GUIDELINES FOR USING QuantiFERON® TB Gold In-Tube

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The Prevention Committee of the Japanese Society for Tuberculosis

QuantiFERON® TB Gold In-Tube (referred to hereafter as QFT-3G), an improved version of the QuantiFERON® TB-2G (QFT-2G), was approved in April 2009 as a diagnostic kit for extracorporeal tuberculosis (TB) infection. QFT-3G was available for purchase in July 2009. Subsequent problems with blood collection tubes led to the withdrawal of QFT-3G sales; however, it was re-released in January 2010 and is widely available at present. Its predecessor, QFT-2G, had been confirmed as a TB diagnostic tool not influenced by BCG immunization. QFT-3G is increased for diagnostic accuracy by the addition of a new specific antigen, TB7.7, along with the other *Mycobacterium tuberculosis*-specific antigens, ESAT-6 and CFP-10. In some areas, QFT-2G tests were difficult because transportation of blood specimens to testing laboratories presented an issue related to distance and time. QFT-3G is more user-friendly because the blood collection tubes are precoated with antigens and can be used in most places. Recently, however, it has been determined that the diagnostic accuracy of QFT-3G may be influenced by the handling of blood collection tubes, amount of blood collected, mixing and storage of collected blood, and subsequent culture conditions. Accordingly, we need to be aware of quality control for this test from the moment blood is drawn.

Another extracorporeal TB infection diagnostic kit, T-SPOT.*TB*® (T-SPOT, Oxford Immunotec Ltd, UK), is used overseas and will become available in Japan in the near future. The principle underlying the T-SPOT test is identical to QFT-3G: a peripheral blood sample is mixed with ESAT-6 and CFP-10 and the test determines the number of blood cells that produce interferon-γ (IFN-γ), a cytokine that regulates the immune response to *M.tuberculosis*. Tests of this type are called IFN-γ release assays (IGRA)[1].

TB is diagnosed based on the detection of *M.tuberculosis* in the patient's blood samples; but in clinical practice, appropriate samples for microbiological diagnosis cannot be obtained many a time, even for patients who are highly suspected for TB. In these cases, IGRAs can be very useful as auxiliary diagnostic tools. IGRA results can be used to diagnose latent tuberculosis infection (LTBI) in persons who were in contact with TB patients by considering the risk of infection based on several factors, including the bacillary excretion status of the index patient, closeness of contact, and history of residence in TB endemic countries. IGRAs can function only as an aid to diagnosis; active TB disease cannot be diagnosed solely on the basis of IGRA results.

The third version of the guidelines for IGRAs, released in June 2010 by the U.S. Centers for Disease Control and Prevention (CDC)[2], states that adequate evidence is currently available regarding the sensitivity and specificity of QFT and T-SPOT tests, but questions remain regarding the following: (1) risk of progression to active TB disease in persons determined as TB positive in contact investigations; (2) diagnostic accuracy in immunosuppressed patients; and (3) diagnostic accuracy in children, particularly those under 5 years of age.

The Prevention Committee of the Japanese Society for Tuberculosis decided to formulate guidelines for the use of QFT-3G, the only IGRA presently available in Japan, based on data and experiences gathered during the period of more than 1 year since its release for clinical use.

In July 2011, the World Health Organization warned against the use of several types of blood tests for the diagnosis of active TB due to low diagnostic accuracy (www.who.int/mediacentre/news/releases/2011/tb_20110720/en/index.html). We point out that these tests involve the measurement of serum levels of *M.tuberculosis* antibody, and thus, differ significantly from the IGRA tests.

＜Notes on terminology＞

QuantiFERON® (QFT) is the registered trademark of a whole blood IFN-γ measurement kit manufactured by Cellestis Ltd, Australia. Various antigens can be used in this system, but QFT-2G and QFT-3G use antigens specific to *M.tuberculosis*. The antigen used in the first generation kit (QuantiFERON® TB) was based on a purified protein derivative rather than antigens specific to *M.tuberculosis*. The first generation kit was not used in Japan because results were influenced by previous vaccination with BCG similarly to the tuberculin skin test (TST). QuantiFERON® TB Gold has been used overseas and noted in scientific papers and corresponds to the second generation kit (QFT-2G) formerly used in Japan. QuantiFERON® TB Gold In-Tube is the third generation kit (QFT-3G) and is available for use in Japan presently. Accordingly, care should be exercised when reviewing research papers to distinguish QuantiFERON® TB Gold overseas from QuantiFERON® TB Gold used in Japan.

As a diagnostic kit, QFT-3G is considered to have equal or greater accuracy than QFT-2G. At several instances in these guidelines, we will refer to QFT without distinguishing between the 2 variants.

Because the second generation kit QuantiFERON® TB-2G is sometimes identified as QFT-2G and the third generation kit QuantiFERON® TB Gold In-Tube as QFT-G, QFT-G sometimes is confused with the first generation kit, which can lead to the

misconception that QFT-2G is newer than QFT-G. To avoid confusion in this paper, we use generational numbering QFT-2G and QFT-3G, which reflect the generations of each test.

1. Use in general contact investigation

The Guidelines for TB Contact Investigation, stated in the Infectious Diseases Control Law (revised fourth edition), recommend wider use of QFT-3G for LTBI diagnosis[3]. This statement originates from a letter by the Director of Division of Tuberculosis and Infectious Diseases, Bureau of Health, Ministry of Health, Labour and Welfare (No. 0607001, June 7, 2007) that states a person diagnosed as an asymptomatic carrier of TB who requires TB treatment shall be deemed as having an LTBI.

[Note] The former policy was "to give chemoprophylaxis (preventive treatment) for persons with tuberculosis infection." However, a statement made by the American Thoracic Society in 2000 changed the wording to "detection and treatment of latent tuberculosis infection" for the same management; this concept has been widely accepted[5].

For the majority of persons infected with *M.tuberculosis* via contact with patients with active TB disease, QFT tests become positive 2−3 months following contact[6][7]. Accordingly, QFT tests should be conducted 2−3 months after the most recent contact. TST results in over diagnosis of LTBI and prescription for treatment, but QFT is free of this problem[8].

When LTBI is first suspected, the possibility of infection and risk of developing active disease is considered based on patient age, underlying disease, and history of BCG inoculation, information regarding the possible source of infection (e.g., TB disease type and level of bacillary excretion), closeness of contact, and whether other contacts have developed infection or active TB.

2. Use in contact investigation of healthcare workers

When a patient is diagnosed with TB disease unexpectedly in a healthcare setting, a list of all the workers who were in close contact with the index case should be made promptly for investigation. Workers who were QFT-negative at the time of employment and experienced only a short period of contact (＜2−3 months required for QFT conversion in infection) should undergo QFT-3G testing immediately to generate baseline data. The QFT-3G test should be repeated after 2−3 months.

3. Use for pre-employment examination of healthcare workers[9]

We recommend all new employees in healthcare facilities undergo QFT-3G testing upon beginning their employment. In particular, we strongly recommend testing on individuals who work with TB patients daily or who work in other areas with elevated risk of exposure to TB infection. Pre-employment testing screens are useful for detecting LTBI and providing a QFT baseline result.

4. Management of immunosuppressed patients

Immunosuppression has many causes, including human immunodeficiency virus (HIV) infection, dialysis, diabetes mellitus, and the use of corticosteroids or tumor necrosis factor-α (TNF-α) inhibitors. The risk of developing active TB is high in immunosuppressed patients; most cases represent endogenous reactivation of LTBI[10]. On the other hand, the QFT response can be suppressed in immunosuppressed patients, and sometimes test results are negative or indeterminate in the presence of TB infection[11]. However, the degree of QFT response suppression is lower than TST response suppression and high sensitivity is maintained, which highlights the usefulness of QFT-2G in these patients[12)~14)]. Therefore, QFT-3G should be performed in immunosuppressed patients despite potential reductions in sensitivity.

[Note] Recently, high incidence of active TB has become apparent in patients who were treated with TNF-α inhibitors for immunological diseases such as rheumatoid arthritis[15][16]. The CDC recommends that patients are tested and treated for LTBI before the TNF-α inhibitor therapy is introduced[17]. In 2005, the Japanese Society for Tuberculosis in conjunction with the Japan College of Rheumatology issued a statement entitled "For more active chemoprophylaxis," which recommended chemoprophylaxis for persons with suspected TB infection based on TST results. QFT-2G has been reported as a useful tool for LTBI diagnosis in patients with rheumatoid arthritis[18]; the adoption of QFT-3G should be discussed as soon as possible.

5. Auxiliary diagnosis of active TB infection

The diagnosis of active TB disease is based on sputum microscopy, culture, gene tests, and chest radiography. However, lesions that are very small for detection and/or insufficient sputum production are common. QFT-3G is useful as an auxiliary diagnostic tool when active TB infection is suspected, but a definitive diagnosis cannot be achieved due to difficulties in *M.tuberculosis* detection.

6. Application to children

The QFT is a useful diagnostic tool with high sensitivity in children with active TB disease[19]. However, several problems related to the diagnosis of LTBI have emerged. The cellular immune response is immature in children, particularly in those less than 5 years of age, and indeterminate results are common due to the weak IFN-γ response against mitogen (PHA) stimulus (positive control [M] in the interpretation criteria [see below]). False negative results also may be due to weak immune responses against *M.tuberculosis*-specific antigens[20)~22)]. Among children who live with smear-positive sources of infection, infants have shown extremely low rates of positive QFT-2G in comparison to older children[23]. Therefore, exclusion of TB infection based solely on negative QFT-2G results is inappropriate. Furthermore, TST reportedly is more sensitive than QFT[24]; thus, TST should be the preferred test for contact investigations on infants and primary school children. QFT-3G is the more appropriate test for secondary

school children and older.

7. Principles of measurements, methods, and interpretations of results

[Methods]

Whole blood is drawn from the peripheral vein and mixed with *M.tuberculosis*-specific antigens (ESAT-6, CFP-10, and TB7.7) that are in the tube already. The concentration of plasma IFN-γ released by lymphocytes is determined by enzyme-linked immunosorbent assay (ELISA).

[Note] The *M.tuberculosis*-specific antigens used in this test are present in all species of *M.tuberculosis*, including *M.tuberculosis*, pathogenic *M.bovis*, and *M.africanum*. In addition, these antigens are present in several nontuberculous mycobacteria (*M.kansasii*, *M.marinum*, *M.szulgai*, *M.flavescens*, *M.gastri*, and *M.leprae*), but not in *M.avium*, *M.intracellulare*, or the BCG strain of *M. bovis*. Accordingly, individuals vaccinated with BCG are not QFT-3G positive in the absence of TB infection.

[Note] The T-SPOT test is based on the enzyme-linked immunospot (ELISpot) measurement technique: mononuclear cells are separated from other components of peripheral blood, and incubated in a 96-well plate with *M.tuberculosis*-specific antigens ESAT-6 and CFP-10 (unlike QFT-3G, TB7.7 is not included) for 20 hours. The cells that secrete IFN-γ are enumerated, which is the primary characteristic of this method.

[Procedure]

- Step 1 (includes blood collection through culture)

Blood (1 mL) is drawn from a peripheral vein and added to each of 3 special tubes that contain reagents (i.e., antigens and controls). Although the sequence of tubes is not specified, it is preferable to collect blood into the negative control tube first, followed by the TB antigen tube and the positive control tube. Immediately following blood collection, each tube is shaken for 5 seconds or 10 times to ensure thorough mixing and the samples need to be transported to the testing laboratory within 16 hours. Care must be taken not to shake the tubes very vigorously when mixing because the layer separating the plasma and blood cells may become compromised.

At the testing laboratory, the collection tubes are incubated upright for 16−24 hours at 37°C. If a relatively long period of time has elapsed between blood collection and incubation, the tubes should be re-shaken for 5 seconds or 10 times immediately prior to incubation.

- Step 2 (ELISA)

Please refer to the manufacturer's instructions for the ELISA procedure (Japan BCG Laboratory, http://www.bcg.gr.jp/).

[Interpretation criteria]

The IFN-γ concentrations (IU/mL) released in response to either TB antigens (A) or mitogen (M, positive control) are calculated as the difference between plasma IFN-γ concentrations from antigen- or mitogen-stimulated blood and blood incubated with saline (nil, N), as shown below:

A (IU/mL) = IFN-γ (A) − IFN-γ (N)

M (IU/mL) = IFN-γ (M) − IFN-γ (N)

Interpretation criteria and explanations are listed in the enclosed Table[25].

When the QFT value, A, exceeds 0.35 IU/mL (the unit is omitted hereafter), the result is considered "positive."

If positive control M is ≧0.5 and A is ＜0.1, the test is interpreted as "negative," whereas a QFT value of 0.1−0.35 is interpreted as "intermediate." This intermediate range represents a buffer zone and assists with comprehensive diagnosis. For example, a person with an intermediate test result who has been in close contact with a smear-positive TB patient is at a high risk for infection, and thus, is considered equivalent to a positive result and is indicated for LTBI treatment.

An indeterminate result occurs in patients with QFT values less than 0.35 and with M values, which are measured simultaneously, less than 0.5. Impaired cellular immune response is suspected in these cases, and thus, the antigen response measurements are unreliable and interpretation is impossible.

[Note] The above interpretation criteria apply to adults and cannot be applied strictly to children under 12 years of age. QFT values may be lower in children than in adults. For children younger than 5 years, the QFT should be used only as a reference and not as the basis of a diagnosis.

[Note] In the United States, QFT-2G test results that include a negative control value that exceeds 0.7 and a TB antigen value of less than 50% of the negative control value are interpreted as "indeterminate" on the basis of high background levels of IFN-γ.

Later studies on QFT-3G interpreted test results with a negative control value of 0.7−8.0 and an A value of 25%−50% of the negative control value as "positive" rather than "indeterminate." In addition, test results with a negative control value of 0.7−8.0 and an A value of less than 25% of the negative control value were interpreted as "negative." Finally, tests with a negative control value of 8.0 or greater are interpreted as "indeterminate" irrespective of M and A values[2].

Negative control values often are elevated in the presence of high circulating levels of interferon, which can occur in patients with rheumatoid arthritis or systemic lupus erythematosus (SLE).

Table Criteria for interpretation of QFT-3G results

Positive control (M)	QFT value (A)	Assessment	Explanation
Any	≧ 0.35	Positive	Suspect TB infection
≧ 0.5	≧ 0.1 and ＜ 0.35	Conditionally positive	Overall assessment considering degree of risk of infection
≧ 0.5	＜ 0.1	Negative	Not infected with TB
＜ 0.5	＜ 0.35	Indeterminate	Cannot be interpreted, possible immune deficiency

(Unit IU/mL for all readings)

Although, this issue is not considered in the approved conditions under which this test can be administered nor is it mentioned in the manufacturer's instructions, it warrants attention.

8. Sensitivity and specificity of QFT-3G tests

Sensitivity

The QFT-3G has a sensitivity of 92.6% (95% confidence interval, 86.4%−96.3%)[26].

[Note] Sensitivity was determined in a study on 100 patients (age range, 20−92 years; age mean, 53.3 years; 73% male) who were confirmed microbiologically with active TB and either untreated or within 1 week of treatment initiation. The head-to-head sensitivity of QFT-2G was determined as 81.4% (95% confidence interval, 72.6%−87.9%), and QFT-3G exhibited significantly greater sensitivity.

Specificity

QFT-2G and QFT-3G both have a specificity of 98.8% (95% confidence interval, 95.1%−99.8%)[26]. Meta-analyses have yielded extremely high specificities for QFT-3G as well[27)28)].

9. QFT-3G and limited access to testing laboratories

For the QFT-3G test, antigen stimulation begins immediately following the addition of venous blood to the special tube. Thus, the first stage of the test can be performed at the point of care if the instructions are observed carefully. Within 16 hours after thorough mixing of the blood sample with the stimulating antigens, the tubes are incubated for 16−24 hours at 37°C. The manufacturer's instructions should be consulted for procedural details (Japan BCG Laboratory, http://www.bcg.gr.jp).

Following incubation, sample cultures that have not been centrifuged can be stored up to 3 days at 2°C−27°C. During this period, the sample should be transported to the laboratory for the second stage of testing. Samples that are centrifuged and separated into plasma and blood cells can be stored at 2°C−8°C for up to 28 days, which improves the degree of freedom for testing.

10. Challenges with QFT-3G

QFT-3G is considered superior to tuberculin testing by many. In Japan, this preference is reflected by the high incidence of BCG immunization, which has led to a low diagnostic value (specificity) for tuberculin testing. On the other hand, considerable evidence from cohort studies demonstrates the usefulness of tuberculin testing; tuberculin-positive individuals exhibit a high risk for progression to active TB, especially in patients with strong responses. Treating tuberculin-positive individuals reduces the risk of progression to active TB[5)29)~32)]. Presently, no comparable evidence exists for QFT.

There is no gold standard for the diagnosis of LTBI. The cutoff values for QFT-3G are based on QFT-2G cutoff values, which in turn are based on results obtained from patients with untreated, active *M.tuberculosis* infection confirmed microbiologically (i.e., by culture[33)]). Different immune responses are evident between patients who have developed active TB and those who have not progressed beyond latent infection, which is the basis of the "intermediate" test in Japanese diagnostic criteria. The use of a separate diagnostic criterion with a somewhat lower cutoff value may be useful for the reduction of false negative results (missing cases of infection) in high-risk situations, such as mass outbreaks. These Guidelines are based on Japanese and international literature and opinions. As new evidence continue to emerge, these Guidelines should be consulted to ensure that maximum benefits are gained from the QFT-3G test.

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