

Introduction to Interferon Gamma Release Assay

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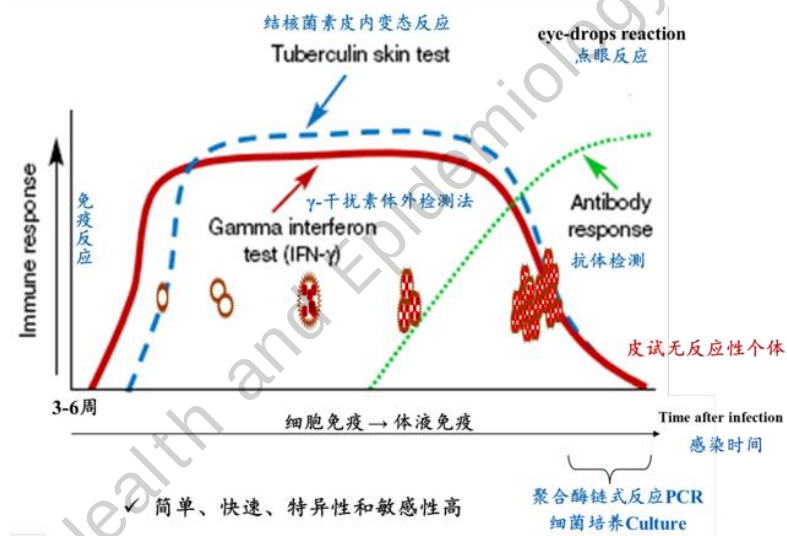
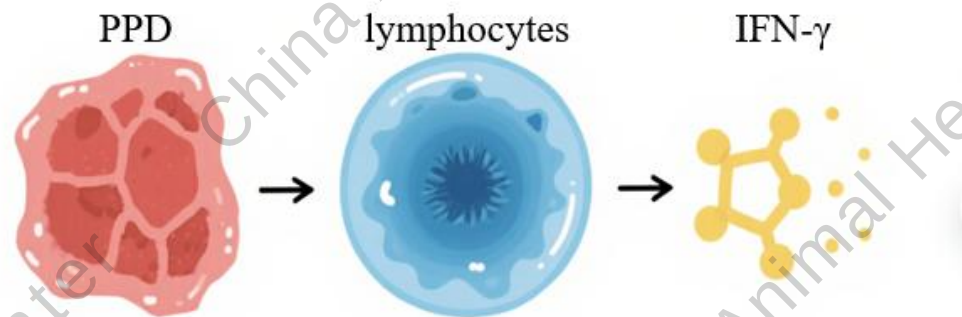
Basic Principle of IGRA Test



IFN- γ Detection

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- IGRA (Interferon gamma release assay).
- After the tuberculin **PPD antigen** is presented to the lymphocytes in the **whole blood** tissue culture.
- The lymphocytes **infected with** *Mycobacterium bovis* can produce IFN- γ .
- While the lymphocytes **not infected with** *Mycobacterium bovis* will not produce IFN- γ .
- Therefore, the detection of IFN- γ is closely related to *Mycobacterium bovis* infection.





IFN- γ Detection

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- The **BOVIGAM[®]** γ -interferon assay kit (catalog Number **63320**).
- This method is a type of **cellular immune response-based** detection.
- For the IFN- γ assay, 5 mL of bovine **anticoagulated blood** needs to be collected.
- In the laboratory, the blood sample is stimulated with bovine PPD and avian PPD.
- A **sandwich ELISA** is used to detect the level of γ -interferon released by the cellular immune response, so as to determine the infection status of the animal.





IFN- γ Detection



- The IGRA assay can identify animals in the **early stage of infection**.
- It has **higher sensitivity but lower specificity** compared with the Single Intradermal Comparative Cervical Test (SICCT).
- In cases of natural infection, if the SICCT is applied, it will affect the test results of the BOVIGAM[®] γ -interferon assay.
- Therefore, some countries recommend an interval between the skin test and the BOVIGAM[®] γ -interferon assay.



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On-Site Test for Bovine Tuberculosis IGRA SOP

- I. Collection of Bovine Whole Blood Samples
- II. Sample Processing
- III. Sample Stimulation and Incubation (16-24 h)
- IV. Harvesting and Preservation of Stimulated Supernatant

Required Reagents and Consumables:

- ✓ Lithium heparin anticoagulant tubes (5 mL)
- ✓ Blood collection needles
- ✓ Scalp needles
- ✓ Bovine PPD (stored at 4°C)
- ✓ Avian PPD (stored at 4°C)
- ✓ PBS (stored at 4°C)
- ✓ RPMI 1640 medium
- ✓ 24-well plates
- ✓ Sterile 1000 µL and 200 µL pipette tips
- ✓ EP tubes
- ✓ Self-sealing bags



On-Site Test for Bovine Tuberculosis IGRA SOP

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Whole Blood Collection

- Collect **5 mL or more** of bovine blood into a lithium heparin anticoagulant tube.
- Gently invert the tube several times to mix the blood and dissolve the heparin.
- Transport the tube to the laboratory **at room temperature** ($22 \pm 5^\circ\text{C}$, avoiding excessively high or low temperatures) and perform the culture within **8 hours** after blood collection.
- Refrigeration at low temperatures is prohibited.

Note:

- Mark the cattle **ID** clearly on the tube.
- If collecting whole blood with a blood collection syringe, avoid pushing the syringe plunger forcefully when injecting blood into the anticoagulant tube to **prevent red blood cell lysis**.
- After injecting blood into the anticoagulant tube, remember to gently invert and **mix the tube several times** to prevent blood coagulation; do not apply excessive force, as this may cause red blood cell lysis.
- During transportation, avoid severe shaking of the anticoagulant tube.



➤ Antigen Dilution:

Taking 90 Head-Doses as an Example:

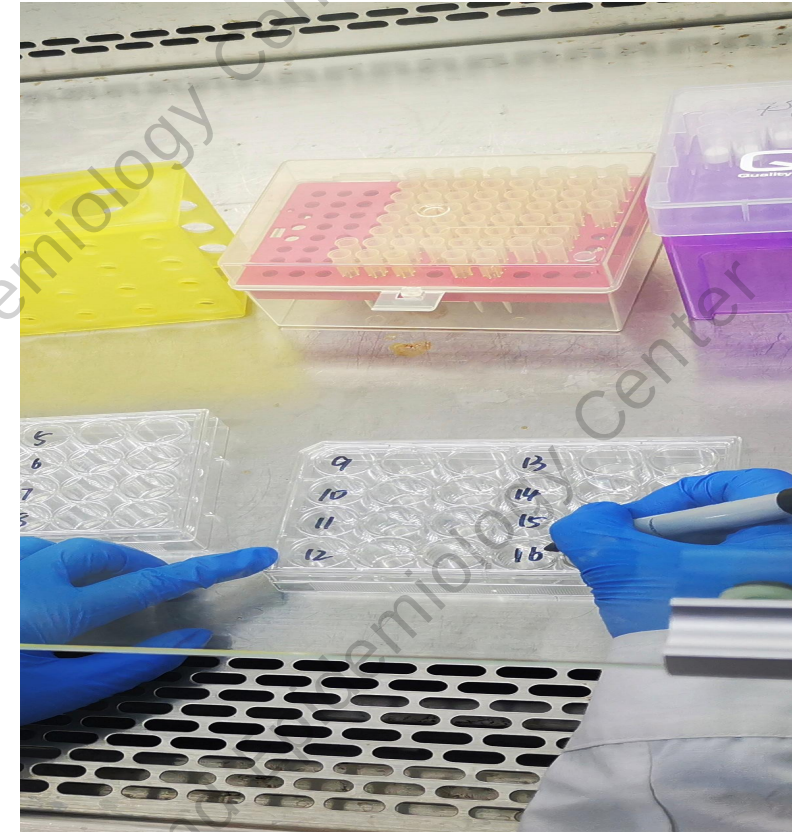
1.6 mL PPD + 8.4 mL RPMI 1640

➤ Label the 24-well plate:

Mark the sequence of samples and stimulating antigens clearly on the lid of the 24-well plate.

➤ Add stimulating antigens:

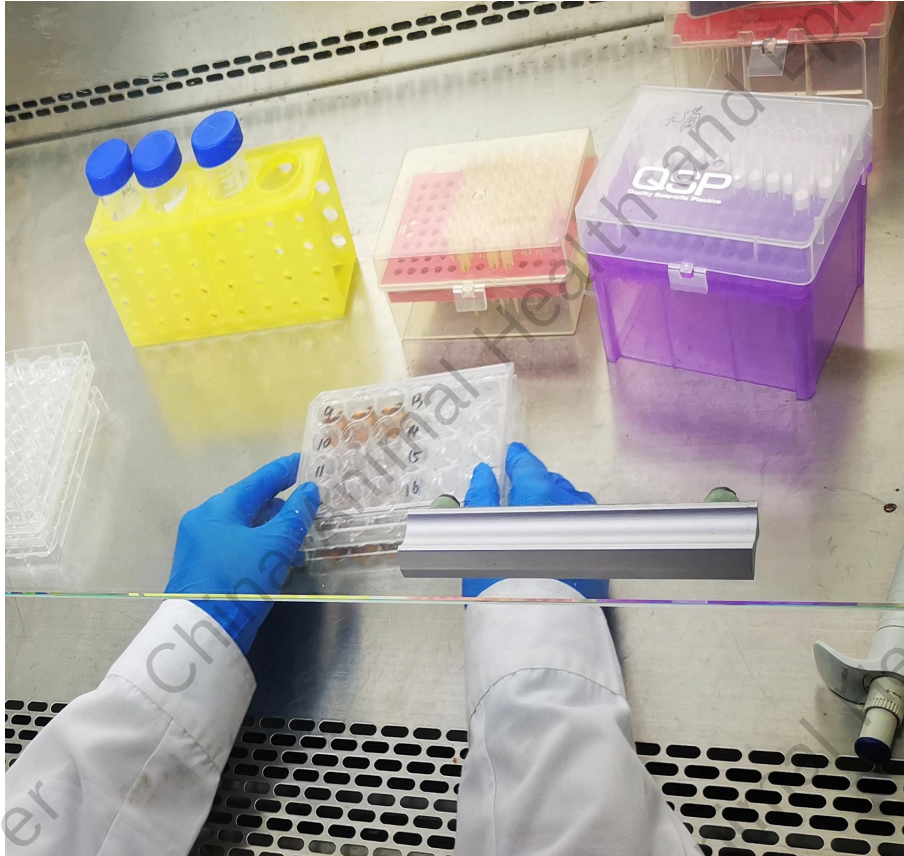
Aseptically add **100 μ L** of bovine PPD, avian PPD, and PBS to the 24-well plate respectively.





On-Site Test for Bovine Tuberculosis IGRA SOP

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➤ Add whole blood:

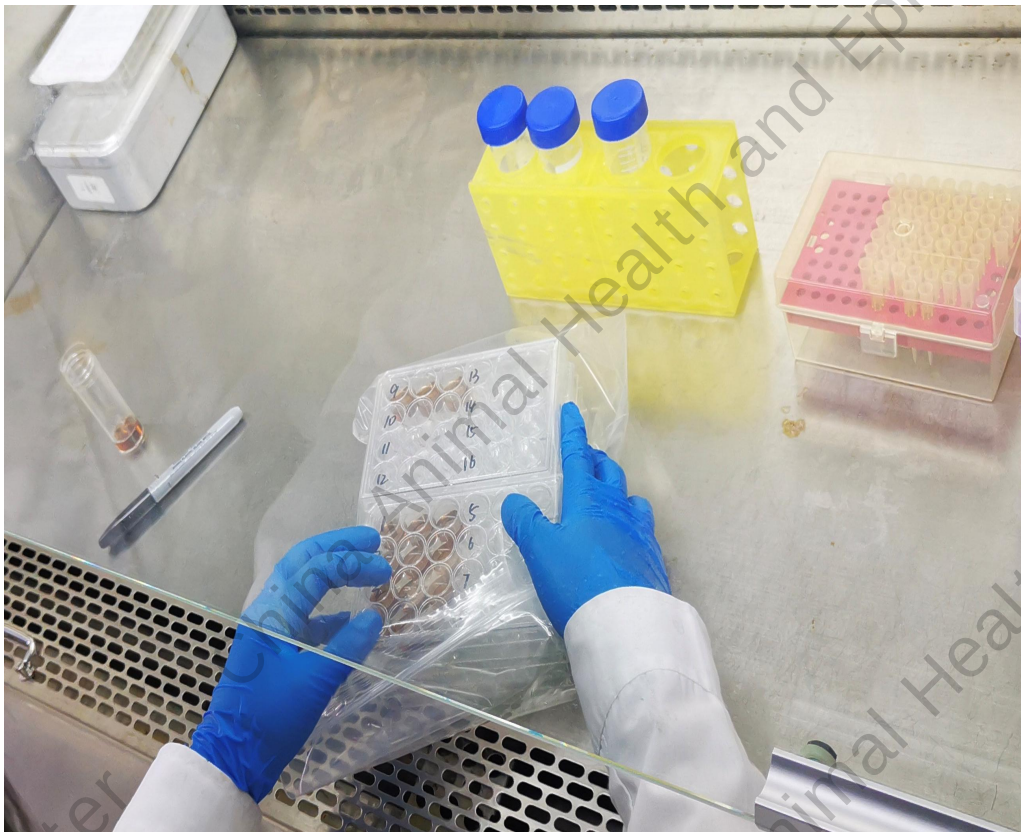
Under sterile conditions, add the anticoagulated blood to the 24-well plate.

For each animal, sequentially add blood to three wells horizontally in a non-contact manner (**750 μ L per well**), then repeat the addition to the same three wells in sequence (750 μ L per well).

Note: Use a sterile 1 mL pipette tip with a filter for each cow.

➤ Sample Mixing:

Gently rotate the cell culture plate 10 times clockwise and 10 times counterclockwise on a smooth surface.



Sample Stimulation and Incubation

- Incubate the tissue culture plate containing blood and antigens in a humidified incubator at 37°C for **16-24 hours**.

Note:

- Stack the 6 cell culture plates into two piles, place them into self-sealing bags, and seal the bags tightly.



Harvesting and Preservation of Stimulated Supernatant

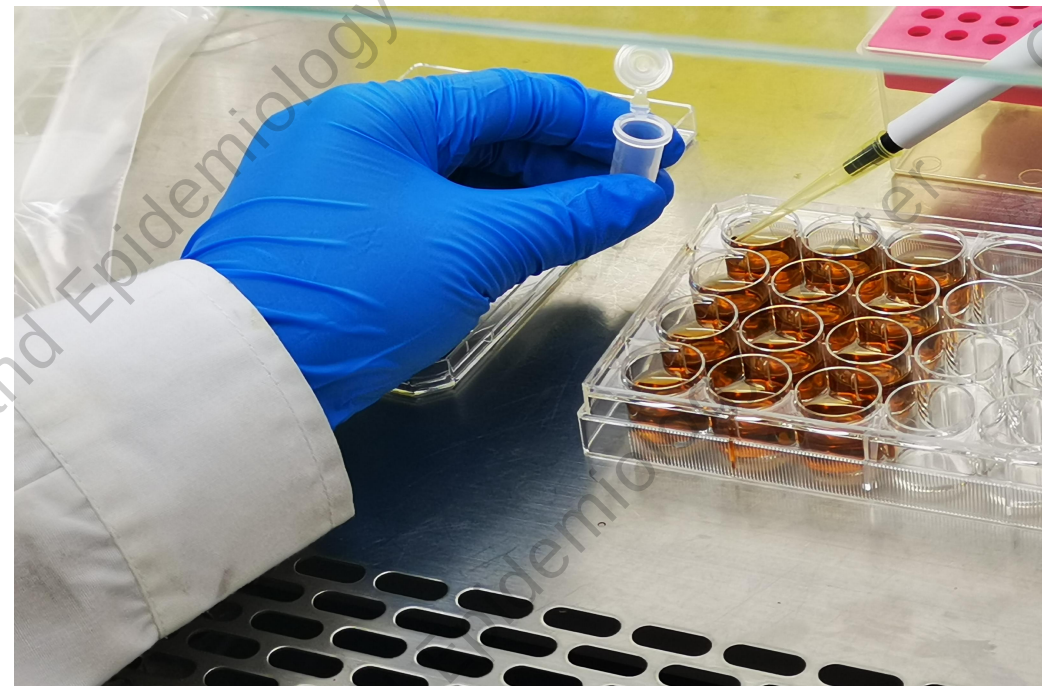
➤ Harvesting of stimulated supernatant :

Carefully aspirate approximately 200 μL of the upper stimulated supernatant along the edge of the well using an adjustable pipette, transfer it into separate 1.5 mL centrifuge tubes, and label them (e.g., 1B, 1A, 1P).

Note : Use a 200 μL pipette tip and aspirate twice or more. When aspirating the plasma, try to avoid drawing in red blood cells.

➤ Preservation of Stimulated Supernatant :

It can be stored at 2-8°C for 7 days, or at -20°C for several months.





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Bovine Tuberculosis Gamma-Interferon ELISA Detection



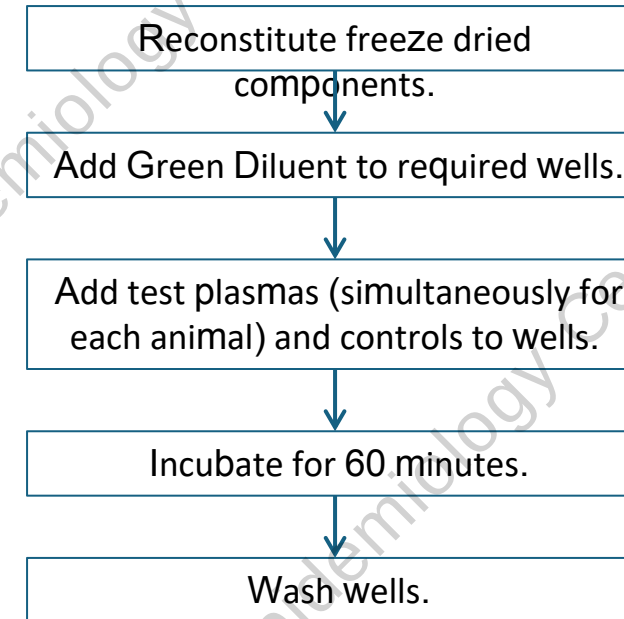
- Perform the enzyme-linked immunosorbent assay (ELISA) for bovine tuberculosis gamma interferon (INF- γ) using the **BOVIGAM® Bovine Tuberculosis Gamma Interferon ELISA Test Kit**, following the operating procedures provided in the kit instructions.





Bovine IFN- γ EIA

1. Reconstitute freeze dried components, if required, while equilibrating other reagents according to the guidelines outlined in “Procedural Notes”.
2. Add 50 μ L of Green Diluent to the required wells.
3. Add 50 μ L of test and control samples to the appropriate wells containing Green Diluent. Control samples should be added last to each plate. Mix thoroughly by vortexing plates for 1 minute on a microplate shaker, or, if not available, by pipetting up and down 5 times.
4. Cover each plate with a lid and shake for 1 minute on a plate shaker at a setting of 600 rpm $\pm$ 50 rpm. Remove plate from shaker and incubate at room temperature (22 $\pm$ 3°C) for 60 $\pm$ 5 minutes.
5. Shake out contents and wash wells 6 times at room temperature. Fill wells with wash buffer taking care not to cross contaminate adjacent wells. Shake out wash fluid and repeat operation a further 5 times. After the sixth wash, place plate(s) face down on clean filter paper and allow to drain, and flick several times over absorbent paper to remove as much remaining wash buffer as possible.



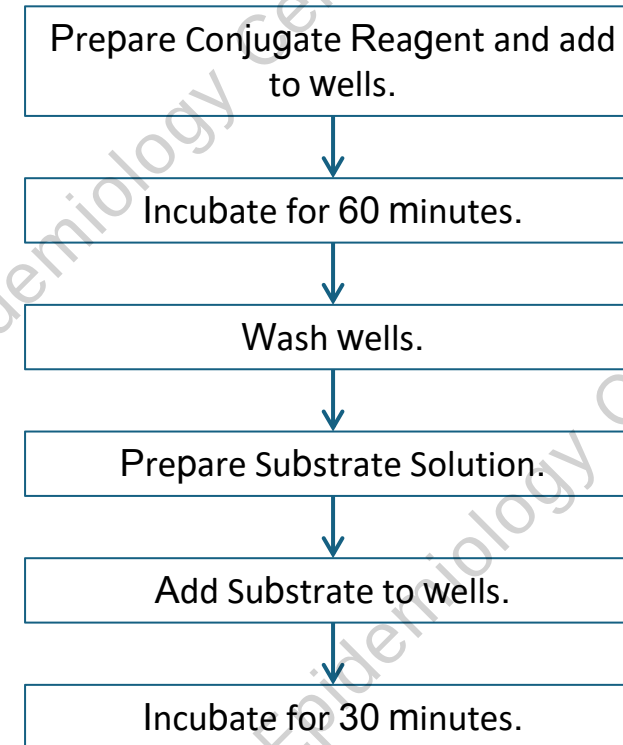


6. Add 100 μ L of freshly prepared conjugate reagent to wells. The reagent is 1x conjugate diluted in working strength Blue Diluent according to the Reagent Preparation Table (Table 1). Mix thoroughly as in Step 3.
7. Cover each plate, shake and incubate as in Step 4 for 60 ± 5 minutes.
8. Wash wells as in Step 5.

Note: The enzyme substrate solution is best prepared after this wash step.

9. Add 100 μ L of freshly prepared enzyme substrate solution to wells. Mix thoroughly as in Step 3.
10. Cover each plate with a lid, shake and incubate as in Step 4 for 30 minutes. Protect from direct sunlight.

Note: Commence incubation time as you add substrate to the first well(s).

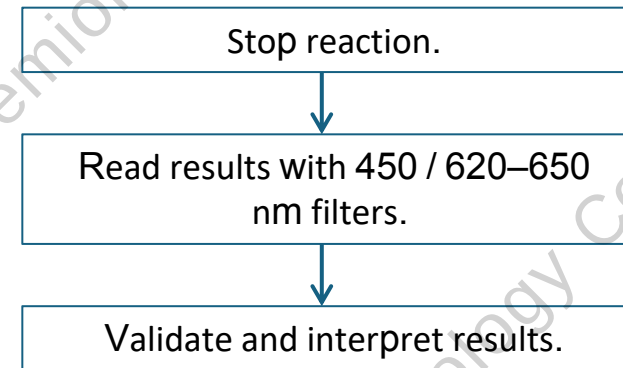




11. Add 50 μ L of Enzyme Stopping Solution to each well, being careful not to transfer chromogen from well to well, then mix by gentle agitation.

Note: The Enzyme Stopping Solution should be added to wells in the same order and at the same speed as the enzyme substrate solution.

12. Read the absorbance of each well within 5 minutes of terminating the reaction using a 450 nm filter with a 620–650 nm reference filter. The absorbance values will then be used to calculate results.





Result Interpretation:

Compare the mean absorbance values of the PBS, avian and bovine PPD stimulated samples for each animal.

Positive = $\text{OD bovine PPD} - \text{OD PBS} \geq 0.1$; and = $\text{OD bovine PPD} - \text{OD Avian PPD} \geq 0.1$

Negative = $\text{OD bovine PPD} - \text{OD PBS} < 0.1$; or = $\text{OD bovine PPD} - \text{OD Avian PPD} < 0.1$

Thank you!
