

Veterinary application of QuantiFERON[®]-TB Gold Plus (QFT[®]-Plus) Blood Collection Tubes

This protocol is designed for stimulation of ruminant blood samples using the QuantiFERON-TB Gold Plus Blood Collection Tubes. For identification of ruminant interferon gamma (IFN- γ), the harvested plasma samples must be tested using the *cattletype*[®] IFN-gamma ELISA Kit according to the respective handbook.

Equipment and reagents

When working with chemicals, always wear a suitable lab coat and disposable gloves. For more information, consult the appropriate safety data sheets (SDSs), available from the product supplier. When handling samples from known tuberculosis (TB) positive herds, additional safety measures may apply.

Equipment

- Incubator set at 37°C (\pm 1°C)
- Tube rack
- Optional (highly recommended): Laminar flow hood (in order to ensure that all necessary pipetting steps, until blood simulation, are carried out aseptically)
- Optional: Microcentrifuge with rotor/inserts for blood collection tubes

Material

- QuantiFERON-TB Gold Plus Blood Collection Tubes (200 tubes, cat. no. 622526)
- Pipettes and pipette tips (pipette tips with aerosol barriers for preventing cross-contamination are recommended)
- Optional (recommended): **Lithium heparin** blood collection tubes (minimum volume 5 ml)
- Optional: Device to store blood filled in lithium heparin blood collection tubes at 2–8°C for up to 48 h
- Optional: Eppendorf[®] reaction tubes for long-term storage of plasma samples

Reagents

- Optional (highly recommended):
 - Pokeweed mitogen (PWM), e.g., Sigma-Aldrich, Lectin from *Phytolacca americana* (pokeweed) suitable for cell culture 5 mg; # 8777-5MG
 - PBS or sterile 0.9% NaCl for reconstituting PWM

Kit to be used at conclusion of this protocol

- *cattle*type IFN-gamma ELISA Kit (cat. no. 270401)

Blood collection

Note: Blood should be collected aseptically.

Option 1: Blood samples collected in lithium heparin tubes and transferred into the QFT-Plus Blood Collection Tubes afterward in the laboratory

- Sampling needle is combined with blood collection tubes capable of collecting at least 5 ml of blood containing **lithium heparin** only.

Option 2: Blood samples collected directly into the QFT-Plus Blood Collection Tubes

- Vacuum in QFT-Plus Blood Collection Tubes draws 1 ml of blood samples. Sampling needle combined with “purge” tube is recommended to ensure that the tubing is filled with blood prior to the QFT-Plus Blood Collection Tubes being used.

Option 3: Blood samples drawn into a syringe and transferred immediately into the QFT-Plus Blood Collection Tubes

- Sampling needle is combined with a single-use syringe.

Note: The sample must be transferred into the QFT-Gold Blood Collection Tubes immediately.

Important points before starting

- QFT-Plus Blood Collection Tubes should be stored at 4–25°C before use and at ambient temperature (17–25°C) at the time of blood filling.

- The blood collection volume of a tube is affected by altitude. QFT-Plus Blood Collection Tubes should be used between sea level and 810 m (2650 ft). If using QFT-Plus Blood Collection Tubes outside this altitude range, blood filling options 1 or 3 are recommended.
- Antigens have been dried onto the inner wall of the QFT-Plus Blood Collection Tubes, so it is essential that the contents of the tubes be thoroughly mixed with the blood.
- The QFT-Plus Blood Collection Tube package provided for human testing includes 4 QFT-Plus Blood Collection Tubes: Nil Tube (negative control, gray cap), Mitogen Tube (positive control, purple cap) and two antigen tubes (TB1, green cap; TB2, yellow cap).
However, the TB2 Tube (yellow cap) is best suited for use in veterinary applications.
Therefore, only the following 3 QFT-Plus Blood Collection Tubes should be used for blood stimulation in the veterinary field.
 - Nil Tube (gray cap)
 - Mitogen Tube (purple cap)
 - TB2 Tube (yellow cap)

Things to do before starting

Mitogen/stimulation control

In order to provide confidence that the samples were handled correctly and are capable of producing interferon gamma, it is highly recommended to use a mitogen/stimulation positive control. The mitogen used in the QFT-Plus Mitogen Tube may not work as reliably in ruminants compared to human blood samples. In order to improve mitogen stimulation, it is strongly recommended to add 5 µg (per 1 ml blood) of pokeweed mitogen (PWM) to the QFT-Plus Mitogen Tubes. However, if the QFT-Plus Mitogen Tube only (without added PWM) is used to serve as stimulation control tube, particular attention has to be paid to the test results obtained. In case of a negative test result for mitogen stimulation as well as for TB antigen stimulation, the respective animal should be re-tested, including a QFT-Plus Mitogen stimulation control with added PWM.

- Reconstitute and aliquot the PWM as follows.
Note: Aseptic working under a laminar flow hood is highly recommended.
 - Reconstitute freeze-dried pokeweed mitogen (PWM) with sterile PBS or sterile 0.9% NaCl in order to provide a 500 µg/ml PWM stock solution.
 - Use 10 µl (corresponding to 5 µg) PWM stock solution per 1 ml blood in the QFT-Plus Mitogen Tube (purple cap) for mitogen stimulation. PWM stock solution should be aliquoted in appropriate volumes in sterile tubes and stored at –30 to –15°C.

Procedure

Blood collection

1. Label Nil, Mitogen and TB2 Tubes appropriately for the respective animal samples.

Make sure that each tube (Nil, TB2 and Mitogen) is identifiable by its label with the given Sample ID once the cap is removed.

2. For each animal sample, collect 1 ml of blood into each of the 3 QFT-Plus Blood Collection Tubes, according to step 2a (Option 1), 2b (Option 2) or 2c (Option 3).

2a. **Option 1: Blood samples collected in lithium heparin tubes and transferred into the QFT-Plus Blood Collection Tubes afterward in the laboratory**

Note: Use **lithium heparin** tubes only. Other anticoagulants may interfere with the assay.

- Fill a **lithium heparin** blood collection tube (minimum volume 5 ml) with blood sample, and gently mix by inverting the tube several times to dissolve the lithium heparin.
- Maintain blood at either 17–25°C, for a maximum of 16 h, or at 2–8°C (preferred), for a maximum of 48 h, before transfer to QFT-Plus Tubes for stimulation at 37°C ($\pm 1^\circ\text{C}$).

Note: Make sure that the blood sample is not subjected to repeated temperature changes during the storage period.

- Before dispensing into QFT-Plus Tubes, equilibrate blood samples to 17–25°C and mix evenly by gentle inversion.
- Remove the caps from the 3 QFT-Plus Tubes, and pipet 1 ml of blood into each tube under aseptic conditions (ensuring appropriate safety procedures). Replace the tube caps securely and proceed immediately to steps 3 and 4.

2b. **Option 2: Blood samples collected directly into the QFT-Plus Blood Collection Tubes**

- Allow the vacuum in the tubes to draw 1 ml blood samples.

The black mark on the side of the tubes indicates the validated range of 0.8–1.2 ml.

If the level of blood in any tube is outside the range of the indicator mark, a new blood sample should be obtained.

As 1 ml tubes draw blood relatively slowly, keep the tube on the needle for 2–3 s once the tube appears to have completed filling. This will ensure that the correct volume is drawn. If a needle-tube combination is being used to collect blood, a “purge” tube should be used to ensure that the tubing is filled with blood prior to using the QFT-Plus Tubes.

- Maintain the filled QFT-Plus Tubes at 17–25°C for up to 16 h, before blood stimulation at 37°C ($\pm 1^\circ\text{C}$).

2c. Option 3: Blood samples drawn into a syringe and transferred immediately into the QFT-Plus Blood Collection Tubes

- For safety reasons, remove the syringe needle, ensuring appropriate safety procedures.
- Remove the caps from the 3 QFT-Plus Tubes, and add 1 ml of blood to each tube (to the center of the black mark on the side of the tube label).
If the level of blood in any tube is outside the range of the indicator mark, a new blood sample should be obtained.
- Maintain the filled QFT-Plus Tubes at 17– 25°C for up to 16 h, before blood stimulation at 37°C ($\pm 1^\circ\text{C}$).

3. Add 10 μl of the 500 $\mu\text{g}/\text{ml}$ PWM stock solution (prepared as described in “Things to do before starting”, page 3) to the 1 ml of blood that is already filled into the QFT-Plus Mitogen Tube (purple cap). Dispensing should be performed aseptically.
4. Immediately after filling tubes, shake them ten times just firmly enough to ensure that the entire inner surface of the tube is coated with blood, to dissolve antigens on tube walls.
Note: Excessively vigorous shaking may cause gel disruption and could lead to abnormal results.
5. After labeling, filling and shaking, the tubes must be transferred to a 37°C ($\pm 1^\circ\text{C}$) incubator as soon as possible, within 16 h of collection (if blood was stored at 17–25°C) or within 48 h (if blood was stored at 2–8°C in lithium heparin tubes before filling the QFT-Plus Tube).

Incubating tubes and harvesting plasma

6. If the blood is not incubated immediately after collection, re-mixing of the tubes by inverting ten times must be performed immediately prior to incubation.
7. Incubate the tubes **upright** at 37°C ($\pm 1^\circ\text{C}$) for 20–48 h.
The incubator does not require CO₂ or humidification.
Note: Extended stimulation time may increase sensitivity in certain species (1).
8. After incubation, blood collection tubes can be held at 4–25°C for up to 3 days prior to centrifugation.
9. After incubation, centrifuge tubes for 15 min at 2000 to 3000 g (RCF).
The gel plug will separate the cells from the plasma. If this does not occur, the tubes should be centrifuged again.
It is possible to harvest the plasma without centrifugation. However, additional care is required to remove the plasma without any cells.
10. Harvested plasma samples can be stored for up to 4 weeks at 2–8°C or below –20°C for extended periods. For adequate test samples, harvest at least 100 μl of plasma.

For identification of ruminant interferon gamma (IFN- γ), the harvested plasma samples must be tested using the *cattletype* IFN-gamma ELISA Kit according to the respective kit handbook.

Cited reference

1. Goosen, W. J., Miller, M. A., Chegou, N. N., Cooper, D., Warren, R. M., van Helden, P. D., and Parsons, S. P. D. (2014) Agreement between assays of cell-mediated immunity utilizing *Mycobacterium bovis*-specific antigens for the diagnosis of tuberculosis in African buffaloes (*Syncerus caffer*). *Vet. Immunol. Immunopath.* **160**, 133–138

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