

QuantiFERON[®]-TB Gold

Filling QuantiFERON-TB Gold blood collection tubes

The QuantiFERON-TB Gold assay (QFT[®]) measures the Interferon-gamma (IFN- γ) response in whole blood stimulated with antigen. The kit uses specialized QFT blood collection tubes. The following is a guide for blood collection into these tubes. (Catalogue No: T0590-0301)

Filling Tubes

One QFT test uses the following three collection tubes:

- | | |
|--------------------------------|--------------|
| Nil tube, negative control | (Grey cap) |
| TB Antigen tube | (Red cap) |
| Mitogen tube, positive control | (Purple cap) |

These procedures should be followed for optimal results:

- Tubes should be at 17–25°C at the time of blood filling.
- Collect 1 mL of blood by venipuncture directly into each QFT blood collection tube, preferably in the order Nil, TB-Antigen and Mitogen.

As 1 mL tubes draw blood relatively slowly, keep the tube on the needle for 2–3 seconds once the tube appears to have completed filling to ensure that the correct volume is drawn.

- The black mark on the side of the tubes indicates the 1 mL fill volume. QFT blood collection tubes have been validated for volumes ranging from 0.8 to 1.2 mL. If the level of blood in any tube is not close to the indicator line, it is recommended to obtain another blood sample. As a guide, the picture on the lower right illustrates the approved fill range.

If a “butterfly needle” is used, prime tubing with a “purge” tube before filling the QFT tubes.



Figure 1. (L-R) Nil, TB Antigen, and Mitogen tubes



Figure 2. Approved fill volume range; image for guidance only



Mixing Tubes

Antigens have been dried onto the inner wall of the blood collection tubes. It is essential that the tubes' contents be thoroughly mixed with the blood. Thorough mixing will dissolve the heparin, preventing clotting, and allow resolubilization of the stimulating antigen. Mixing is performed by shaking the tubes ten (10) times just firmly enough to ensure that the entire inner surface of the tube is coated with blood.

- The tubes must be transferred to a $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ incubator as soon as possible, and within 16 hours of collection. Prior to incubation, maintain tubes at room temperature ($22^{\circ}\text{C} \pm 5^{\circ}\text{C}$) Do not refrigerate or freeze the blood samples. Tubes should be incubated **upright**.

If the blood is not incubated immediately after collection, mixing of the tubes by inverting 10 times must be performed immediately prior to incubation.

- Over-energetic shaking may cause gel disruption and could lead to aberrant results.



Figure 3. A correctly mixed tube

Further information on QFT tube handling can be found at www.QuantiFERON.com.

Full instructions for use can be found in the QFT Package Insert, available in up to 25 different languages, at www.QuantiFERON.com.

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For more information, please contact the office nearest you or visit www.QuantiFERON.com.

Cellectis, a QIAGEN Company

World Headquarters ■ Cellectis International ■ +61 3 8527 3500 ■ info@cellectis.com
Asia/Pacific ■ QIAGEN Singapore PTE Ltd ■ +65 6854 8100 ■ asiapac@cellectis.com
Australia/New Zealand ■ Cellectis International ■ +61 3 8527 3500 ■ anz@cellectis.com
Europe/Middle East ■ Cellectis GmbH ■ +49 6151 428 590 ■ europa@cellectis.com
Japan/Korea ■ QIAGEN KK ■ +81 3 6890 7300 ■ jp.kr@cellectis.com
North America/South America ■ Cellectis Inc ■ +1 661775 7480 ■ customer.service@cellectis.com



Specimen collection and shipment for QuantiFERON-TB Gold IT (QFT-GIT) assay

**Blood collection tubes MUST be checked to ensure they are not expired
Check fill-volume to ensure they are between 0.8-1.2 mL.**

Please follow these guidelines for shipping sets of QuantiFERON-TB blood collection tubes containing processed patient serum to the DSHS Austin Laboratory:

- Collect 1mL patient blood by venipuncture into each QFT blood collection tube (Grey, Red, and Purple cap), **SHAKE them ten (10) times** just firmly enough to ensure the entire inner surface of the tube is coated with blood to dissolve antigens on tube walls.
 - **NOTE: Over-energetic shaking may cause gel disruption and could lead to invalid results.**
- **As soon as possible**, transfer the tubes to a 37°C±1°C incubator, and incubate the tubes **UPRIGHT** for 16 to 24 hours. Incubation must occur within 16 hours of collection, or results may be compromised. Do not refrigerate specimens prior to incubation.
 - **NOTE: Re-mix tubes by inverting 10 times immediately prior to incubation.**
- After the incubation at 37°C, centrifuge tubes for 15 minutes at 2000 to 3000 RCF (g). The gel plug will separate the cells from the plasma. If this does not occur, the tubes should be re-centrifuged at a higher speed.
 - **NOTE: Once centrifuged, tubes should be refrigerated (4°C - 8°C) before shipment to DSHS.**
- Deliver/ship to the DSHS Lab with cold packs within 28 days from the time removed from the incubator. Shipment must be received cold.
 - **NOTE: Do not freeze the samples in QFT blood collection tubes**

Submit completed G2A form and before shipping to DSHS lab,
Check / Circle “Yes” to indicate that incubation has been completed.
Check / Circle “Yes” to indicate that centrifugation is completed

Contact DSHS lab at 512-776-7760 or 7514 or 2450 for shipping guidelines.

Implemented April, 2009
Revised, effective November 10, 2009
Revised, effective April 1, 2010
Revised, effective December 19, 2011
Revised, effective December 1, 2013

QFT NEWS

Issue 4, 2010

“Changing the way the world looks at TB”

Highlights

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In Focus

QFT for Student Health: *We’re not (just) in Kansas anymore*

With increasing numbers of individuals studying abroad, the focus on tuberculosis (TB) screening in international students has increased substantially in recent years. This issue focuses on how uptake of QFT is helping colleges and universities around the world tackle TB.

The recent United States (US) National TB Controllers Association (NTCA) annual conference in Atlanta set the stage for not only the release of the **new CDC TB Testing guidelines**, but also a focused interest in international student TB screening.

Dr Neil Schluger, Chair of the Expert Committee overseeing guideline development, previewed the updated CDC guidelines, which were officially published on June 25 in *Morbidity and Mortality Weekly Report (MMWR)*. Dr Schluger highlighted three paradigms for **understanding and improving TB control** in the US. **Firstly, trends show that foreign-born (ie. non-US-born) individuals mostly develop TB disease via reactivation of latent TB infection, while a substantial percentage of US-born individuals who develop TB disease do so through recent transmission.** Secondly, TB control strategies aimed solely at interrupting

transmission are unlikely to reduce disease below a certain threshold. **Thirdly, TB control efforts should be expanded to include effective treatment of latent TB infection.** The updated guidelines have clearly been developed with these paradigms in focus; they aim at breaking the transmission cycle with IGRA technology and now recommend IGRAs for many populations, including the BCG-vaccinated and those unlikely to return for skin test reading (see **QFT News Special Issue** for more details).

These principles are central to improving TB control throughout the US, and the updated guidelines will surely encourage wider-spread IGRA use across the US. Kansas is one US state that is already paving the way for successful IGRA implementation. Thor Elliott, Microbiologist from the Kansas Department of Health and Environment in Topeka, presented the NTCA delegates with a synopsis of his department’s experience implementing QFT for two highlighted populations—incoming high-risk (usually BCG-vaccinated international) students at state universities and TB contacts identified in state-wide contact investigations. The primary objective of the program was to establish a QFT service in Kansas that could meet the rural US state’s public health needs in a cost-effective way. ►

Over the course of the three-year study period:

- Kansas' QFT program grew from processing less than 200 tests in '08 to a projected 3000 tests this year,
- Overall TB Control program costs decreased as a result of fewer required follow-ups for BCG-vaccinated contacts or those who had previous questionable screening results,
- Costs to the laboratory were neutral after negotiations, and
- Kansas Public Health TB Laboratory became a validated provider of the QFT service.

The need for highly-accurate TB screening in students is implicit, with an estimated **500,000 international entrants** to US colleges and universities annually. **In Kansas, between 15 and 20% of active TB cases each year occur among international students attending state schools. Recognizing the importance of accurate testing, three local Kansas universities have already adopted QFT into their respective TB screening policies:** **Wichita State University** now requires all international students to undergo QFT testing, the **University of Kansas** has required QFT for new students since August 2006, and **Emporia State University** implemented QFT as the test for new high-risk students in August 2008.

The positive experiences with and demand for QFT in Kansas have even led to state legislation requiring QFT testing in state college and university students. Senate Bills No. 62 (2009) and 565 (2010) which mandate testing and cost structures, respectively, have now been passed into law. These laws can ensure the strong future of TB control in Kansas.

QFT use in the education sector is buoyed by the **American College Health Association TB testing guidelines**, in effect from July 2008, which provide the choice of IGRA or TST for TB screening. This choice is particularly important for international students as BCG vaccination, which is common in this group, can cause higher numbers of false-positive TST results compared with IGRAs. A recent study from Canada, a country with low TB incidence, showed that only 72 of 298 (24.2%) TST-positive post secondary school students were QFT-positive (Kunimoto *et al* *IJTL* 2009).

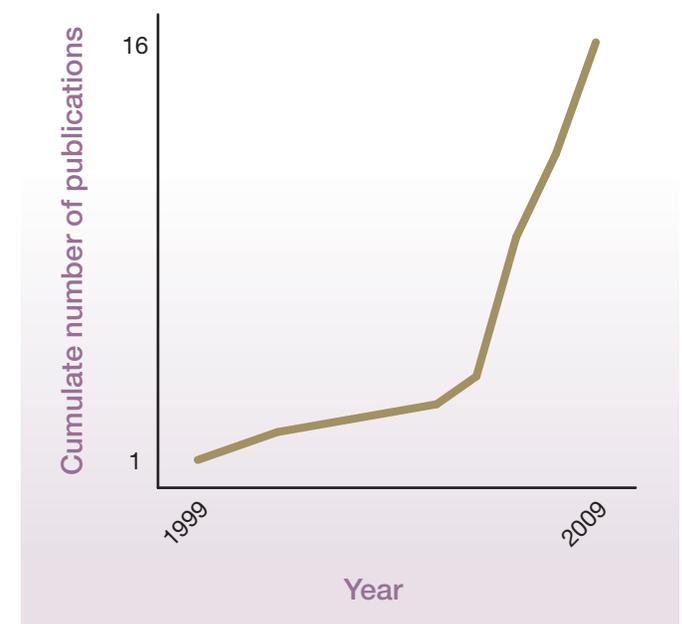
In fact, QFT's overall performance in university students is significantly better than TST's. Kang *et al* (JAMA 2005) found a QFT specificity of 96%—compared to TST's 49% specificity—in a group of 99 healthy, BCG-vaccinated medical students in Korea. An earlier report by Mazurek *et al* (JAMA 2001) showed a QFT specificity of 98.1% in 216 BCG-vaccinated Japanese nursing students at low risk for TB infection. The accuracy figures in students are congruent to the statistics in the wider population. A recent meta-analysis reported QFT specificity and sensitivity as 99.2% and 84.5%, respectively (Diel *et al* *CHEST* 2010). In contrast, the TST may have much lower sensitivity (78.0%, Pai *et al* *Ann Intern Med* 2008) and specificity (71.5%, Diel *et al* *CHEST* 2010). A recent study from Sweden reported a specificity of the TST (≥ 6 mm) of only 95% in non-BCG vaccinated students and an alarming 41% in BCG vaccinated students (Fjällbrant *et al* *IJTL* 2010).

The amount of clinical data supporting QFT performance in Student Health should increase as the list of educational institutions utilizing QFT grows (see graph for current trend). ▶

“In Kansas, a successful, cost effective and growing QFT service has been established.”

Conclusion of abstract, *Collaborative effort between TB control program and TB laboratory leads to successful implementation of Quantiferon Gold In-Tube service* by Elliot & Griffin, presented at NTCA Atlanta, June 2010. [View presentation of QFT experience in Kansas.](#)

Other schools have also adopted QFT. At the University of Tennessee, QFT testing has been successfully implemented and well-accepted in campus-wide TB surveillance program (Veaser *et al*. *J Am Coll Health* 2007). Whereas the first schools to adopt QFT were mostly medical and health science schools where students may have greater occupational exposure to TB, countless colleges and universities around the world are adopting broader QFT screening strategies for international students as well as health science students (see list, inset).



QFT-related publications on college/university screening and/or contact investigations

The effect of this may well be increased clinical confidence in QFT. Combined with already reduced confidence in TST results—a Columbia University study found that international medical graduates would treat as few as 63% of BCG-vaccinated individuals who return a positive TST result and would treat their own positive TST results less than 50% of the time (Salazar-Schicchi *et al* IJTLID 2004)—increased confidence in QFT can lead to broader implementation and hopefully more accurate diagnosis of TB.

The Kansas study presented at the NTCA meeting clearly shows the benefits of implementing accurate TB testing such as QFT for individuals, schools, and local communities. This, in addition to the CDC TB testing guidelines' endorsement of IGRAs, will encourage more schools, states, and countries to adopt IGRAs. With the start of the northern-hemisphere school year upon us, the most accurate TB testing possible is essential. ■

An increasing number of colleges and universities world-wide are adopting QFT in their TB testing policies. Here are some of those universities that had policy information freely available on the web at the time of writing:

Carroll University

City College of San Francisco

Columbia University

Deakin University (Australia)

Edith Cowan University (Australia)

Indiana State University

Loras College

Massachusetts Institute of Technology

Monash University (Australia)

Northwestern University

San Francisco State University

St Catherine University

St George School of Medicine

St George School of Veterinary Medicine

Stanford University and Stanford Hospitals

University of California, San Francisco

University of Hartford

University of Iowa

University of Michigan

University of Nebraska–Lincoln

University of Nevada, Reno

University of Toledo (Ohio)

University of Virginia

University of Washington

University of Western Australia



QFT
FERON-TB Gold

You give them the tools to succeed.

We give you the cutting edge tool for tuberculosis screening.

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Latest News

Publications & Guidelines update

CDC Guidelines 2010

As mentioned in the InFocus article, the CDC has issued updated guidelines for TB testing. The CDC now state that IGRAs are now the **preferred** method for TB testing in many populations.

- ▷ For more information on how these changes might affect you, please see the [guidelines publication](#), [CDC Guideline Pocket Book](#), or www.cellestis.com

Guidelines updated in Denmark

[Tuberculosis Control in Denmark—A national tuberculosis program].
Seersholm *et al.* *May 2010*.

This recent update to the Danish guidelines for the first time describes IGRA and QFT use for TB infection and states:

[Detection of infection by interferon-gamma release assays (igra) or with tuberculin skin test (Mantoux) can complement the microbiological studies and used for environmental studies and prior treatment with immunosuppressive drugs.]

- ▷ Read the full Danish Guidelines (in Danish) at www.ssi.dk

Guidelines Updated in Norway

In June, the Norwegian Institute of Public Health (NIPH) updated its TB testing guidelines. In addition to its May update (**[Regulation changes from 1 March 2009 with Significance for tuberculosis work] in Norwegian**), the NIPH published its “[Tuberculosis Guide],” an e-book of guidelines and recommendations which states:

[IGRA tests are recommended as a supplement to tuberculin skin test performed in risk groups (see Chapter 5 and Chapter 6). By repeated testing (for example, of health workers), one can consider using the IGRA test directly without first TST.

- When a positive TST to rule out false positive test (NTM/BCG vaccine).
- When negative TST suspected to be false negative because of the T-cell anergy.

QFT is recommended as first choice.]

- ▷ Link to the updated guidelines e-book (in Norwegian) at www.fhi.no



CME course on updated CDC Guidelines

With the recent release of the 2010 CDC Guidelines update, Medscape has developed a CME course called, “CDC Issues Updated Guidelines for Testing for Tuberculosis Infection”. Alerts were sent through different Medscape Pulse outlets including Public Health and Prevention, Pathology and Lab Medicine, and Infectious Diseases. If you have any questions regarding this short course or would like to sign up, please visit Medscape (www.cme.medscape.com/medscapetoday).

New England Journal of Medicine’s Interactive Medical Case features QFT

The NEJM has launched a new Interactive Medical Case on IGRAs. Featuring QFT, the case presents an evolving patient history and a series of questions and exercises designed to test physicians’ diagnostic and therapeutic skills. Interactive Medical Cases in general provide immediate feedback on answers and treatment, peer-to-peer score comparisons, and interactive multi-media content regarding mechanisms, diagnostic tests, and treatments. Each case can also lead to a CME examination, where the user may claim up to 2 AMA PRA Category 1 Credits.

- ▷ Explore NEJM IGRA Interactive Medical Case at www.nejm.org/doi/full/10.1056/NEJMimc1000140

Now is the time for heightened focus on the TB threat. www.cnbc.com guest blog by Dr Tony Radford. Published online, 28 June 2010.

Included in the increased media activity following the publication of the updated CDC guidelines, CNBC.com published a guest editorial by Dr Tony Radford, CEO of Cellestis. The op-ed piece focuses on the modern-day challenges of controlling TB in the United States.

- ▷ Read the full article at www.cnbc.com

Voice of America online TB video series

Visit www.voanews.com to view the following video content.

World AIDS Conference to Focus on HIV/AIDS, Tuberculosis 12 July 2010



Dual infections of TB and AIDS make each harder to treat 5 July 2010



“...TB is everybody’s problem, and to control TB anywhere, you have to control TB everywhere.”

Prof Lee Reichman, Pulmonologist and Director of the New Jersey Medical School's Global Tuberculosis Institute, as told to Voice of America

Experts: Too many people are ignorant about TB 28 June 2010



Van Voorhees, AS. *Skintillating revelations: Smarter meds call for smarter screening.* *Skin & Aging, Vol 18 (5), May 2010.*

This first instalment of a new column in the journal, *Skin & Aging*, focuses on the importance of TB testing for psoriasis patients taking immunosuppressant medications. Assoc Prof Van Voorhees, Director of the Psoriasis and Phototherapy Treatment Center at the University of Pennsylvania Health System, described her experiences using QFT and TST in the article:

“The advantages of [QFT] have prompted me to switch my patients on immunosuppressive therapies to baseline and annual [QFT] screenings. This has been good for both me and my patients. ...This is an exciting new development in my practice, allowing for enhanced accuracy and convenience for patients, and reduced hassles for everyone. Do consider checking with the labs that you use about whether this test is available to your patients, and if so, I strongly recommend that you consider using it for your patient monitoring.”

▷ Read more at www.skinandaging.com

Air travellers with TB triple since 2006

by Tom Blackwell, National Post (Canada). 25 May 2010.

This article reports on a publication by the Public Health Agency of Canada that appeared in *Travel Medicine and Infectious Disease* and describes the substantial increase in TB infection in the Canadian airspace. In 2009, official reports were made regarding 65 individuals who very likely flew into or out of Canada while infected with active TB, compared to 104 between 2006 and 2008. Post-flight diagnoses indicated that nine of these people had strains resistant to one or more antibiotics and four had multi-drug resistant TB.

▷ Read more about air travel with TB at www.nationalpost.com

Product Updates

QuantiFERON-CMV

The 23rd International Congress of the Transplantation Society was an important meeting for promoting the benefits of the QuantiFERON-CMV (QF-CMV) test for monitoring cytomegalovirus (CMV) in the transplant setting.

Program highlights included the exploration and discussions on therapeutic immunosuppression, immunology of organ rejection, and prediction of complications including CMV. The meeting ran from Aug 15 to 19 in Vancouver.



Preceding the congress was the 4th International Transplant Infectious Diseases Conference, a one-day meeting that included in-depth review of the recently-released International Consensus Guidelines on CMV Management. Another related topic was new immunologic tools for diagnosing and managing CMV disease in a transplant setting. The potential utility of QuantiFERON-CMV to monitor levels of anti-CMV immunity in persons at most risk of developing CMV disease received considerable discussion.

▷ Visit www.transplantation2010.org for more congress details.

▷ Visit www.cellestis.com/cmV for more information on QF-CMV.



New tube cap color for Mitogen and HA Mitogen blood collection tubes

You may have noticed a slight color change on our Mitogen and High-Altitude Mitogen blood collection tube caps and labels.

New images will soon be available in the Gnowee image bank.



QFT Blood Collection and Handling Wall Chart

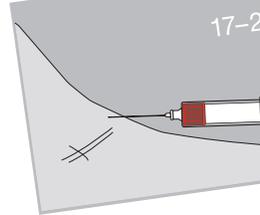
This simple wall chart outlines QFT blood collection and tube shaking/handling techniques at-a-glance—perfect for your medical practice, clinic, laboratory, or blood collection center.

QFT The

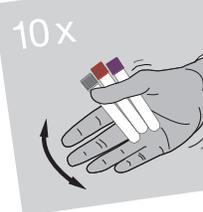
Blood collection

QuantiFERON®-TB Gold (QFT) is used to identify people infected with Mycobacterium tuberculosis (TB). QFT is a Tuberculin Skin Test (TST) and is unaffected by BCG.

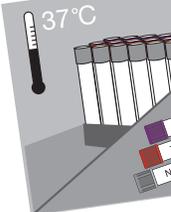
1. Blood Collection



2. Tube Shaking



3. Incubation



For further information, visit www.cellestis.com or call 1-800-445-2345.

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M05995193B Sept 2010

New QFT support tools available!

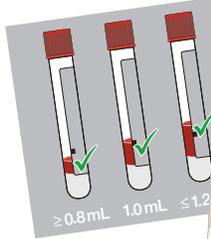
We are pleased to introduce a range of new QFT-related materials

To order any of these materials, please contact your local Cellestis representative or email info@cellestis.com

Simple blood test for TB infection and tube handling technique

QFT® is a simple blood test that accurately identifies *Mycobacterium tuberculosis*, the causative agent of tuberculosis, as a modern alternative to the 110-year-old Tuberculin Skin Test (TST) or the Bacillus Calmette-Guérin (BCG) vaccination.

Collect 1 mL into each tube.
Hold tube on needle for
2–3 seconds after flow
ceases. Repeat tube if not
close to black fill line.



Immediately after filling tubes, shake them ten (10) times just firmly enough to ensure entire inner surface of tube is coated with blood, to solubilize antigens on tube.

Caution

Tubes should be between 17–25°C at time of blood filling. Over-energetic shaking may cause gel disruption and could lead to aberrant results.

Option 1: Incubate at collection site. Incubate tubes at collection site (upright at 37°C for 16–24 hours) then ship to lab at 4–27°C. Record as “incubated”.

or –

Option 2: Incubate at lab at 17–27°C (blood must be collected as possible and within 16 hours “not incubated”).

For more information, contact your local Cellestis representative, or visit www.cellestis.com

CDC Guidelines Pocket Book

The Pocket Guide is a quick reference summarizing important points in CDC’s new recommendations for using IGRAs in screening for TB infection. A free copy of the CDC Pocket Guide can be found on www.cellestis.com



QFT Instructional DVD

This complete training resource details step-by-step instructions on blood collection, handling of tubes post-collection, and ELISA and data analysis for clinics, laboratories and other facilities using the QuantiFERON-TB Gold In-Tube test.

QFT News CDC Guidelines Special Edition

This issue (No. 3, 2010) focuses on new CDC guidelines, recommended results reporting, and American Association of Pediatricians guidelines for TB testing in children. Click here to read the April QFT News or other past QFT News issues.

QFT NEWS

Issue 3, 2010

“Changing the way
the world looks at TB”

CDC GUIDELINES SPECIAL ISSUE

For an in-depth review of the ground-breaking meta-analysis published in *Chest* last month, please see the April issue of QFT News

In Focus

New CDC Guidelines Intensify “Race” for QuantiFERON Gold

The US Centers for Disease Control and Prevention (CDC) today released updated guidelines for TB testing, which will have a major impact on the screening for TB infection in special populations.

Imagine homeless Shelter A in Big City, USA—the “front line” of American life. Thirteen residents of the 600+–bed TB over several months, and quick identification of those residents with TB infection is paramount to limit future exposure to others. Immediately a contact investigation begins. Three days of questionnaires, blood sampling, medical exams and histories, chest X-rays, and treatment prescription later, some startling results emerge: Six additional active TB cases are identified, requiring admission to local hospital. The known TB infection rate is 41% at conclusion of the investigation.

Shelter A is that Shelter A, its contact investigation, and TB cases are real and not imagined. Last year Shelter A, in Fulton County, Georgia, was the epicentre of a contact investigation and subsequent analysis that was

run by a team from the CDC (Powell et al. Shelter-based on-site active case-finding during a tuberculosis outbreak among homeless persons—Fulton County, Georgia 2009 [Abstract only]. *IAATLD*, Mar 2010). One of the largest ever (n = 311) to use QuantiFERON® (QFT) in a US-born population, this contact investigation followed the CDC’s own contact investigation guidelines, which allow for the use of QFT in place of the tuberculin skin test (TST) during contact investigations. The major outcome of such a large and dangerous outbreak was the successful containment of the outbreak using the active case-finding method (vs traditional name-based methods). QFT was fundamental to the success of this method. Of the 286 participants, including residents and staff, tested with a valid QFT, 117 (41%) were QFT-positive. ▶

CDC TB Testing Guidelines Update

Using IGRAs for
TB screening in
your patients

June 2010

A full copy of the US Centers for Disease Control and Prevention (CDC) guidelines is available for viewing at www.cdc.gov/mmwr/pdf/r/r5905.pdf

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Upcoming Events

Events calendar

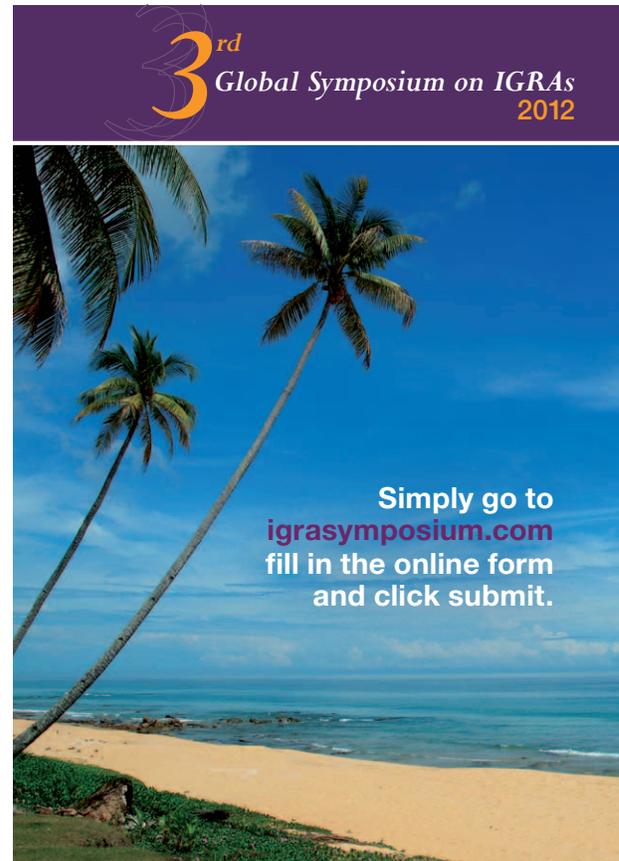
For upcoming QFT or TB-related events around the world, visit the Cellestis Events Calendar at www.cellestis.com

USA events

For information on TB-related events in the USA, please visit <http://tb-usaevents.com>

3rd IGRA Symposium

Register for information updates on the 3rd IGRA Symposium that is planned for 2012.



3rd Global Symposium on IGRAs
2012

Simply go to igrasymposium.com
fill in the online form
and click submit.

If you have any questions, comments or would like to have QFT-News mailed to your Inbox, contact us at news@cellestis.com

World Headquarters
Cellestis Limited
Email: info@cellestis.com
Tel: +61 3 8527 3500

North America / South America
Cellestis Inc.
Email: customer.service@cellestis.com
Tel: +1 661 775 7480 (outside USA)
Toll free: 800 519 4627 (USA only)

Europe / Middle East / Africa
Cellestis GmbH
Email: europe@cellestis.com
Tel: +49 6151 428 59 0

Japan / Korea
Cellestis Asia KK
Email: quantiferon@cellestis.com

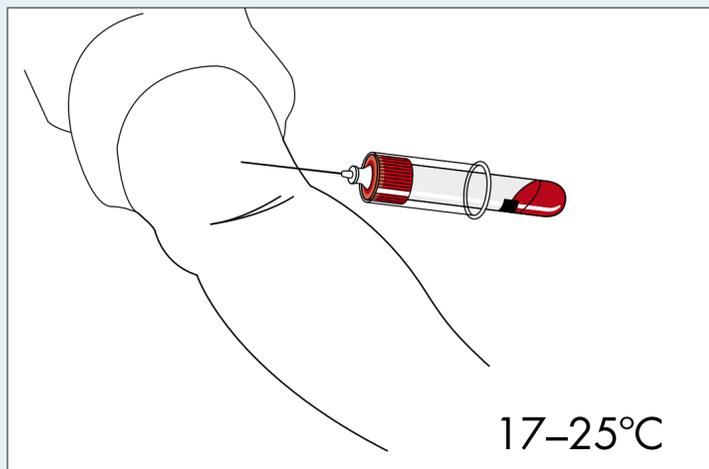
Australia / New Zealand
Cellestis International
Email: quantiferon@cellestis.com
Tel: +61 3 8527 3500

Asia / Pacific
Cellestis AP Pte Ltd
Email: asiapac@cellestis.com
Tel: +65 6322 0822

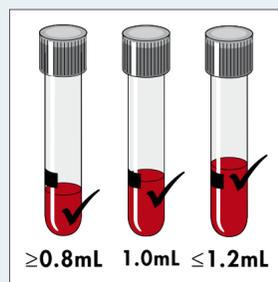
Blood collection and tube handling technique

QuantiFERON®-TB Gold

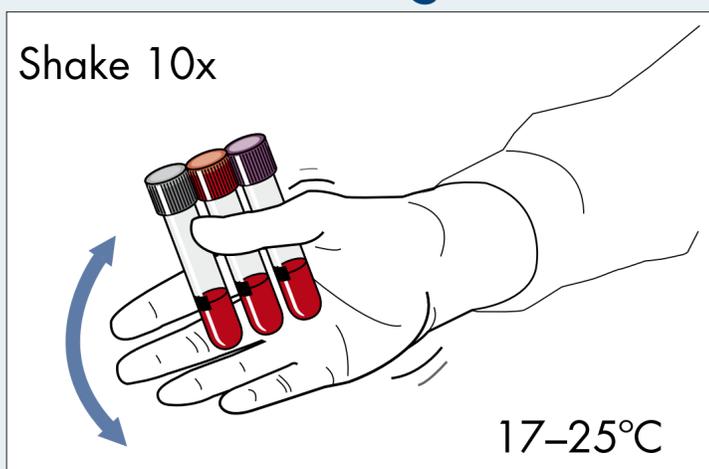
1. Blood Collection



Collect 1 mL into each tube. Hold tube on needle for 2–3 seconds after flow ceases. Repeat tube if not close to black fill line.



2. Tube Shaking



Immediately after filling tubes, shake them ten (10) times, just firmly enough to ensure entire inner surface of tube is coated with blood, to solubilize antigens on tube wall.



Tubes should be between 17–25°C at time of blood filling.

Over-energetic shaking may cause gel disruption and could lead to aberrant results.

3. Incubation / Shipping



Option 1: Incubate at collection site. Incubate tubes at collection site (upright at 37°C for 16–24 hours) then ship to lab at 4–27°C. Record as “incubated”.

Option 2: Incubate at laboratory. Ship tubes to laboratory at 17–27°C (blood must be incubated at 37°C as soon as possible and within 16 hours of collection). Record as “not incubated”.

Comprehensive instructions for use can be found in the QuantiFERON®-TB Gold Package Insert, which is available in 25 different languages, on www.QuantiFERON.com.

Contact us today or visit www.QuantiFERON.com



Option 2: Incubate at Laboratory



1. Blood Collection

Collect 1 mL blood by venipuncture into each QFT blood collection tube.

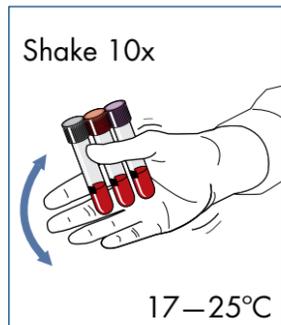


Tubes should be at 17–25°C at the time of blood filling.

Tubes fill slowly—hold tube on needle for 2–3 seconds after flow ceases. If blood level is not close to the black mark on the side of the tube label, obtain another sample.

Technical Tip:

Butterfly needles—prime tubing with a “purge” tube (not supplied) before filling QFT tubes.



2. Blood Collection

Immediately after filling, shake tubes ten (10) times just firmly enough to ensure that the inner surface of the tube is coated in blood (to dissolve antigens on tube walls).



Over-energetic shaking may cause gel disruption and could lead to aberrant results.

Label tubes appropriately.



3. Shipping and Incubation - Incubate at Laboratory

Ship tubes to laboratory at 17–27°C.

Blood must be incubated at 37°C as soon as possible (and within 16 hours of collection).

Re-mix tubes by inverting 10 times immediately prior to incubation.

Technical Tip:

Label tubes as “Not Incubated”.



WARNING: Standard blood handling precautions apply.

Please see reverse for instructions if incubating tubes at Collection site.

For comprehensive instructions for use, please refer to the Package Insert, available in up to 25 different languages, on www.QuantiFERON.com.

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World Headquarters • Cellestis International • +61 3 8527 3500 • info@cellestis.com



Option 1: Incubate at Collection Site



1. Blood Collection

Collect 1 mL blood by venipuncture into each QFT blood collection tube.

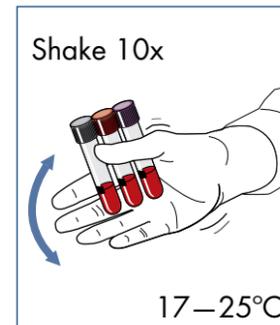


Tubes should be at 17–25°C at the time of blood filling.

Tubes fill slowly—hold tube on needle for 2–3 seconds after flow ceases. If blood level is not close to the black mark on the side of the tube label, obtain another sample.

Technical Tip:

Butterfly needles—prime tubing with a “purge” tube (not supplied) before filling QFT tubes.



2. Blood Collection

Immediately after filling, shake tubes ten (10) times just firmly enough to ensure that the inner surface of the tube is coated in blood (to dissolve antigens on tube walls).



Over-energetic shaking may cause gel disruption and could lead to aberrant results.

Label tubes appropriately.



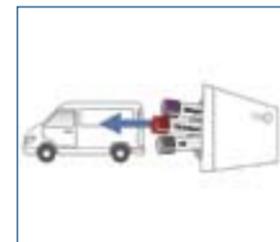
3. Shipping and Incubation - Incubate at Collection site

Blood must be incubated as soon as possible (and within 16 hours of collection).
Incubate tubes **upright** at 37°C for 16–24 hours.

Humidity/CO₂ not required.

Portable incubators are available from Cellestis.

If tubes are not incubated at 37°C soon after collection, re-mix tubes by inverting ten (10) times immediately prior to incubation.



4. Shipping and Incubation

Ship incubated tubes to testing laboratory (within 3 days, if not centrifuged)

Maintain tubes at 4 – 27°C .

Technical Tip:

Label tubes as “Incubated”.



WARNING: Standard blood handling precautions apply.

Please see reverse for instructions if incubating tubes at Laboratory.

For comprehensive instructions for use, please refer to the Package Insert, available in up to 25 different languages, on www.QuantiFERON.com.



Frequently Asked Questions

QuantiFERON[®]-TB Gold

Laboratory Professionals



Questions and Answers

These Frequently Asked Questions (FAQs) relate to the QuantiFERON®-TB Gold (QFT®) assay. The answers provided are meant to act as a guide only. We recommend that the QuantiFERON-TB Gold Package Insert be used as the reference for test procedures, as well as for all other enquiries relating to the use or performance of the assay.

Test Principle

The QuantiFERON-TB Gold assay is an *in vitro* diagnostic laboratory test that aids in the detection of infection with *Mycobacterium tuberculosis*. It uses human whole blood, with patented assay technology based on the measurement of Interferon-gamma (IFN- γ) secreted from stimulated T-cells previously exposed to *M. tuberculosis*.

The QFT assay is a straightforward laboratory test that involves the following steps:

- Collection of blood into QFT blood collection tubes.
- Incubation at 37°C.
- Detection of released IFN- γ in harvested plasma using an ELISA.
- Analysis and results using the QFT Analysis Software.

Blood Collection

The blood hasn't reached the black mark on the side of the QFT blood collection tube. Is this important?

The black mark on the side of the tubes indicates the 1 mL fill volume. QFT blood collection tubes have been validated for volumes ranging from 0.8 to 1.2 mL. If the level of blood in any tube is not close to the indicator mark, it is recommended to obtain another blood sample.

How important is the tube mixing process?

The antigen mixing process ensures even distribution of stimulating antigens to allow white blood cells to ingest and process antigen for presentation to T-cells, thus leading to IFN- γ secretion. It is a very important step in the QFT assay—poor mixing may lead to erroneous results.

Immediately after filling the tubes, shake them ten (10) times just firmly enough to ensure that the entire inner surface of the tube has been coated with blood, to solubilize antigens on the tube wall. Thorough mixing is required to ensure proper mixing of the blood with the tube's contents. Some blood frothing is expected and will not adversely affect the performance of the test. Universal blood handling precautions should be used. Tubes should be between 17–25°C at the time of blood filling.

Technical notes on blood handling procedures are available on www.cellestis.com.

Can the blood collection tubes be transported lying down?

Yes. QFT blood collection tubes can be transported lying down, but only after the tube shaking has been performed. The tubes should be mixed again by inverting 10 times immediately prior to being placed upright in the 37°C ± 1°C incubator.

At what temperature can the blood be transported to another site, or held prior to incubation at 37°C?

Blood should be held and transported at room temperature (17–27°C). Do not refrigerate the blood or place on ice.

Blood Incubation / Plasma Harvesting

What if 37°C incubation starts more than 16 hours after the time of blood collection?

If the blood is not incubated immediately after collection, re-mixing of the tubes by inverting 10 times must be performed immediately prior to incubation.

Blood samples incubated more than 16 hours after collection are likely to exhibit a decreased IFN- γ response due to cellular breakdown (death), leading to loss of sensitivity and inaccurate results.

Can I incubate the blood collection tubes lying down?

QFT blood collection tubes must be kept upright during incubation at 37°C.

Do I have to centrifuge the tubes before I can harvest the plasma?

While it is recommended to centrifuge the tubes to assist with harvesting, it is possible to harvest the plasma from the tubes without centrifugation. However, additional care is required to remove the plasma without disturbing the cells.

Do I have to centrifuge the tubes immediately after removal from the incubator?

QFT blood collection tubes may be held between 4°C and 27°C for up to 3 days before centrifugation or harvesting.

The gel plug hasn't moved during centrifugation. What should I do?

After incubation of tubes at 37°C, the plasma is separated from the cells by centrifuging for 15 minutes at 2000–3000 RCF (g). The gel plug should move to separate the cells from the plasma. If this does not occur, the tubes should be re-centrifuged at a higher speed.

After centrifugation, avoid pipetting up and down or mixing plasma by any means prior to harvesting. At all times, take care not to disturb material on the surface of the gel.

- Plasma samples should only be harvested using a pipette.

- Plasma samples can be loaded directly from centrifuged blood collection tubes into the QFT ELISA plate, including when automated ELISA workstations are used.
- Plasma samples can be stored for up to 28 days at 2-8°C or, if harvested, below -20°C for extended periods.

The plasma doesn't appear the colour it normally does. Is this OK?

Plasma from the QFT blood collection tubes can appear more red than usual—this is normal. It should be noted that the colour of plasma, even those without any red blood cell contamination, can vary from almost colourless to shades of yellow/pale brown; some plasma samples even have an opaque appearance. These qualities have been found not to affect QFT results.

Do I require a Class II Biohazard Cabinet in which to perform plasma harvesting?

Ideally, all work with blood should be performed in a Biohazard Cabinet to minimize the risk of infection (eg HIV, Hepatitis-B) from potentially infectious blood samples. However, as long as aseptic techniques are used, plasma harvesting can be performed outside of a Biohazard cabinet. 'Safe Laboratory Practices' should be followed, including the use of protective clothing such as gloves, gown, safety eyewear, etc, as suggested by relevant regulators.

What volume of plasma do I need to harvest from above the sedimented red blood cells or gel plug? Is this important?

As little as 100 µL of plasma is sufficient, as only 50 µL of plasma is required to perform the ELISA. Two-hundred µL will leave sufficient plasma for reference (re-testing) purposes, if required. It is generally possible to take greater than 300 µL. Validation studies have shown that IFN-γ is evenly distributed in the plasma and the volume removed is not critical. The volume of plasma available can vary from patient to patient.

The QuantiFERON method also allows for direct sampling of plasma from the blood collection tubes using an automated system. This allows the plasma harvesting and ELISA to be performed with minimal operator input.

Note: After centrifugation and prior to harvesting, avoid pipetting up and down or mixing plasma by any means, manual or automated.

I want to maximize the cost-effectiveness of the QuantiFERON-TB Gold assay by batching my samples. What is the stability associated with the harvested plasma?

Harvested plasma (or plasma stored in the blood collection tubes after centrifugation) can be stored at 2–8°C for up to 28 days, or below -20°C for extended periods. Plasma kept at -70°C are less likely to form clots. For short-term storage (less than 28 days), it is better to refrigerate plasma samples rather than freeze them, due to possible fibrin clot formation.

What should I do if clots form in my plasma samples during frozen storage?

Upon thawing, frozen plasma samples may require centrifugation to sediment the clots that can form during the freeze/thaw process. A guide to dealing with clotted plasma samples is outlined in the Package Insert.

Do I need to use microtubes when storing harvested plasma? Can I use more cost-effective microtitre plates in this instance?

Uncoated low-binding microtitre plates, with an appropriate adhesive covering to prevent evaporation, can be used to store harvested plasma.

Interferon-gamma (IFN- γ) ELISA

What is the stability associated with—

a) Kit Standard?

Reconstituted IFN- γ kit standard may be kept for up to 3 months if stored at 2–8°C (the date of reconstitution should be noted). Reconstituted kit standard should be equilibrated at Room Temperature (17–27°C) for 1 hour before use.

b) Conjugate 100X Concentrate?

Once reconstituted, the Conjugate 100X Concentrate must be used within 3 months or discarded. Working strength conjugate (Conjugate 100X Concentrate mixed with Green Diluent) must be used within 6 hours of its preparation. Any unused Conjugate 100X Concentrate must be returned immediately to 2–8°C following its use.

c) Wash Buffer?

Working strength Wash Buffer may be stored at room temperature (17–27°C) for up to 2 weeks.

Can I use the QuantiFERON ELISA plates immediately after their removal from the fridge?

No. Sealed ELISA plates should be allowed to equilibrate at Room Temperature for at least 1 hour before opening the foil bag.

Do I require an automated Plate Washer?

No. Although an automated plate washer is recommended, manual washing can be performed following the procedure as outlined in the Package Insert.

How important is washing during the QuantiFERON ELISA?

As with most ELISAs, inadequate or incorrect washing is the single most common cause of QuantiFERON ELISA error. If you have any such problems, please check the following—

- If bubbles and froth form during the wash steps, the flow rate of the wash cycle should be adjusted (usually lowered) to prevent this from occurring.
- Wash volumes should allow the wash buffer to reach the top of each well (preferably with a positive meniscus forming over the rim of each well).
- Ensure all wells receive sufficient and equal wash buffer. Blocked washer probes can be cleaned according to the manufacturer's instructions.
- Six complete washes are suggested as a minimum in the Package Insert, however additional washes can be performed without affecting the performance of the assay.
- A soak period of at least 5 seconds between each cycle is recommended.

Data Analysis

I have very high Nil control values? What may be the problem?

Under most circumstances, the expected IFN- γ concentration range for the Nil control is below 5 IU/mL (however, values up to 8 IU/mL can be acceptable). If Nil control values are much greater than this, the result may be due to a technical error. In such instances judgement should be used in determining whether to re-test. If re-testing is required, it is recommended that all of the subject's plasma samples be re-assayed. If the Nil result remains high—and contamination of the sample plasma is unlikely—the Nil result is valid. A good practice is to check all Nil control values following each test to make sure they fall within, or are close to, the expected range.

A patient's TB Antigen value is very high (possibly above the detectable limit of the plate reader). Is this OK?

In some cases the patient's TB Antigen IFN- γ level may be above the limit of the microplate reader—such an occurrence will have no impact on the test interpretation, provided the result for that patient's Nil Antigen is below 8 IU/mL.

Can the amount of IFN- γ measured be correlated to the stage or degree of TB infection?

No. Individuals displaying a response greater than or equal to 0.35 IU/mL above the Nil control (and greater than or equal to 25% of the Nil value), for the TB Antigen, are likely to be infected with *M. tuberculosis*. No correlation between their response to these antigens and the stage or degree of infection, their level of immune responsiveness, or their likelihood for progression to active disease can be made, based on currently available data.

Is there an easy way of calculating and interpreting QuantiFERON-TB Gold test results?

An outline of the Data Analysis and Test Interpretation method for the QuantiFERON assay is provided in the Package Insert. Calculation of QuantiFERON results can be performed using a spreadsheet program.

Alternatively, QuantiFERON Analysis Software is available from www.cellestis.com to analyse assay raw data, and calculate QuantiFERON results.

The QuantiFERON Analysis Software allows the simple transfer of raw data (ODs) directly from microplate reader software (or from any spreadsheet program).

The software performs—

- Calculation of a Standard Curve.
- Quality Control check of the standard replicates and curve.
- Calculation of all sample IFN- γ concentrations (IU/mL) from the Standard Curve.
- Reporting of a diagnostic result for each patient, according to the 'Interpretation of Results' guidelines outlined in the QuantiFERON-TB Gold Package Insert.
- Please ensure that you are using the most current QuantiFERON-TB Gold software for your region.

Troubleshooting

My results are not as I had anticipated. What could be the problem?

General ELISA problems—with the possible causes and appropriate solutions—are listed in the following tables.

Low Absorbance

Possible Cause	Solutions
Standard dilution error	Ensure that dilutions of the Kit Standard are prepared correctly as per Package Insert.
Pipetting error	Ensure that pipettes deliver correct volume.
Wash Buffer dilution error	Ensure that a 1-in-20 dilution of the wash buffer concentrate is performed to prepare the working strength wash buffer.
Incubation temperature too low	Incubation of the ELISA should be performed at room temperature, 17–27°C.
Incubation time too short	Incubation of the plate with the conjugate, standards and samples should be for 120 ± 5 minutes. The Enzyme Substrate Solution is incubated on the plate for 30 minutes.
Incorrect plate reader filter used	Plate should be read at 450 nm with a reference filter of between 620 and 650 nm.
Reagents are too cold	All reagents, with the exception of the Conjugate 100X Concentrate, must be brought to room temperature prior to commencing the assay. This takes at least one hour.
Kit/Components have expired	Ensure kit is used within the expiry date. Ensure that reconstituted Standard and Conjugate 100X Concentrate are used within three months of the reconstitution date.

Standards Duplicate Variability

Possible Cause	Solutions
Poor mixing	Mix reagents thoroughly by inversion or gentle vortexing prior to their addition to the plate.
Incomplete washing of the plate	Wash the plate at least 6 times with 400 µL/well of wash buffer. More than 6 washing cycles may be required depending on the washer being used. A soak time of at least 5 seconds between cycles should be used.
Inconsistent pipetting technique or interruption during assay set-up	Sample and standard addition should be performed in a continuous manner. All reagents should be prepared prior to commencing the assay.

High Background

Possible Cause	Solutions
Incomplete washing of the plate	Wash the plate at least 6 times with 400 µL/well of wash buffer. More than 6 washing cycles may be required depending on the washer being used. A soak time of at least 5 seconds between cycles should be used.
Conjugate reconstitution/dilution error	Conjugate 100X Concentrate should be reconstituted with 300 µL of distilled water. Working strength conjugate is prepared by diluting the Conjugate 100X Concentrate 1/100 in Kit Green Diluent as per the Package Insert.
Incubation temperature too high	Incubation of the ELISA should be performed at room temperature, 17–27°C.
Components have expired	Ensure that reconstituted Kit Standard and Conjugate 100X Concentrate are used within 3 months of the reconstitution date.
Enzyme Substrate is contaminated	Discard substrate if blue colouration exists. Ensure clean reagent reservoirs are used.
Mixing of plasma in centrifuge tubes before harvesting	After centrifugation, avoid pipetting up and down or mixing plasma by any means prior to harvesting. At all times, take care not to disturb material on the surface of the gel. Plasma samples should only be harvested using a pipette. Plasma samples can be loaded directly from centrifuged blood collection tubes into the QFT ELISA plate, including when automated ELISA workstations are used. Plasma samples can be stored for up to 28 days at 2-8°C or, if harvested, below –20°C for extended periods.

Poor Standard Curve

Possible Cause	Solutions
Incomplete washing of the plate	Wash the plate at least 6 times with 400 µL/well of wash buffer. More than 6 washing cycles may be required depending on the washer being used. A soak time of at least 5 seconds between cycles should be used.
Standard dilution error	Ensure that dilutions of the Kit Standard are prepared correctly as per Package Insert.

Non-specific Colour Development

Possible Cause	Solutions
Incomplete washing of the plate	Wash the plate at least 6 times with 400 μ L/well of wash buffer. More than 6 washing cycles may be required depending on the washer being used. A soak time of at least 5 seconds between cycles should be used.
Cross-contamination of ELISA wells	Take care when pipetting and mixing samples to minimize risk.
Components have expired	Ensure that the kit is used within the expiry date. Ensure that reconstituted Standard and Conjugate 100X Concentrate are used within 3 months of the reconstitution date.
Components are contaminated	Avoid contamination of components.
Mixing of plasma in centrifuge tubes before harvesting	After centrifugation, avoid pipetting up and down or mixing plasma by any means prior to harvesting. At all times, take care not to disturb material on the surface of the gel. Plasma samples should only be harvested using a pipette. Plasma samples can be loaded directly from centrifuged blood collection tubes into the QFT ELISA plate, including when automated ELISA workstations are used. Plasma samples can be stored for up to 28 days at 2-8°C or, if harvested, below -20°C for extended periods.

QFT has been CE marked. QFT is approved by the US FDA

QFT is approved by FDA as an *in vitro* diagnostic aid for detection of *Mycobacterium tuberculosis* infection. It uses a peptide cocktail simulating ESAT-6, CFP-10 and TB7.7(p4) proteins to stimulate cells in heparinized whole blood. Detection of IFN- γ by ELISA is used to identify *in vitro* responses to these peptide antigens that are associated with *M. tuberculosis* infection. FDA approval notes that QFT is an indirect test for *M. tuberculosis* infection (including disease) and is intended for use in conjunction with risk assessment, radiography and other medical and diagnostic evaluations. QFT Package Inserts, available in up to 25 different languages, can be found at www.cellestis.com.

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Cellestis, a QIAGEN Company

World Headquarters ▪ Cellestis International ▪ +61 3 8527 3500 ▪ info@cellestis.com
Asia/Pacific ▪ QIAGEN Singapore PTE Ltd ▪ +65 6854 8100 ▪ asiapac@cellestis.com
Australia/New Zealand ▪ Cellestis International ▪ +61 3 8527 3500 ▪ anz@cellestis.com
Europe/Middle East ▪ Cellestis GmbH ▪ +49 6151 428 590 ▪ europe@cellestis.com
Japan/Korea ▪ QIAGEN KK ▪ +81 3 6890 7300 ▪ jp.kr@cellestis.com
North America/South America ▪ Cellestis Inc ▪ +1 661 775 7480 ▪ customer.service@cellestis.com

