

Procedures for application of the QuantiFERON-TB Gold In-Tube (QFT-GIT) system in pleural fluid

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Abstract

This document aims to standardize the protocol for processing of the pleural fluid and its application to QuantiFERON-TB Gold In-Tube (QFT-GIT; Cellestis Limited, Australia) system.

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Guidelines

Goal:

This document aims to standardize the protocol for processing of the pleural fluid and its application to QuantiFERON-TB Gold In-Tube (QFT-GIT; Cellestis Limited, Australia) system.

General considerations:

- All procedures in this protocol should be performed using personal protective equipment (PPE) including N95 medical mask, and the handling must always be carried out within the biosafety cabinet.
- Plastic materials, such as tips and polypropylene tubes must be virgin and sterile.
- Tubes temperature provided by the QFT-GIT kit (Nil [negative control], Antigen-specific TB [ESAT-6, CFP-10 e TB7.7], Mitogen [positive control] should be between 17 - 25°C (63 - 77°F) at the time of sample tube filling.
- Overly vigorous shaking may cause gel disruption and could lead to misinterpretation of the results.
- Additional material: thermal box for sample transportation.

Procedures:

1. The collection of pleural fluid occurs during the procedure called *Thoracentesis* which is

performed by a physician trained. After identification of the region of the pleural effusion in the patient, guided by chest ultrasound, asepsis and local anesthesia are performed. Then, under monitoring of vital signs, the physician aspirates the pleural fluid by using a surgical syringe;

2. Immediately after collection, the pleural fluid is stored in polypropylene tube without additives;
3. The samples are then transported in a thermal box to the laboratory for processing.
4. Pleural fluid is homogenized in the polypropylene tube and then 1 mL of sample is pipetted into each of the three tubes of QFT-GIT.
5. QFT-GIT Tubes are homogenized by inversion 10 times, carefully, for proper homogenization of the pleural fluid with the compounds in the respective tubes.
6. The tubes are then incubated at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ as soon as possible for 16 - 24 hours in an incubator. The incubator does not require CO_2 or humidification.
7. After incubation at 37°C , harvesting of pleural fluid supernatants is facilitated by centrifuging tubes for 15 minutes at 2000 to 3000 RCF (g) at 25°C . Samples are collected using a pipette in aliquots for each tube (Nil, Antigen, Mitogen) and stored at -20°C or -80°C until the immunoenzymatic assay (ELISA) be performed. Samples can be loaded directly from centrifuged collection tube into the QFT ELISA plate.

References:

<https://www.quantiferon.com>

https://www.qiagen.com/kr/resources/technologies/qft_technology-spotlightpages/

Protocol