en

Immunoassay

REF CMT0101/CMT0102/CMT0103/ CMT0104

*14 tests/28 tests/28*2 tests/28*5 tests*

TB-IGRA CLIA Microparticles

This assay is based on a chemiluminescent microparticle immunoassay (CLIA Microparticles) using specific antigen representing to Mycobacterium tuberculosis peptides to stimulate cells in fresh peripheral venous anticoagulant blood to release interferon-γ (interferon gamma release assay, IGRA). Detection of interferon-γ is used to identify specific T-cells immunological reaction to those peptide antigens that are associated with Mycobacterium tuberculosis infection.

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Key to Graphical Symbols Used

LOT	batch code		use by
	manufacturer	Σ	contains sufficient for <n> tests
IVD	*in vitro* diagnostic medical device		temperature limitation
REF	catalogue number		consult instructions for use
EC REP	authorized representative in the European Community		

EC REP

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CE IVD

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Introduction

Tuberculosis is a chronic infectious disease caused by the Mycobacterium tuberculosis complex. It is mainly transmitted through the air to the body's multiple organ system, especially the lung accounting for various organs. It can also affect organs such as liver, kidney, brain and lymph nodes. [1] Tuberculosis patients often have fever, night sweats, fatigue, weight loss, loss of appetite, and cough blood. One third of the world's people currently have M. tuberculosis infection. And about 10% of them will develop into active tuberculosis. [2]

In recent years, interferon gamma release assay (IGRA) has been developed. The ELISA and ELISPOT (ELISA or ELISPOT) method adopts for the quantitative detection of IFN-γ level in whole blood and peripheral blood monocytes under the specific stimulation of tuberculosis antigens and was an aid in the diagnosis of tuberculosis.[3]

Measurement Principle

This assay is based upon one-step sandwich method. Firstly, the supernatant collected after the incubation, Microparticles Solution, Enzyme labeled IFN-γ antibody conjugate are added. IFN-γ present in the supernatant collected after the incubation is allowed to react simultaneously with the two antibodies, thus a complex is generated by immunological reactions. Chemiluminescent Substrate are then added and catalyzed by this complex, resulting in a chemiluminescent reaction. The resulting chemiluminescent reaction is measured as RLUs. The RLUs is proportional to the concentration of IFN-γ in the patient sample.

Materials Provided

1. Calibrators

6 vials lyophilized Calibrator A through F. The matrix is Tris-HCl buffer containing a selection of preservatives. Reconstitute each lyophilized calibrator with 1.0 mL of distilled water. Then invert the calibrator gently to mix it completely.

2. Positive Control Medium

The Positive Control Medium contains NaCl.

Σ	14tests	28tests	28*2tests	28*5tests
Positive Control Medium	1.7mL	3.2mL	3.2mL*2	3.2mL*5

Reagent provided ready to use.

3. Negative Control Medium

The Negative Control Medium contains NaCl.

Σ	14tests	28tests	28*2tests	28*5tests
Negative Control Medium	1.7mL	3.2mL	3.2mL*2	3.2mL*5

Reagent provided ready to use.

4. Test Medium

The Test Medium contains Nacl.

Σ	14tests	28tests	28*2tests	28*5tests
Test Medium	1.7mL	3.2mL	3.2mL*2	3.2mL*5

Reagent provided ready to use.

5. 3-hole Cell Culture Strip

Each contains 3 holes.

Σ	14tests	28tests	28*2tests	28*5tests
3-hole Cell Culture Strip	14	28	28*2	28*5

Plate provided ready to use.

6. Reagent pack

Reagent pack provided ready to use.

Σ	14tests	28tests	28*2tests	28*5tests
Microparticles Solution	1.2mL	2.3mL	2.3mL*2	2.3mL*5
Enzyme Conjugate	3.0mL	5.5mL	5.5mL*2	5.5mL*5

- Microparticles Solution

Mouse monoclonal Anti-IFN-γ coated microparticles in PBS (phosphate buffered saline) buffer, contains a selection of preservatives.

- Enzyme Conjugate

Horseradish-peroxidase labeled mouse monoclonal Anti-IFN-γ in Tris-HCl buffer, contains a selection of preservatives.

Assay Analyzers on which the kit can be used

- AutoLumo A2000 Plus
- AutoLumo A2000 Plus B
- AutoLumo A1000

The chemiluminescent microparticle immunoassay (CLIA Microparticles) is intended for use on Assay Analyzers which are AutoLumo A2000 Plus, AutoLumo A2000 Plus B and AutoLumo A1000.

Materials Required but not Provided

1. Assay Analyzer
2. Reaction vessel(s) for sample and reagent reaction
3. Sample tube(s) or cup(s) for sample containing
4. Micropipette
5. Chemiluminescent Substrate
6. System Wash for washing the pipetting needle
7. Wash Buffer used in washing procedure
8. Distilled or deionized water
9. Biological safety cabinet
10. Electro-thermal incubator

Metrological Traceability of Calibrators

The measurand or analyte in the calibrators is traceable to the manufacturer's working calibrators. Traceability process is based on EN ISO 17511. The assigned values were established using representative samples from this lot of calibrator and are specific to the assay methodologies of the reagents. Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

Warnings and Precautions

1. For professional use only.
2. Follow the instruction for use carefully. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this instruction for use.
3. Refer to the material safety data sheet and product labeling for any chemical hazards that may be present in this assay.
4. Handle the potentially contaminated materials and wastes safely according to local requirement.
5. CAUTION: This assay contains materials of animal origin. Bovine components originate from countries where bovine spongiform

encephalopathy (BSE) has not been reported.

6. Some reagents contain ProClin 300® may cause sensitization by skin contact, which must be avoided to contact with skin. This material and its container must be disposed in a safe way. If swallowed, seek medical advice immediately.
7. Do not smoke, drink, eat or use cosmetics in the working area.
8. Wear protective clothing and disposable gloves when dealing with samples and reagents. Wash hands after operations.
9. Use caution when handling patient samples to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
10. Conduct the assay away from bad ambient conditions. e.g. ambient air containing high concentration corrosive gas such as sodium hypochlorite acid, alkaline, acetaldehyde and so on, or containing dust.
11. Do not mix or use components from kits with different batch codes.
12. When storing the calibrators, be certain the vials are securely sealed.
13. Ensure the microparticles are resuspended before loading it on the analyzer.
14. Avoid foam formation in all reagents and sample types (samples, calibrators and controls).
15. Do not substitute any reagent in this kit from other manufacturers or other lots.
16. When any damage to the protective packaging or any change of analytical performance is observed, do not use the kit.
17. Do not use reagents beyond the labeled expiry date.

Storage

1. Store the kit at 2-8 ℃. Do not freeze. Avoid strong light.
2. Refrigerate the reagent pack at 2-10 ℃ for a minimum of 2 hours prior to use.
3. The expiry date and date of manufacture are shown on the container label.
4. Store the unsealed reagents pack upright on the analyzer or 2-10℃ for a maximum of 28 days. After 28 days, the reagent pack must be discarded. Once they are removed from the analyzer, store them at 2-10℃ in an upright position.
5. The reconstituted calibrators can be used for 3 days at 2-8 ℃. The Negative Control Medium, Positive Control Medium and Test Medium can be used for 7 days at 2-8 ℃. They should be opened in the biological safety cabinet and be sealed in time to prevent bacteria contamination. For longer use, they all need to be aseptically packed as needed. Frozen at -20 ℃, and ensure the freeze-thaw cycles to no more than once.

Sample

1. Collect peripheral venous anticoagulant blood samples in accordance with correct medical practices. The anticoagulants sodium heparin and lithium heparin has been tested and used with this assay.
2. Do not use heat-inactivated samples. Do not use sodium azide preservative in samples.
3. Do not use samples with obvious microbial contamination.
4. Insufficient processing of sample or disruption of the sample during transportation may cause depressed results.
5. Avoid grossly hemolytic, lipemic or turbid samples.
6. The whole blood sample should not be frozen in ice bath to prevent cell death.
7. Add the whole blood sample to the cell culture plate containing culture medium in the order of N, P, T within 8 hours after collection and incubate at 37℃. The whole blood for more than 8 hours cannot be used.
8. Store the supernatant collected after the incubation at room temperature for no more than 8 hours, for longer use supernatant should be stored at 2-8 ℃ up to 48 hours. Or freeze the samples that need to be stored or transported for more than 48 hours at -20 ℃. Avoid multiple freeze-thaw cycles to more than 2 times. Mix thawed samples thoroughly by low speed vortex. Visually inspect the samples, if sediment is observed, it shall be removed by centrifugation. After thawing, bring to room temperature and mix well by gently shaking.
9. For optimal results, inspect all samples for supernatant. Remove bubbles with a tip prior to analysis. Use a new tip for each sample to prevent cross contamination.

Prior to use

1. Collection: Use aseptic heparin sodium or heparin lithium anticoagulant vacuum blood-collecting tube to collect whole blood samples by intravenous puncture, the amount of the whole blood samples is no less than 4mL.
2. Medium dispensing: Add 100μL Negative Control Medium (N), 100μL Positive Control Medium (P) and 100μL Test Medium (T) respectively to the corresponding hole of the 3-hole Culture Strip in the biological safety cabinet.
3. Whole blood dispensing: Gently reverse the whole blood tube for 3-5 times after collection. Dispense 1.0mL whole blood sample respectively to the corresponding hole of the 3-hole Culture Strip within 8 hours in the safety biological cabinet.
4. Incubation: Cap the 3-hole Culture Strip, then put it into the electro-thermal incubator at 37℃ for 16-24 hours. The 3-hole Culture Strip shall be upright during the incubation.
5. Supernatant collection after incubation: Keep the 3-hole Culture Strip on the flat table for 1 minute after incubation. Then open the cap, drain the supernatant from each cell culture plate hole for IFN-γ detection. Avoid the supernatant under the cell layer of hemolysis.

Measurement Procedure

1. **Check the consumable materials**

- Verify adequate volume of consumable materials is present prior to running the test.
- Refer to the Assay Analyzer's operation manual.

2. **Load the kit**

- Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the analyzer. Avoid foam formation in all reagents. Don't invert the open (punctured) packs. If necessary, shake gently to mix horizontally after the first loading.
- Read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Refer to the Assay Analyzer's operation manual.

3. **Order tests**

- Place the sample tube(s) or cup(s) on the sample holder. 100μL of supernatant of sample for each test. But consider the sample container and 150 μL of system dead volumes, which can be refer to the appropriate Assay Analyzer manuals for the minimum sample volume required.
- Load the sample holder and input the sample information on the system software interface.
- Select "run" to start the test, the analyzer automatically operates tests. It performs the following functions:
  - Moves the sample to the set point
  - Loads a reaction vessel into the process path

- Aspirates and transfers supernatant of sample into the reaction vessel
- Adds Microparticle Solution and Enzyme Conjugate to the reaction vessel
- Mixes, incubates and washes the reaction mixture
- Adds Chemiluminescent Substrate
- Measures chemiluminescent emission to determine the quantity of IFN- γ in the sample
- Discards the used reaction vessel
- Calculates the result

- Refer to the Assay Analyzer's operation manual.

4. **Calibrate the curve**

- Analyzer can read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Transfer the calibrators into the sample tube (s) or cup(s) and place them on the sample holder. Conduct duplicate detection on the system.
- Load the sample holder and input calibrators' information on the system software interface.
- Select "run" to start the test and generate the calibration curve, calibration is required every 28 days.
- Once a calibration curve is accepted and stored, all subsequent samples may be tested without further calibration unless:
  - Controls are out of range after repeated measurements
  - A reagent kit and Chemiluminescent Substrate with new batch code is used
  - Beyond the expiration date of a calibration curve
  - Important parts of the analyzer are replaced or repaired
- Refer to the Assay Analyzer's operation manual.

Measurement Results

The sample test results are determined automatically by the system software. The amount of IFN- γ in the samples is determined from the measured light production by means of the stored calibration data. Sample test results can be reviewed using the appropriate screen. Refer to the Assay Analyzer's operation manual on reviewing sample results. The default unit for this assay is IU/mL.
The table below shows Interpretation of Results:

N (IU/mL)	T minus N (IU/mL)	P minus N (IU/mL)	Result	Interpretation
≤10.0	<0.438	≥0.625	Negative	Tuberculosis may not infect T cell.
	≥0.438 and <25% N	≥0.625		
	≥0.438 and ≥25% N	Any	Positive	Tuberculosis may infect T cell.
	<0.438	<0.625	Indeterminate	Likelihood of Tuberculosis infecting T cell is cannot be determinated.
	≥0.438 and <25% N	<0.625		
>10.0	Any	Any		

Note: N: Test value of negative control culture hole; P: Test value of positive control culture hole, T: Test value of testing culture hole.

Control Procedure

Controls for the various concentration ranges should be run individually when the test is in use, once per reagent kit, and following each calibration.
The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.
Follow the applicable government regulations and local guidelines for quality control.

Limitations of the Procedure

1. This assay is intended as an aid for the clinical diagnosis. Conduct this assay in conjunction with clinical examination, patient's medical history and other test results.
2. The negative results cannot be completely ruled out the possibility of tuberculosis. If the results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
3. Heterophilic antibodies and rheumatoid factors in samples may interfere with test results. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis. This kind of samples is not suitable to be tested by this assay.
4. Because of pulsatile secretion, samples obtained within the same day from the same patient may fluctuate widely within the reference interval, reflecting physiological variation rather than errors in technique or methodology.
5. Lipemia, hemolysis, turbidity samples must not be used since the accuracy of their test results has not been validated.
6. Patients with impaired immune function or undergoing immunosuppressive therapy, such as HIV patients or patients undergoing immunosuppression after organ transplantation. The reference value of antigen testing is limited and may lead to erroneous medical explanations.
7. Improper handling of whole blood samples, such as violent manipulation, whole blood for more than 8 hours, result in damage to cells or hemolysis. The abnormal test result may occur.
8. Due to limitation of methodology or immunological specificity and other reasons, the test results from different manufacturers' reagents for the same sample may be different, so such results should not be directly compared with each other, so as to avoid the wrong medical explanation. It is recommended that the characteristics of the different manufacturers' reagents should be indicated when reported to the clinician.

Performance Characteristics

1. Measurement Precision

2 internal controls (Q1 and Q2) were assayed, using 1 batch of reagents, in replicates of 10. Data from this study are summarized in the following table.

Internal Controls	Batch	n	Mean	Within-run Precision SD	%CV
Q1	1	10	0.53	0.02	3.77
Q2	1	10	2.30	0.10	4.35

2 internal controls (Q1 and Q2) were assayed, using 1 batch of reagents, in replicates of 10, once per day across 3 testing days. Data from this study are summarized in the following table.

Internal	Batch	n	Mean	Between-run Precision

Controls				SD	%CV
Q1	1	30	0.53	0.01	1.89
Q2	1	30	2.34	0.09	3.85

2. Limit of Blank

Limit of Blank: 0.02IU/mL.

3. Analytical Specificity

Cross reaction: this assay was evaluated for potential cross-reactivity for samples from individuals with medical conditions unrelated to TB infection. The substances with the concentration of 40ng/mL, such as Human interleukin-la (IL-la), Human interleukin-lb (IL-lb), Human interleukin-1RA (IL-lRA), Human interleukin-3 (IL-3), Human interleukin-5 (IL-5), Human interleukin-8(1-72)-(IL-8 1-72), Human interleukin-8 (1-77) (IL-8 1-77), Human interleukin-12 (IL-12), Human interferon alpha (IFN-α), Human interferon beta (IFN-β) are found no cross reaction with the test.
Interference: this assay is designed to have an acceptable interference with the substances listed below, at the concentration levels listed.

Interferent	Concentration
Bilirubin	40 mg/dL
Triglyceride	3000 mg/dL
Hemoglobin	125 mg/dL

4. High dose HOOK Effect

A sample spiked with IFN-γ up to 200IU/mL was determined, the concentration result obtained was no sample misclassification.

Literature References

1. Pai M, Jr R L J. Interferon-gamma assays in the immunodiagnosis of tuberculosis: a systematic review[J]. Lancet Infectious Diseases, 2004, 4(12):761-776.

2. Tsiouris S J, Coetzee D, Toro P L, et al. Sensitivity Analysis and Potential Uses of a Novel Gamma Interferon Release Assay for Diagnosis of Tuberculosis[J]. Journal of Clinical Microbiology, 2006, 44(8):2844-2850.

3. Sharma S K, Vashishtha R, Chauhan L S, et al. Comparison of TST and IGRA in Diagnosis of Latent Tuberculosis Infection in a High TB-Burden Setting:[J]. Plos One, 2017, 12(1):e0169539.