

## "Gamma-Interferon" Assay for the diagnosis of *M. bovis* infections

### Requirements:

1. 1 Heparin blood tube (10 ml) per animal
2. 8 sterile disposable plastic tubes ( $\pm 2$  ml) per animal
3. Equipment for:
  - o sterile dispensing of 1.5 ml blood (micropipette, tuberculin syringe or pasteur pipette)
  - o sterile dispensing of tuberculin (25, 30 and 60 microliter)
  - o sterile harvesting of plasma ( $\pm 0.3$  ml)

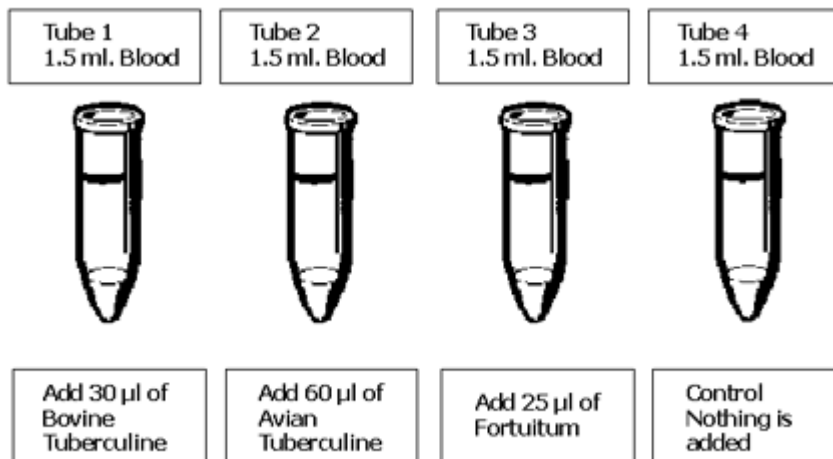
### Field procedure:

1. Collect 10 ml blood in heparin per animal
2. Keep blood at ambient temperature (optimum: 15 – 25°C) during transport
3. Submit blood to the laboratory within **8 hours after collection**

### Laboratory procedure:

#### Day 1 = day of blood collection:

1. Tilt blood tube several times to ensure proper mixing
2. Dispense 1.5 ml blood into each of 4 sterile plastic tubes per animal
3. Label tubes and add antigens as follows:



4. After adding the antigens close and tilt tubes several times to ensure sufficient mixing of the content
5. Incubate tubes at 37 °C for 20 – 24 hours

#### Day 2:

1. Transfer plasma (supernatant) into sterile plastic tube (correct labelling!)

(If separation of plasma and red blood cells is not sufficient the tubes should be centrifuged in a microcentrifuge at low speed (1200 rpm) for 10 minutes

2. Freeze plasma at - 20°C and send to Tuberculosis Laboratory Onderstepoort Veterinary Institute, Old Soutpan road, Tel: +27 (0)12 529 9149 or 529 9452