- For each patient collect 1 mL of blood by venipuncture directly into each of the **ichroma™ IGRA-TB Tube**.
- Collect 1 mL of blood in the order the ichroma™ IGRA-TB Nil tube (gray), TB antigen tube (red), and Mitogen tube (purple), and shake 10 times vigorously so that the additive and blood are well mixed.
- Fill in the information on the person who has collected blood on the label and place it in the incubator (37 ± 1 ℃) within 16 hours (2 hours recommended).
- If not cultured immediately after collection, the tubes are lightly mixed 10 times before incubation.
- Incubate the tube vertically at 37 ± 1 ℃ for 16-24 hours.
- After incubation, centrifuge the blood collection tube at 2,000 ~ 3,000 RCF (g) for 15 minutes.
- **Sample storage;**
  - Plasma samples in tube can be stored for up to 4 weeks at 2 ℃ to 8 ℃ or, if harvested, below -20 ℃ for 2 months.
  - Once the sample was frozen, it should be thawed one time and only for test, because repeated freezing and thawing can result in the changed test values.
  - 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 (Gray > Red > Purple)	Collect 4 ml of blood into single Li-Heparin coated tubes and distribute it into each of ichroma™ IGRA-TB Tubes (Gray > Red > Purple)

↓

Shake tubes by 10 times

↓

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

↓

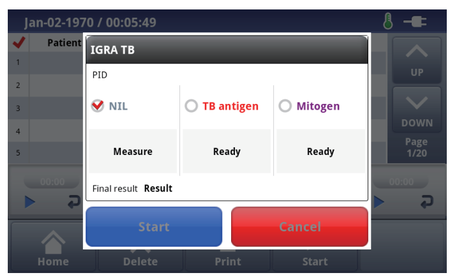
Centrifuge tubes at 2000-3000 RCF (G) for 15 min.

TEST SETUP

- Check the contents of **ichroma™ IGRA-TB 25**: Sealed Cartridges, Detector tubes, Detector Diluent and ID chip.
- Ensure that the lot number of the cartridge matches that of the ID chip as well as the diluent.
- Keep the Detector tubes and Diluent (If stored in refrigerator) at room temperature for at least 30 minutes just prior to the test. Place the cartridge on a clean, dust-free and flat surface.
- Turn on the Instrument for **ichroma™ II**
- Insert the ID chip into the ID chip port of the instrument for **ichroma™ II**.
- Touch the 'Multi Mode' on the instrument for **ichroma™ II**.
(Please refer to the 'Instrument for **ichroma™ II** Operation Manual' for complete information and operating instructions)

TEST PROCEDURE

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. Transfer 150 µL of Detector Diluent using a pipette to the Detector tube
3. Transfer 100 µL of sample using a pipette to the Detector tube
4. Mix thoroughly by shaking 10 times
5. Pipette out 75 µL of a sample mixture and dispense it into the samplet well on the cartridge
   * ①Nil, ②TB antigen and ③Mitogen samples are loaded sequentially
6. Leave the sample-loaded cartridge at room temperature for 15 minutes.

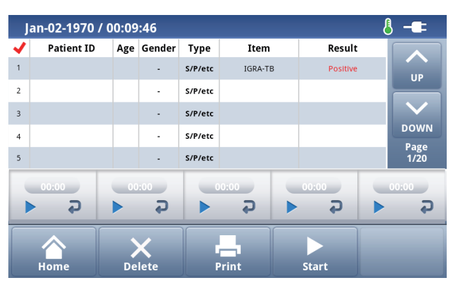


7. Insert Nil tube sample-loaded cartridge into the cartridge holder of the ichroma™ II first and touch the "Start" button. Make sure the pop-up display of IGRA-TB-specific test mode.
8. Confirm the value of TB antigen tube-test (IU/mL) and then insert TB antigen tube sample-loaded cartridge into the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

9. 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

10. 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

10-1. If you want to re-test among 3 tubes, touch the tube type, insert the cartridge, and touch the "Start"



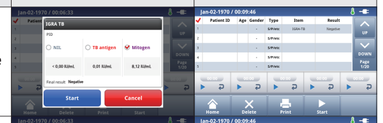
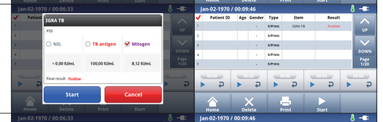
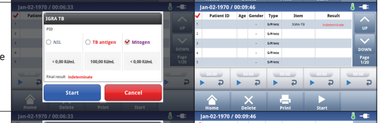
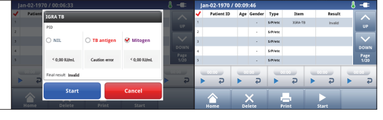
11. Insert new Nil tube sample-loaded cartridge into the holder and touch the "Start" to carry out new IGRA-TB test

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

INTERPRETATION OF TEST RESULT

Nil (IU/mL)	TB Antgen 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% Nil value	≥ 0.5		
	≥ 0.35 and ≥ 25% Nil value	Any	Positive	M. tuberculosis infection likely
	≥ 0.35 and < 25% Nil value	< 0.5	Indeterminate	Results are indeterminate for TB-Antigen responsiveness
	< 25%	< 0.5		
> 8.0	Any	Any		

- Display of the ichroma™ II 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
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.
- The control tests should be performed immediately after opening a new test lot to ensure the test performance is not altered.
- Quality control tests should also be performed whenever there is any question concerning the validity of the test results.
- Control materials are not provided 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 instruction for use of control material.)

PERFORMANCE CHARACTERISTICS

- Analytical sensitivity
  - Limit of Detection (LoD) 0.1 IU/mL
  - Limit of Qualtification (LoQ) 0.2 IU/mL
- Analytical specificity
  - Cross-reactivity

    There was no cross-reactions with clinical category such as Human IL-2, Human IL-4, Human IL-10, Human IL-27, Human IL-17E, Human TGF-beta 1, Human IL-23, Human TNF-alpha, Human IL-6 with the ichroma™ IGRA-TB 25 test measurement.
  - Interference

    There was no significant interference from these material with the ichroma™ IGRA-TB 25 test.

Materials	Concentration
Heparin	100,000 mIU/mL
Bilirubin	400 μM
Hemoglobin	2 mg/mL
Triglycerides	1.5 mg/mL
Cholesterol	20 mM
Albumin	60 mg/mL

- Precision
  - Between LOT

    One person tested three different lots of **ichroma™ IGRA-TB 25**, ten times at each concentration of the control standard.
  - Between person

    Three different persons tested same LOT of **ichroma™ IGRA-TB 25**, five times at each concentration of the control standard.
  - Between day

    One person tested one LOT of **ichroma™ IGRA-TB 25** for three days, five times at each concentration of the control standard.
  - Between site

    One person tested one LOT of **ichroma™ IGRA-TB 25** in three different space, five times at each concentration of the control standard.

Sample	Between LOT		Between person		Between day		Between site	
	Positive /NO. of tests	Positive rate	Positive /NO. of tests	Positive rate	Positive /NO. of tests	Positive rate	Positive /NO. of tests	Positive rate
Negative	0/10	0%	0/5	0%	0/5	0%	0/5	0%
High	10/10	100%	5/5	100%	5/5	100%	5/5	100%
Mid	10/10	100%	5/5	100%	5/5	100%	5/5	100%
Low	10/10	100%	5/5	100%	5/5	100%	5/5	100%

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

	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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