DRAFT SUMMARY OF SAFETY AND EFFECTIVENESS

Device Summary

Device & Classification

a) Generic Name

Cellestis Limited is not aware of any generic name for this particular EIA test.

b) Device Trade Name

Trade Name: QuantiFERON®-TB

c) Device Class

Device Class: III

d) Classification

Panel: 83 (Microbiology)

e) Applicants Name &Address

Name: Cellestis Limited

Address: 9/149 Fitzroy St,
St Kilda, Melbourne
Victoria 3 182
AUSTRALIA

f) Contract Manufacturer

Name: CSL Limited

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Intended Use

The QuantiFERON-TB test is intended as an aid in the detection of latent *Mycobacterium tuberculosis* infection

Contraindications, Limitations, Warnings and Precautions

Contraindicated Population

- There are no identified contraindications for the QuantiFERON-TB test.

Limitations

A negative QuantiFERON-TB result does not preclude the possibility of TB infection.

As for tuberculin skin testing, some individuals may not be able to mount a measurable cellular immune response to tuberculin. This will result in low IFN-γ readings and may prevent the QuantiFERON -TB test yielding a conclusive result. This may include:

- Individuals who are immunocompromised such as those with HIV infection, AIDS, leukemia, lymphoma, Hodgkin's Disease or other disorders that compromise the immune system.
- Individuals who are immunosuppressed such as those taking immunosuppressive drugs (eg. corticosteroids, methotrexate, Immuran, chemotherapy).
- Individuals who do not have a cellular immune response to tuberculin.

A low IFN-γ reading may prevent a valid result from being obtained with the QuantiFERON-TB test. Therefore caution must be exercised when interpreting results from these population groups.

In the addition the safety and effectiveness of QuantiFERON-TB has not been established in the following populations:

- Individuals under the age of 17 years.
- Pregnant women.

Warnings

For *in vitro* diagnostic use.

Deviations from the package insert may yield erroneous results.

Handle human blood as if potentially infectious, Observe universal blood handling precautions (ref CDC).

Blood must be cultured within 12 hours of collection to obtain valid results.

Handle chromogen solution with care. Avoid contact with skin and wear gloves, *This reagent contains dimethyl sulphoxide (DMSO), which is readily absorbed through the skin and may cause skin irritation. If Chromogen Solution comes into contact with skin, wash the affected area immediately with copious quantities of water and seek medical attention.*

Enzyme stopping solution is a strong acid. *Wipe spills up immediately and flush with water. If the stopping solution contacts the skin or eyes, flush with copious quantities of water and seek medical attention.*

Thimerosal; *Thimerosal is used as a preservative in some reagents. It may be toxic upon ingestion, inhalation or skin contact.*

Precautions

Correct laboratory procedures should be adhered to at all times.

Store kit components at 2°C to 8°C. Do not store kit at room temperature.

Blood must be cultured within 12 hours of collection to obtain valid results.

Do not use kit if any reagent bottle shows signs of damage or leakage prior to use.

Do not mix or use reagents from other QuantiFERON-TB kit batches.

Do not use kit standards or conjugate after three months from reconstitution.

Discard unused reagents and biological samples in an appropriate manner.

Do not use kit after the expiry date.

Transport blood samples at 17°C to 27°C. Do not transport blood on ice or refrigerate.

Bring all components, except conjugate concentrate, to room temperature (17°C - 27°C) before use.

Store conjugate 100 X concentrate at 2°C to 8°C at all times.

Disease Description

In the United States, the vast majority of TB cases in humans are caused by *Mycobacterium tuberculosis*, sometimes referred to as the tubercle bacillus. *M. tuberculosis* and two very closely related mycobacterial species *(M. bovis, M. africanum)* can cause tuberculous disease and, along with *M. microti* they compose what is *known as* the *M. tuberculosis* complex. *M. bovis* and *M. africanum* are very rare in the United States and *M. microti* does not cause disease in humans.

Transmission

M. tuberculosis is usually spread from person to person through the air. When a person with pulmonary or laryngeal *M. tuberculosis* disease coughs or sneezes, droplet nuclei containing *M. tuberculosis* are expelled into the air. If another person inhales air containing droplet nuclei, transmission may occur.

Pathogenesis

When a person inhales air that contains particles expelled by an infectious person, most of the larger particles become lodged in the upper respiratory tract, where infection is unlikely to develop. However, the droplet nuclei may reach the alveoli, where infection begins.

Initially, the tubercle bacilli multiply in the alveolar macrophages. A small number of tubercle bacilli spread through the lymphatic channels to regional lymph nodes and then through the bloodstream to more distant tissues and organs, including areas in which *M. tuberculosis* disease is most likely to develop. These areas include the apices of the lungs, the kidneys, the brain, and bone. Within 2 to 10 weeks after infection, the immune system usually intervenes, halting the multiplication of the tubercle bacilli and preventing further spread.

Persons who are infected with *M. tuberculosis* but who do not have active *M. tuberculosis* disease are not infectious to others. *M. tuberculosis* infection in a person who does not have *M. tuberculosis* disease is not considered a case of TB and is often referred to as latent TB infection.

Persons with *M. tuberculosis* infection progress to disease when tubercle bacilli overcome the defenses of the immune system and begin to multiply. Infection can progress to disease either very quickly or many years after infection. In the United States, in approximately 5% of persons who have been recently infected with *M. tuberculosis,* TB disease will develop within two years of infection. In another 5%, disease will develop later in their lives. Thus approximately 10% of persons infected with *M. tuberculosis* will develop TB disease at some point. The remaining 90% will stay infected, but free of disease, for the rest of their lives.

Epidemiology

Tuberculosis is the largest single microbial killer in the world. The annual death toll is estimated at over three million, and one third of the world's population may be infected with *M. tuberculosis* (Raviglione et al, 1995). While most *M. tuberculosis* infections are latent, they can progress to active and contagious disease. The number of people with latent infection in the United States is estimated by the Centers for Disease Control and Prevention

at between 10 and 15 million (Centers for Disease Control, 1995) with a large number of active cases of tuberculosis arising from this substantial pool of latent infected people.

From a public health perspective, it is important to identify people with latent infection, as available therapy is effective in preventing the development of active disease, thereby minimizing further spread of tuberculosis (American Thoracic Society, 1994).

Explanation of how the device functions

QuantiFERON-TB is a test of Cell Mediated Immune (CMI) reactivity. Individuals infected with *M. tuberculosis* have lymphocytes in their blood that recognize specific mycobacterial antigens in tuberculin Purified Protein Derivative (PPD) preparations due to prior exposure of the lymphocytes to *M. tuberculosis.* This lymphocytic recognition process involves the generation and secretion of the cytokine, interferon-gamma (IFN-y). The QuantiFERON-TB test measures the amount of IFN-γ secreted in 24-well microplates by *M. tuberculosis* sensitized lymphocytes in response to the stimulation antigens.

QuantiFERON-TB relies upon the stimulation of T cells previously sensitized by exposure to mycobacteria.

The QuantiFERON-TB test is a laboratory test that is conducted in two stages as illustrated below.

First, small aliquots of heparinized whole blood are incubated individually with Human PPD, Avian PPD, Mitogen and a Nil control. After overnight incubation, plasma is removed and in the second stage, the amount of IFN-γ in each plasma sample is quantified by EIA.

The Mitogen is used to non-specifically stimulate an IFN-γ response and acts to validate interpretation of PPD responses. Only blood samples taken from patients capable of generating detectable amounts of IFN-γ in response to the Mitogen are sufficiently immunocompetent for analysis using this test. The relative levels of IFN-γ detected in plasma samples indicate whether an individual is likely to be infected with TB organisms.

The Nil control is a solution containing phosphate buffered saline (PBS) and 0.0 1% w/v Thimerosal as a preservative. It acts in the test to measure background activity and is subtracted from the other readings to correct for any background present.

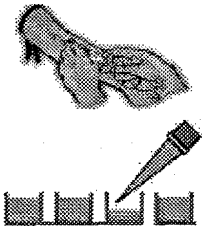
The QuantiFERON-TB test is conducted in two stages as follows:

1 *Incubation of Blood Samples*

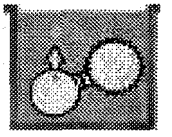
1.a Patient blood is collected into heparin and gently mixed to prevent coagulation.

1.b Blood is then dispensed into 4 wells of a 24 well tissue culture plate.

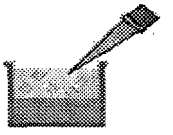
1.c Three drops of each of the stimulating antigens (Human Tuberculin PPD, Avian Tuberculin PPD, Mitogen antigen and Nil Control) are added to the four wells



respectively to stimulate T cells that recognize the specific stimulating antigen and secrete the cytokine IFN-γ.



1.d The blood is then incubated overnight to allow for the secretion of detectable levels if IFN-y.



1.e After incubation, plasma samples from each of the four wells are harvested for IFN-γ quantification.

Note: Plasma samples may be tested immediately in the EIA or can be stored for 14 days at 2°C to 8°C or up to 3 months at -20°C before testing

2 Human IFN-γ EIA

2.a The plate(s) are equilibrated to room temperature.

2.b The recombinant Human IFN-γ Standards are reconstituted with distilled or deionised water.

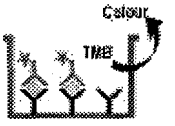
2.c The Green Diluent is brought to room temperature and mixed thoroughly to ensure homogeneity.

2.d The freeze-dried Conjugate 100X Concentrate is reconstituted with distilled or deionised water.

2.e Working strength conjugate is prepared by mixing appropriate volumes of Green Diluent and reconstituted Conjugate 100X Concentrate. 50µL is added to all wells. Then 50µL of plasma samples and standards are added to appropriate wells.

2.f The solid phase antibody and the diluted conjugate are incubated for 60 minutes in order to capture and bind the IFN-γ in the plasma sample.

2.g After incubation of the microplates, the wells are washed with Wash Buffer to remove unbound material.



2.h Enzyme Substrate solution is added to each well for 30 minutes to react with the bound conjugate to produce color.

2.i Adding Stopping Solution is added to terminate the reaction.

2.j The amount of color development is measured spectrophotometrically. The rate of conversion of substrate is proportional to the amount of bound IFN-γ. Using the Standards provided, the concentration of IFN-γ in each plasma supernatant is quantified. The sample must be read within 5 minutes of terminating the reaction.

Data is obtained from the microplate reader. Calculations of this data are performed using standard software and spreadsheet packages that are not supplied with the kit. The IU/mL of IFN-γ present in each sample is determined from a standard curve produced from the kit standards.

The algorithms outlined below are then used to convert the raw quantified IFN-γ readings (corrected for the Nil control values) into the % Human Response and % Avian Difference for each patient. These values are then used in concert with the labeled cut-off to interpret the test result.

The IU/mL IFN-γ values are used in the following calculations:

$$\% \text{ Human Response} = \frac{H - N}{M - N} \times 100$$

$$\% \text{ Avian Difference} = \frac{[(H-N)-(A-N)]}{G - W} \times 100$$

H = IFN-γ (IU/mL) for Human PPD well; M = IFN-γ (IU/mL) for Mitogen Control well, N = IFN-γ (IU/mL) for Nil Control well

MTB infection is indicated when the following results are obtained:

1. Both H-N and M-N are ≥ 1.5 IU/mL	(i.e. T cells are capable of producing IFN-γ)
2. % Human Response ≥ 15%	(i.e. There is significant response to PPD)
3. % Avian Difference ≥ - 10%	(i.e. Between -10% and positive values, predominant response is to *M. tuberculosis* PPD)

Summary of Alternative Practices

Diagnosis of Mycobacterium Tuberculosis (TB)

The definitive diagnosis of active tuberculosis (tuberculosis disease) and other mycobacterial diseases is dependent on the isolation and identification of the etiologic agent (Tenover et al, 1993). Microscopic examination, even though relatively insensitive (requiring at least $5x10^3$ organisms per mL of specimen for detection), is the method used for laboratory screening. The number of organisms present in pulmonary secretions is directly related to the risk of transmission and 50 to 80% of patients with pulmonary tuberculosis will have positive smears (American Thoracic Society, 2000 and Saceanu et al, 1993). Legally marketed devices for the identification (presumptive and definitive) of Mycobacterium spp. in the USA are available from various manufacturers using the following technologies:

1) Nucleic acid hybridization. This method is FDA approved only as an adjunctive test for evaluating pulmonary specimens from patients showing signs and symptoms consistent with active pulmonary tuberculosis who are not on therapy. It is not approved for use in general population screening or for aiding in the clinical determination of an individual's mycobacterium infection or disease status when there are no signs and symptoms consistent with active tuberculosis. It must be used in conjunction with mycobacterial cultures.

2) Microscopic screening. Direct microscopic screening for acid-fast bacilli using fluorescent microscopy with auramine-rhodamine staining is able to detect 10^5 organisms/ml of specimen. Although it is considered definitive for mycobacterium, it is not able to differentiate between the different species in the mycobacterium complex nor is it able to distinguish *M tuberculosis* from MOTT species. Cytocentrifugation of the specimen has also been reported to enhance sensitivity (Saceanu et al, 1993).

2) Conventional culture. Conventional culture is able to detect as few as 10 bacteria/ml of digested, concentrated material (American Thoracic Society, 2000) and is the definitive diagnosis for tuberculosis when *M. tuberculosis* is identified. During specimen collection of patients with pulmonary TB, patients produce an aerosol that may be hazardous to health care workers or other patients in close proximity. For this reason, precautionary measures for infection control must be followed during sputum induction or bronchoscopy.

3) Tuberculin skin test. A positive skin test does not necessarily signify the presence of disease, but will support the diagnosis of tuberculosis in patients with suggestive clinical signs. Conversely a negative skin test does not always indicate the absence of TB disease. On average, 10% to 25% of patients with TB disease have negative reactions when tested with a tuberculin skin test (Heubner et al, 1993).

Summary of Marketing History

In September 1995, QuantiFERON-TB was launched in Australia. The kit has been widely sold in all States, with the exception of Tasmania and the Territories.

International sales began in 1996/97 to New Zealand, Japan and the United Kingdom. From July 1997 to the present, sales of QuantiFERON-TB continued to the above-mentioned countries, as well as Spain, Malaysia, Denmark, Ethiopia, Kenya, Slovenia, Germany, Italy and Bulgaria. The kits sent to Ethiopia were used in a trial conducted by the John Hopkins University. In total there have been 736 kits sold outside of the United States with only 5 reported complaints. The complaints reported were; incorrect component in kit (1), poor component cap seal (1), higher than expected OD reading (2), and standards give higher than expected OD reading (1).

There has been NO marketing of the device in the US.

QuantiFERON-TB has not been withdrawn from the market in any country for any reason related to the safety or effectiveness of the device.

Potential Adverse Effects of the Device on Health

Possible Adverse Effects of the Device on Patient Health

The only direct adverse effects on patient health are those associated with venipuncture, namely a slight risk of bleeding, hematoma, and infection. Pain and redness at the site of injection occur and some people become dizzy and/or faint when blood is drawn. A false-negative result could lead to an individual developing active TB disease, adversely affecting their health and like the TST, a false-positive response could lead to an individual being administered unnecessary prophylaxis for tuberculosis infection.

Possible Adverse Effects of the Device on Public Health

An incorrect diagnosis giving a false-negative result could lead to an individual developing active TB disease, adversely affecting their health and possibly facilitating the spread of *M. tuberculosis* to other individuals in the community. Tuberculosis is contagious and can be readily transferred from one person to another via air particles expelled from an infectious individual while coughing.

Summary of Preclinical Studies

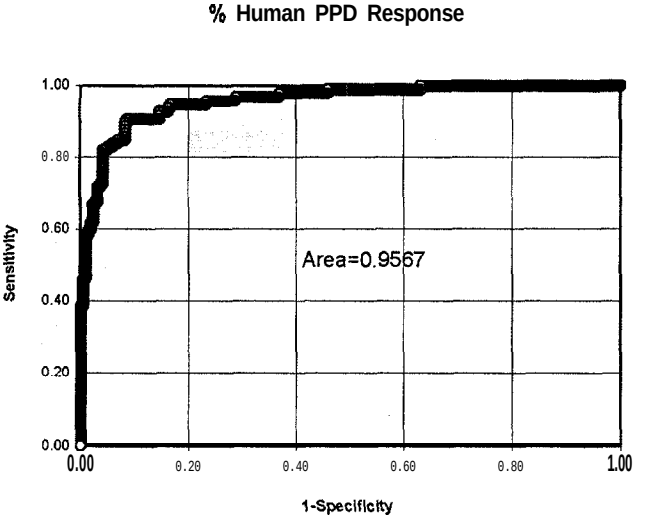
Preclinical studies with QuantiFERON-TB

Studies were conducted to confirm and establish the performance limits of the QuantiFERON-TB test and to develop Package Insert requirements, The following is a summary of the studies conducted.

Analysis to determine optimal methodfor cut-off point selection

Receiver Operating Characteristic (ROC) curves were generated from QuantiFERON-TB results from 599 individuals using American Thoracic Society (ATS, 1990) classification as the gold standard (Class 0, Low exposure risk, n=4 17 for specificity and Class 2, LTBI, n= 182 for sensitivity). ROC analysis identified the % Human PPD Response as the optimal method for distinguishing infected individuals from non-infected individuals (Figure 7.1).

Figure 7.1. ROC Plot for 599 individuals using % Human PPD Response (Streeton et al, 1998)



Establishment of the diagnostic cut-off criteria

Two diagnostic cut-offs were identified for the QuantiFERON-TB test. The % Human PPD Response cut-off was established to distinguish between individuals who are infected and those who are not infected. The % Avian Difference cut-off was established to help identify individuals whose positive % Human PPD Response is likely to be due to infection with a mycobacterium other *than M. tuberculosis* (MOTT).

Human PPD Cut-off- Reactivity to M. tuberculosis PPD

Heparinized whole blood samples from 952 consenting volunteers were tested to determine the Human PPD cut-off threshold (Streeton et al, 1998).

The % Human PPD Response, expressed as the % Human PPD/Mitogen using IU/mL IFN-γ estimates, was identified as the most sensitive and specific method for assessing an individual's Human PPD induced IFN-γ response.

Analysis was performed comparing QuantiFERON-TB and TST results in all ATS classes. Paired QuantiFERON-TB and TST results' were available for 58 1 of the 952 individuals. The TST reactions were assigned positive status for induration diameters ≥ 10mm or ≥ 15mm if BCG vaccinated.

When the % Human PPD Response cut-off was set at 15%, 88.2% of TST positive individuals were positive in the QuantiFERON-TB test. There was no statistical difference between the performance of the TST and the QuantiFERON-TB test using the 15% Human PPD Response cut-off (kappa = 0.786; p = 0·9, McNemar χ^2 test). Therefore the most appropriate % Human PPD response cutoff threshold was determined to be 15%.

The utility of the 15% Human Response cutoff was further validated using data from the CDC clinical study referred to in section 7.13 below. Using data from Group 1 and Group 2 individuals in the CDC study, it was determined that the maximum accuracy for QFT, combining issues of sensitivity and specificity, was obtained with a %Human Response cut-off of 13.6%. The 15% cut-off as a rounded, easy to use value produces very close to maximum accuracy for the QFT.

% Avian PPD Cut-of- Reactivity to non-tuberculous mycobacteria

A feature of the QuantiFERON-TB test is that it compares responses to tuberculin to that of *M. avium* PPD to distinguish sensitization due to mycobacteria other than tuberculosis (MOTT) from sensitization due to TB infection. MOTT is a significant cause of false positive TST responses and as the incidence of tuberculosis declines in the US population the effect of MOTT on TST accuracy will increase.

A - 10% Avian Difference cut-off was validated in a study testing 60 individuals with past culture confirmed *M. avium* complex (MAC) lymphadenitis (n=10) or *M. tuberculosis* disease (n=50). Patients with past culture proven *M. avium* infection were used as a representative group for individuals with MOTT reactivity.

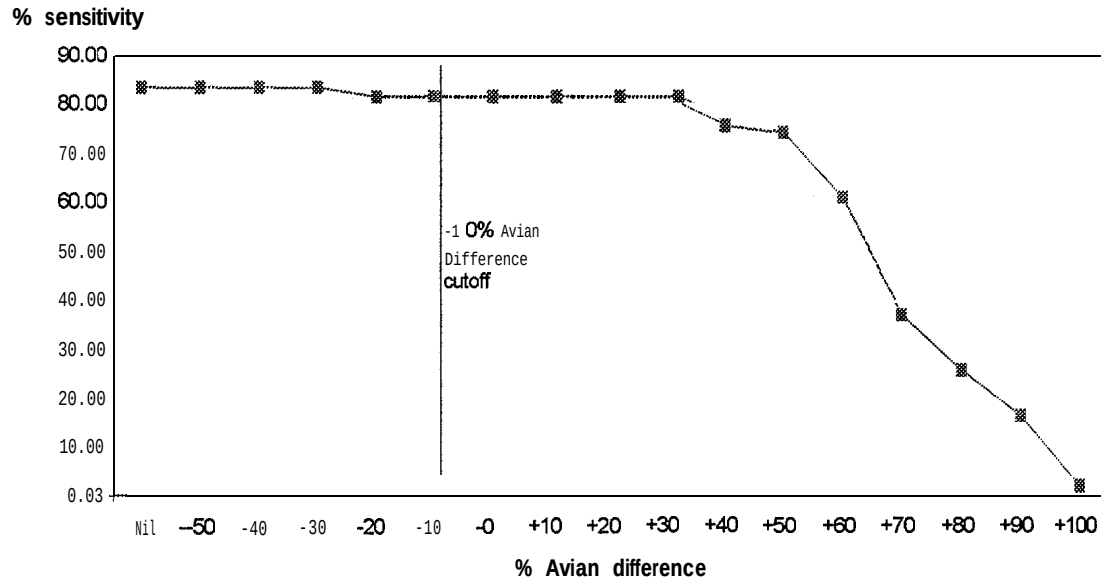
The data was analyzed by direct comparison of the % Avian Differences for each individual. Using the -10% cut-off criterion, 96% of tuberculosis patients had a % Avian Difference of > -10% and 100% of MAC patients had a % Avian Difference of < -10%. This showed that a % Avian Difference of −10%, calculated as [((Human PPD – Avian PPD)/Human PPD) X 100], was a useful cut-off to aid the discrimination of *M. tuberculosis* infection and reactivity due to MOTT. Therefore the most appropriate % Avian Difference cutoff to use in conjunction with the 15% Human PPD response cutoff was determined to be -10%.

To further validate the % Avian Difference cutoff, data from the CDC study was analyzed as described below. QuantiFERON-TB demonstrated a sensitivity of 8 1% (44 out of 54 patients) for TB disease in culture confirmed tuberculosis patients from Group 3 of the CDC study. Analysis of QuantiFERON-TB data from these 54 individuals was performed to ascertain what effect altering the %Avian Difference cutoff would have on the tests performance (Figure 4.1).

In this group of TB patients, where we had the gold standard of culture of *M. tuberculosis,* there was no significant loss of sensitivity observed by employing the secondary cutoff for QuantiFERON-TB of greater than – 10% Avian Difference. Examination of different cut points for the %Avian Difference demonstrated that there was no significant loss of sensitivity for the QuantiFERON-TB test until the %Avian Difference was raised to +30%. At cutoffs greater than +30% the sensitivity of QuantiFERON-TB declined rapidly (Figure 1). This analysis indicates that the %Avian Difference cutoff of -10% has no significant effect on the sensitivity of QuantiFERON-TB in truly infected subjects, and is set substantially below a level where an effect on sensitivity may be expected for individuals with LTBI.

There was one case of culture confirmed *M. avium* infection in Group 3 individuals from the CDC study. This individual was correctly identified by QuantiFERON-TB as being not infected with MTB complex organisms as their %Avian Difference was –90%, but was positive in the TST with an induration of 1 lmm.

figure **4.1. Effect of %Avian Difference on QFT sensitivity in culture confirmed tuberculosis**



Effect of Avian cut-off in high and low risk groups

For Group 1 and 2 (low and high risk) individuals in the CDC study there were 227 people with a positive TST, of which 80 had a negative QuantiFERON-TB result. Thirteen of these 80 had a % Human PPD Response greater than 15% but were classified as negative in the QuantiFERON-TB test due to their % Avian Difference being less than -10% (i.e. the QuantiFERON-TB test detected them as having MOTT reactivity). This represents 5.7% of the TST positive group, but only 1.2% of the population in total. Given that the estimates of false positive TST reactions due to MOTT range up to 3% of the general US population

(Margileth, 1994; Huebner et al, 1993; von Reyn et al, 1993) this would accord with known data on MOTT reactivity in TST.

Within the WRAIR study), 4 of the 29 (13.8%) TST positive QuantiFERON-TB negative subjects were classified as MOTT reactive by QuantiFERON-TB.

Utility of Avian Difference cut-off Sensitivity analysis

Further validation of the -10% Avian difference cut-off emerges from sensitivity analysis of the clinical trial data in low and high-risk groups. Sensitivity analysis was performed to test the effect of altering the sensitivity cut-off to 0% or -20%, or removing the Avian comparison completely .

Examining both the CDC and WRAIR low risk Croups, alteration of the % Avian Difference cutoff from 0%, -10% or -20% has little to no effect on the specificity of the test and its concordance with TST. Thus the -10% Avian Difference cut-off provides a suitable measure. Elimination of the % Avian Difference comparator resulted in loss of specificity for QuantiFERON-TB and a major increase in QuantiFERON-TB positive TST negative discordance.

For higher risk groups in both studies the alteration of the cut-off from 0%, -10% or -20% has little to no effect on the performance of the test and concordance with the TST remains similar.

Within the high risk CDC Croup 2, elimination of the % Avian Difference comparator results in higher levels of QuantiFERON-TB positive subjects, as would be expected, although concordance with the TST as measured by Kappa is only slightly reduced due to the number of TST positive QuantiFERON-TB positive subjects increasing.

Specificity and Interfering Substances

Listed below is a brief description of the non-clinical studies that were conducted to determine the test's specificity and investigate the effect of interfering substances.

Blood collection and storage before incubation

Objective: To determine the length of time which blood can be stored prior to initiation of incubation with antigens, studies were performed investigating time points from 1 to 14 hours post-collection.

Method: Blood from 18 individuals was incubated with the QuantiFERON-TB stimulation antigens at regular intervals up to 14 hours post-collection.

Conclusion: The length of time at which blood can be stored prior to initiation of incubation with antigens and controls is between 0 to 12 hours.

Blood storage temperature prior to incubation

Objective: To validate the storage temperature for blood prior to incubation with the stimulation antigens.

Method: Temperature limits studies were conducted using duplicate blood samples from 5 donors. The blood was stored at 17°C, 22°C and 27°C for 2 and 8 hours prior to incubation with Human PPD, Avian PPD, Mitogen and Nil control antigens.

Conclusion: There was no significant difference in results from blood stored at the temperature range of 22 ± 5°C.

Incubation Time of Blood with Stimulation Antigens

Objective: To determine the length of time that blood should be incubated with the stimulation antigens.

Method: In the first study blood was collected from 4 donors and incubated for 10, 12, 16, 18, 20, 24, and 36 hours at 37°C with each of the stimulating antigens. A second study involved collecting blood from 4 donors and incubating for 12, 13, 14, 15, 16, 17, 18, and 20 hours at 37°C with each of the stimulating antigens.

Conclusion: A labeled incubation time for blood with antigens of 16 to 24 hours is supported.

Comparison of different brands of tissue culture plates

Objective: To determine if the brand of the tissue culture plate had an effect on the performance of the test.

Method: Testing was conducted using donor blood from 4 individuals with four different tissue culture plate manufacturers.

Conclusion: Tissue culture plates manufactured by Costar, Corning and Greiner companies are all suitable for use in the QuantiFERON-TB test.

Position of tissue culture plate in the incubator

Objective: To determine if the position of the tissue culture plate in the incubator had an effect on the performance of the test.

Method: Testing was conducted using donor blood from one reactive individual and incubating the blood and antigens in 3 different ways in the incubator – single plate, stack of 3 plates and stack of 6 plates.

Conclusion: The stacking of culture plates on top of each other during incubation can lead to erroneous results. Thus culture plates must not be stacked in the incubator.

Storage of plasma samples

Objective: The stability of IFN-γ in sets of plasma samples was investigated after storage at 2-8°C for up to 28 days and at -20°C for 6 months.

Method:: Blood from 2 donors (approximately 40mL each) was dispensed into 1mL aliquots and incubated with stimulating antigens for 18-24 hours. Plasma supernatants were then removed and placed into 1mL polypropylene tubes and capped. Half the plasma supernatants were then stored at 2-8°C for up to 28 days and the other half stored at -20°C for 6 months before assaying.

Conclusion: Plasma samples can be stored at 2-8°C for 14 days or 3 months at -20°C with no loss of detectable IFN-γ.

Centrifugation of Plasma

Objective: To investigate the effect of centrifuging the plasma sample on the IFN-γ in the plasma sample as measured by QuantiFERON-TB test.

Method: Blood was collected from 4 donors and stimulated using the four antigens. After incubation half of the samples were centrifuged and half were not. Samples were then tested using QuantiFERON-TB.

Conclusion: Plasma samples can be centrifuged, as plasma samples harvested either prior to or after centrifugation gave equivalent results.

Interference by cytokines other than IFN- y

Objective: To establish that the EIA is specific for IFN-γ and that other cytokines are not detected.

Method: Denatured human IFN-γ , natural human interleukin-2 (IL-2), recombinant human IL-4, IL-.5, IL-6, IL-10 or IL-12 were diluted in pooled human plasma and tested in the EIA for cross-reactivity.

Conclusion: Results showed that the EIA did not detect significant quantities of denatured human IFN-γ, natural human IL-2 (200 IU/ml), or recombinant human IL-4 (5 ng/ml), IL-5, IL-6, IL-IO, or IL-12 (100 ng/ml), being cytokines likely to be present in cultured blood plasma.

Interference by either hemolysis or erythrocyte contamination

Objective: To investigate if the QuantiFERON-TB test was affected by the presence of either hemolysis or erythrocyte contamination.

Method: Two donor blood samples, from a known reactive donor and non-reactive donor were incubated overnight with Human PPD and Mitogen, and the plasma harvested into 1 mL aliquots. One set of plasma samples, from each donor, was then modified by adding various amounts of erythrocytes, whilst the other was modified by varying the degree of hemolysis, and a third set not modified and used as a control.

Conclusion: Results demonstrated that the QuantiFERON-TB test will tolerate slight to moderate levels of hemolysis and erythrocytic contamination. However it is recommended that hemolysis and erythrocytic contamination of the plasma sample be avoided.

The use of internal controls in the Quantiferon-TB test

Objective: To quantify and compensate for interference in the QuantiFERON-TB test by including internal controls.

Cross-reactive Autoimmune or Heterophile Antibodies: The Fc fragment of immunoglobulin is generally the target of cross-reactive autoimmune antibodies or heterophile antibodies. The use of mouse anti-human IFN-γ monoclonal $F(ab')_2$ fragments as the solid-phase capture antibody and the inclusion of unconjugated mouse antibodies (normal mouse serum) in the Green Diluent effectively remove the possibility of such factors interfering with the IFN-y EIA (Desem and Jones, 1998). The unconjugated mouse antibodies (normal mouse serum) prevent any cross bridging from forming between the solid phase capture antibody (mouse anti-human IFN-γ monoclonal $F(ab')_2$ fragments) and the HRP conjugated antibody (mouse anti-human IFN-γ monoclonal).

Nil and Mitogen controls: The Nil control antigen is used to detect any background IFN-γ or interference that may occur in a sample. The nil value is subsequently corrected for in calculating the % Human PPD Response and the % Avian Difference. Conversely, the test's positive control (Mitogen) is used to quantify an individual's capacity to generate an IFN-γ response.

Conclusion: Known sources of interference in the test are quantified and taken into consideration by the calculation of the results and the test algorithm and interpretation used.

Performance Characteristics

Analytical Sensitivity of the EIA

Concentrations of IFN-γ of less than 1.5 IU/mL are not distinguishable from zero concentrations. Therefore individuals demonstrating a value of less than 1.5 IU/mL for their Mitogen-Nil value are deemed as having an indeterminate result for the QuantiFERON-TB test. Moreover, an individual's response to Human PPD-Nil must be greater than or equal to 1.5 IU/mL for that individual to be deemed as likely to be infected with *M. tuberculosis*.

Linear Range of the EIA

The linear range of the QuantiFERON-TB test for detecting the concentration of IFN-γ is between zero and 200 IU/mL (correlation coefficient > 0.99, n=75x4).

Prozone or High-Dose Hook Effect Studies for the EIA

No prozone or hook effect has been detected with the QuantiFERON-TB EIA for concentrations of IFN-γ up to 100,000 IU/mL. No clinical samples tested have yielded IFN-γ levels in excess of 1000IU/ml. Samples with concentrations of IFN-γ that exceed the upper limit of the standard curve will generate an off-scale reading in the EIA. Samples with off-scale readings should be diluted in normal human serum and re-tested in the EIA. Hence,

samples should not give a false-negative result in the assay due to a prozone or high-dose hook effect.

Reproducibility

Reproducibility of the IFN-γ EIA

Numerous studies have been performed to corroborate the reproducibility of the QuantiFERON-TB test.

The use of a single EIA test well for IFN-γ assessment was established after the study of Desem and Jones (1998) assessed accuracy by spiking four replicates of pooled human plasma with recombinant human IFN-γ (150, 75, 37.5, 18.8, 9.4, and 4.7 IU/ml). This was done on two occasions by two operators with two batches of reagents. Average accuracy for the known concentrations was 105% +/- 11.4%.

Further validation of the reproducibility of the EIA testing came from the study of Stapledon et al, which validated the utility of the %Avian Difference. In the Stapledon study, replicates of every sample were used to determine a mean response figure. The replicate testing showed extremely high reproducibility in the replicates of 60 subjects tested for all four Nil, Human PPD, Avian PPD and mitogeri plasma samples. As would be expected, the relative differences were greatest at the lowest levels of IFN-γ, and thus Nil plasma samples showed the greatest variation. The intra-class correlation (ICC) using two-way ANOVA was 0.966, 0.995, 0.998 and 0.997 for Nil; Human PPD, Avian PPD, and Mitogen respectively. All these ICCs are very high and show excellent reproducibility of the tests.

Reproducibility of Antigen Stimulation

Results demonstrate that there is a high degree of reproducibility (Intraclass correlation coefficient (ICC) $\geq$ 0.949) in the level of IFN-γ expressed during the incubation of bloods with stimulating antigens in the QuantiFERON-TB test.

Two batches of Human PPD were demonstrated to have strong agreement with one another, with a kappa chance adjusted agreement statistic of 92% and a correlation coefficient of 0.97.

Reproducibility of QuantiFERON-TB Between Testing Sites

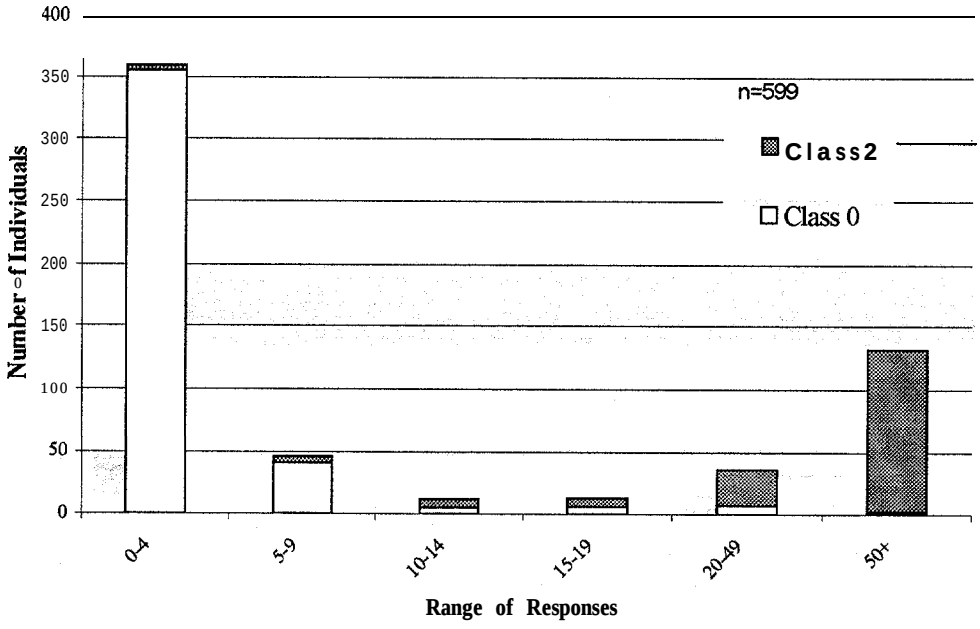
Reproducibility between sites was evaluated in a blind study using two test sites and 50 replicate blood samples (n= 2 x 50). The reproducibility (agreement) of the test between sites was found to be greater than 98% with a kappa chance adjusted agreement statistic of 90% and an ICC of 0.948.

Reproducibility of an Individual's QuantiFERON-TB Test Results Over Time

Thirty-six (36) individuals were tested over a period of 12 weeks. Variation in the % Human PPD within an individual was studied and found to-have' an ICC of 0.84. This level of reproducibility is high, however variation in an individual's response could, in some circumstances, lead to an individual having a different test interpretation over time. The

possibility of discordant interpretations is limited to those individuals whose % Human PPD Response is close to the 15% Human PPD Response cut-off.

Figure 7.2. % Human PPD Responses for Class 0 (Low risk) and Class 2(LTBI; ATS classification) Individuals in Streeton et al (1998).



Storage and Shipping, Time and Temperature Studies

Shelf life

Stability studies (2 - 8°C) carried out revealed no significant loss in performance of the QuantiFERON-TB test kit for up to 30 months (2.5 years) post manufacture. The results support a. shelf life of two years when stored at 2 - 8°C.

Shipping

Shipping studies have shown that the QuantiFERON-TB package can withstand shipping and handling conditions, when stored at 2 - 8°C, from Australia to the US and return under airfreight conditions. Results support airfreight of the product to the US at temperature of 2 - 8°C.

Animal Studies

Bovine TB as an animal model of human TB

In cattle, the bovine TST, using bovine tuberculin PPD has been used extensively for the diagnosis of bovine tuberculosis. This test is essentially the same as the TST in humans and was the forerunner to the human test. Bovine tuberculosis has many parallels with the human disease and a strong argument can be made that it provides the best animal model available. The spectrum of immune response of cattle to *M.* bovis infection and disease is remarkably similar to that for *M.* tuberculosis-in humans. Both species mount a strong CMI and IFN-γ response following exposure and generally control their infection as long as this response is maintained. As for humans, antibody responses are usually only detected in cattle which have progressed to active disease and both bovine TST and IFN-γ responses can be absent in diseased cattle (Wood and Rothel, 1997).

The bovine IFN-γ test (BOVIGAM™), the bovine version of the QuantiFERON-TB test, was developed during the late 1980's and extensive field trials of the test, involving over 100,000 cattle, have now been conducted in many countries throughout the world. Most experimental parameters relating to the performance of the bovine IFN-γ test correlate with the performance characteristics of the QuantiFERON-TB test. Summary of findings 'from the bovine animal model of QuantiFERON-TB

The bovine IFN-γ EIA was able to detect as little as 80 pg/mL of recombinant bovine IFN-γ and exhibited improved detection limits for bovine IFN-γ when compared to the traditional bioassay technique. It could be used in conjunction with a simple whole blood culture technique to discriminate specific CMI reactivity in cattle experimentally infected with different mycobacteria including *M. bovis.* The level of IFN-γ detected in plasma from blood stimulated *in vitro* with nil antigen (PBS control), avian PPD or bovine PPD correlated with the species of mycobacteria used to infect the cattle. Blood'from *M. bovis* infected cattle generated greater levels of IFN-γ in response to bovine PPD than to the other antigens. Conversely, avian PPD generated the greatest amounts of IFN-γ in blood from *M. avium* or *M. kansasii* infected cattle.

Extensive field studies in Australia and elsewhere involving more than 100,000 cattle have shown the sensitivity of IFN-γ assay for the detection of bovine tuberculosis to be approximately 90% which is independent of the source of tuberculin PPDs used to stimulate the blood samples. Most studies reported comparable sensitivities between the IFN-γ assay and the skin test, althoughin Australia and Northern Ireland, the IFN-γ assay was the significantly more sensitive test,

The suitability of the bovine IFN-γ assay for tuberculosis in cattle is further supported by its accreditation by the Standing Committee on Agriculture (Australia) as an Australian Standard Diagnostic Technique for bovine tuberculosis in Australia (Rothel et al, 1993), and by its approval by the Chief Veterinary Officer of New Zealand as an official test for the diagnosis of tuberculosis in cattle testing positive to the caudal fold skin test (O'Neil, 1997). The BOVIGAM assay has recently been approved by the US Department of Agriculture for use in the USA.

CLINICAL STUDIES

CDC Study: Evaluation of the QuantiFERON-TB Test for the Detection of Mycobacterium tuberculosis Infection.

Aim: The primary objective of this clinical trial was to establish the clinical performance of the QuantiFERON-TB when compared to the TST in their ability to detect *M. tuberculosis* infection. Towards this aim, TST and the QuantiFERON-TB test were to be simultaneously performed on 1500 individuals in four groups categorized by their risk of *M. tuberculosis* infection.

The study objectives for CSL Limited (and now Cellestis Limited) were to describe the performance concordance (kappa coefficient) of QuantiFERON-TB and TST among individuals at low to high risk of LTBI, and to determine QuantiFERON-TB "apparent" specificity in individuals with low risk of TB exposure. A further objective was to provide an estimate of the sensitivity of QuantiFERON-TB for detecting individuals with culture confirmed TB disease (Group 3 below).

Study Design: Adults being screened for *M. tuberculosis* infection with TST by staff of the participating institutions were candidates for this study. These individuals were categorized into 4 groups based on their risk of *M. tuberculosis* infection:

- **Group 1: Low Risk** (300): Those who were not believed to be infected or at risk of infection with *M. tuberculosis* because they are from areas or populations of low risk.
- **Group 2: Intermediate Risk** (850): Contacts of patients with tuberculosis, individuals from countries where tuberculosis is prevalent, shelter residents, intravenous drug users, and other individuals determined to be at increased risk by prior local investigations.
- **Group 3: High Risk (150):** Individuals with diagnostic evaluation of pulmonary symptoms compatible with tuberculosis disease.
- **Group 4: Confirmed** (200): Individuals who have completed an effective course of therapy for culture confirmed tuberculosis disease.

After obtaining informed consent, study volunteers completed a questionnaire related to their possible risk factors for TB exposure and were allocated to the appropriate study Group. A blood sample was collected for the QuantiFERON-TB test, prior to the placement of a TST. 48 to 72 hours later, TST responses were measured. The QuantiFERON-TB test was performed as per the manufacturer's instructions.

Individuals in Group 1 and 2 (combined) are representative of the Intended Population for QuantiFERON-TB. That is, those with a low to high risk of having latent TB infection.

Results and Outcomes:

A total of 1,471 persons were enrolled, and information conforming to the study protocol was available for 1,226. For 133 subjects the TST was not placed, read, or recorded as specified. For 97 subjects the QuantiFERON-TB was not performed or recorded as specified. For two subjects, complete data for both the TST and QuantiFERON-TB were unavailable. Other critical information, including results of mycobacterial culture, were missing for 11 subjects. Data from two subjects with an indeterminate QuantiFERON-TB result were not included in the analysis. Subjects included in the analysis ranged in age from 18 to 87 years (mean age = 39 years). Half of the subjects were female, 38 % were white, 35 % were black, 13 % were Hispanic, 12 % were Asian, and 1 % were other races. Eighty-seven subjects had culture-confirmed tuberculosis and had completed treatment within the prior two years (Group 4). Ninety-four subjects were suspects who were being evaluated for active tuberculosis and who had received anti-tuberculosis therapy for less than 6 weeks (Group 3). There were 1042 individuals in the Intended Population; 944 subjects who related situations that put them at risk of infection with *M. tuberculosis* (Group 2), and 98 subjects who related no risks for *M. tuberculosis* infection (Group 1).

TST was interpreted as positive for 390 subjects based on induration and perceived risk as per the ATS/CDC guidelines for interpretation (ATS, 1990). Significant digit preference (rounding off TST measurements to the nearest multiple of 5mm) was observed in reporting a TST response of 10 mm at three study sites (sites A, B, and C), and for reporting a TST response of 15mm at three sites (sites B, C, and D).

For those being screened for LTBI (Groups 1 and 2), agreement of QuantiFERON-TB with TST was 84.8 % (kappa = 0.55) (Table 7.2). Within this group, agreement was 88.1 % for subjects with no history of BCG vaccination, and 70.1% for those who had received BCG. An examination of TST and QuantiFERON-TB results for these persons revealed that BCG vaccination was associated with a disproportionate number of TST positive / QuantiFERON-TB negative results,

Table 7.2. QuantiFERON-TB vs TST results for the Intended Population

		QuantiFERON-TB		TOTAL
		Positive	Negative	
Positive TST	Count	147	80	227
	% of Total	14.1%	7.7%	21.8%
Negative TST	Count	78	737	815
	% of Total	7.5%	70.7%	78.2%
TOTAL	Count	225	817	1042
	% of Total	21.6%	78.4%	100.0%

Kappa = 0.554, McNemar's p = 0.937

Logistic regression analysis was performed to identify other factors associated with TST and QuantiFERON-TB discordance within the Intended Population. The only factor significantly associated with a positive TST but negative QuantiFERON-TB was history of BCG vaccination. Those with prior BCG vaccination were more than six times as likely to be TST

positive only, than those unvaccinated. Males were almost twice as likely to have a negative TST but positive QuantiFERON-TB response than females (Mazurek et al 2001).

Reactivity to mycobacteria other *than M. tuberculosis* (MOTT) can cause false-positive TST reactions. To aid discriminating these individuals from those with TB infection, the QuantiFERON-TB test includes PPD produced from *M. avium.* Of the 80 individuals with TST positive, QuantiFERON-TB negative responses, 13 had a % Human PPD Response greater than 15%, but were classified as negative in the QuantiFERON-TB test due to their % Avian Difference being less than -10% (i.e. the QuantiFERON-TB test detected them as having MOTT reactivity).

In the CDC study, QuantiFERON-TB was found to be equivalent to TST in its ability to detect latent *M. tuberculosis* infection.

Evidence was found for the TST, but not QuantiFERON-TB, being affected by prior BCG vaccination and/or reactivity to MOTT.

For individuals in Group 3 (TB disease suspects) who were positive for culture of *M. tuberculosis,* 44 or 54 (8 1.5%) were positive by QuantiFERON-TB.

Results from this study have been accepted for publication: Mazurek GH, LoBue PA, Daley CL, Bernardo J, Lardizabal AA, Bishai WR, Iademarco MF, Rothel JS. Comparison of an Interferon-gamma Assay with Tuberculin Skin Testing for Detecting Latent *Mycobacterium tuberculosis* Infections. JAMA. 200 1.

WRAIR Study: A comparison of QuantiFERON-TB interferon-gamma test with the TST for detection of M. tuberculosis infection in military recruits.

Aims: The primary objective was to provide data on the performance of the QuantiFERON-TB test in low risk individuals (akin to Group 1 in the CDC study). A second objective was to compare the concordance between results obtained from the QuantiFERON-TB test to those obtained from the TST.

Study Design: The trial was conducted during June 1999. Subjects were male and female Naval recruits in-processing for basic training during the study period. No recruits were excluded. After granting informed consent, recruits completed a questionnaire related to possible risk factors for TB exposure. The next day, blood samples were collected (for the QuantiFERON-TB test) prior to the placement of a TST. Forty-eight to 72 hours later TST responses were measured. Of the 1,961 recruits who gave informed consent to the study, valid results for both QuantiFERON-TB and TST were available for 1,695. Individuals were stratified into groups according to their identified risk factors for TB exposure. Individuals with known risk factors (akin to Group 2 in the CDC study) were further stratified into those with a Primary or Secondary risk (Table 7.3).

Table 7.3. Number of individuals in each of the three stratified Groups of the WRAIR study

Group	Risk Factors for TB Exposure	Number of subjects
Low Risk	None identified	397
Primary Risk	Born or lived in country with TB rate >10/100,000 or, reported as a contact of a TB case	232
Secondary Risk	Born or recruited from a US State with a TB rate >10/100,000	1066
	TOTAL	**1695**

Results and Outcomes: Analysis of the total data set demonstrates a kappa coefficient of 0.179, which represents low agreement between QuantiFERON-TB and TST. For the 1695 individuals, 1561 (92.1%) had concordant results (Table 7.4). For the remaining 134 individuals 29 had a positive TST, negative QuantiFERON-TB response profile and 105 had the inverse profile. These results indicate that the QuantiFERON-TB test identified more individuals as being positive for latent TB infection than did the TST.

Table 7.4'. Comparison of QuantiFERON-TB and TST results for the I695 individuals with valid results

		QuantiFERON-TB		TOTAL
		Positive	Negative	
Positive TST	Count	18	29	47
	% of Total	1.1 %	1.7 %	2.8%
Negative TST	Count	105	1543	1648
	% of Total	6.2%	91.0%	97.2%
TOTAL	Count	123	1572	1695
	% of Total	7.3%	92.7%	100.0%

Kappa = 0.179, McNemar's $p < 0.000$

Stratification of the study population into Low, Primary and Secondary risk groups for *M. tuberculosis* exposure demonstrated low to moderate agreement between the QuantiFERON-TB test and the TST by the kappa statistic. For the 397 individuals with no identified risk factors, 379 (95.5%) had concordant results although the kappa statistic, largely due to the small number of individuals (2) with both QuantiFERON-TB and TST positive results, was 0.168 indicating low agreement (Table 7.5). It should be noted that kappa has little value in such low prevalence situations.

Table 7.5. Comparison of QuantiFERON-TB and TST results for individuals with no identified TB risks

		QuantiFERON-TB		TOTAL
		Positive	Negative	
Positive TST	Count	2	2	4
	% of Total	0.5 %	0.5 %	1.0%
Negative TST	Count	16	377	393
	% of Total	4.0%	95.0%	99.0%
TOTAL	Count	18	379	397
	% of Total	4.5%	95.5%	100.0%

Kappa = 0.168, McNemar's p = 0.002

For the 232 individuals with a Primary risk of having latent TB infection (contacts, foreign-born etc.) 194 (83.6%) had concordant results with 11 individuals being positive to both tests, 14 individuals being TST positive and QuantiFERON-TB negative and 24 having the inverse response profile. The kappa statistic for these Primary risk data was 0.276, indicating low to moderate agreement between the two tests.

For the 1066 Secondary risk individuals (born in or recruited from a US State with a TB rate ≥ 10/100,000), 988 (92.7%) had concordant results, However, there were 5-fold more individuals (65) demonstrating a QuantiFERON-TB positive, TST negative response profile than the inverse. The Kappa coefficient for this group of individuals (0.089) indicated poor agreement between the two tests, although once again, the number of individuals with positive results to both tests was low and thus kappa has little value.

The WRAIR study evaluated QuantiFERON-TB responses for 397 individuals with no identified risk factors. It is important to note that, although these individuals had no identified risk factors, there remains a possibility that some could have latent TB infection.

In the population studied, the specificity of the QuantiFERON-TB test was estimated to be 95.5% (95% confidence intervali 92.9% to 97.1%).

Overall concordance between TST and QuantiFERON-TB was 92.1% in navy recruit volunteers.

Conclusions from the Preclinical and Clinical Studies

Animal model studies

Bovine tuberculosis has many parallels with the human disease and a strong argument can be made that it provides the best animal model available. The spectrum of immune response of cattle to *M. bovis* infection and disease is remarkably similar to that for *M. tuberculosis in* humans. Both species mount a strong CMI and IFN-γ response following exposure and generally control their infection as long as this response is maintained. As for humans, antibody responses are usually only detected in cattle that have progressed to active disease and both TST and IFN-γ responses can be absent in diseased cattle. The recorded sensitivity of the tuberculin skin test in tuberculous cattle parallels reports on that for the TST in humans.

A major benefit of the bovine animal model is that culture of TB bacterium,, from culled animals can be used as a gold standard for TB infection. Using this definitive gold standard, the bovine IFN-γ assay has been consistently shown to be more sensitive than the bovine TST for the detection of TB infection.

Extensive clinical trials of the bovine IFN-γ assay have been completed in many countries worldwide and it is approved as an official test for bovine tuberculosis in Australia, New Zealand, Romania, and in the USA. Well over 100,000 cattle have now been tested with the sensitivity of the assay ranging from 77 to 93.6% as compared with 65.5 to 84.4% for the bovine TST. Cattle, which are only positive to the TST, are highly unlikely to be culture positive for *M. bovis* (2/53; 3.8%; Wood et al, 1991), whereas animals which are only positive to the IFN-γ test have a high probability of being culture positive (37/67; 55.2%). These results have been confirmed in a number of studies conducted worldwide.

Data from the bovine model of TB, clearly demonstrates that the QuantiFERON-TB methodology is superior to the TST for the diagnosis of TB infection in cattle. It is likely that this finding is similar to the human TB situation, given the close relationship between the pathology and immune responses of cattle and man to TB infection.

Clinical studies of QuantiFERON-TB

The protocol for the CDC multi-center clinical trial recognized the limitations of the TST and chose to assign subjects to TB risk groups based on their known exposure risks. Comparison of QuantiFERON-TB with TST results was the primary method of analysis. Data from over 1,000 individuals representing the Intended Population for QuantiFERON-TB in this study demonstrated good agreement between the two tests (kappa = 0.554). Logistic regression analysis of the data from individuals giving discordant results and taking into account known confounders of the TST suggested that the QuantiFERON-TB test is less affected by prior BCG vaccination than the TST and also suggested that reactivity to non-tuberculous mycobacteria is a cause of false-positive TST reactions. Data was available from only 98 individuals (90 [91.8%] were QuantiFERON-TB negative) with no identified risk factors for TB, and therefore this estimate of the specificity of QuantiFERON-TB was not strong.

The overall conclusion from the CDC multi-center clinical trial was that QuantiFERON-TB was equivalent to TST in its ability to detect latent *M. tuberculosis* infection.

Physicians at the Walter Reed Army Institute of Research, initiated a study in Navy recruits at the Great Lakes Naval Station, Illinois, in 1999. Over 1,500 recruits were tested with both QuantiFERON-TB and "me TST. Subjects were stratified into risk-groups for TB exposure and again, results for the two tests were compared. In this study, overall concordance was 92.1%. The QuantiFERON test detected more people as positive for LTBI than the TST. Individuals with no identified risk factors for *M. tuberculosis* exposure were used to estimate the specificity of QuantiFERON-TB. Of the 397 subjects in this category, 95.5% (95% confidence interval: 92.9% to 97.1%) were negative for TB infection in the QuantiFERON-TB test.

The QuantiFERON-TB test has many logistic advantages over the TST. A patient needs to be seen only once for the QuantiFERON-TB test whereas for the TST the patient needs to return 48 to 72 hours later to have their adverse reaction measured. In some situations, as many as 65% of individuals fail to return to have their TST read (Chaisson et al, 1996). Whatever the merits or accuracy of the TST itself, the failure to obtain a result for the test, in such a large proportion of individuals, has considerable public health implications. A test for LTBI, which has equivalent performance to the TST and does not require subjects to return to have the test read, has obvious public health benefits and can only lead to more truly infected individuals being treated than is currently the case.

QuantiFERON introduces a control for atypical mycobacterial sensitization to the diagnosis of tuberculosis infection. This is highly significant, as atypical mycobacteria cause widespread sensitization in the US and such individuals can cross-react with the TST. The effect of reactivity to atypical mycobacteria on false-positive TST responses can only be compounded by the reduction in TB incidence over the last 30 years. As TB infection rates reduce, the proportion of false positive TST responses (as compared with true responses) due to atypical mycobacterial reactivity can only increase.

The QuantiFERON-TB assay eliminates the subjectivity of placing, reading and interpreting the TST, and can be completed in less than 24 hours.

Whole blood IFN-γ testing for TB has proven far superior to skin testing in a robust animal model. Overall, the QuantiFERON-TB test has been demonstrated to have utility for the detection of both LTBI and active TB disease: Clinical studies have shown QuantiFERON-TB is equivalent to TST in the detection of LTBI.

Proposed Restrictions or Training Requirements

The kit should be used in a laboratory environment and the user should be trained in serology and the use of EIA. They should be familiar with the Package Insert and have sound general knowledge of the disease, serology and performing enzyme immunoassays.