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## Appendix 2- Clinicians Guide to QuantiFERON®-TB

### 1) What is QuantiFERON®-TB?

QuantiFERON®-TB is an *in vitro* diagnostic test combining a lymphocyte culture stage (whole blood) and an Enzyme Immunoassay. It is based on the stimulation of whole blood with tuberculin and control antigens and the subsequent detection of IFN- $\gamma$  secreted by human lymphocytes previously sensitized by exposure to *Mycobacterium tuberculosis*. The QuantiFERON®-TB test is intended for use only with blood specimens.

### 2) What is the scientific basis of the QuantiFERON®-TB test?

The QuantiFERON®-TB assay parallels the tuberculin skin test (TST) in that it relies upon the stimulation of T-cells previously sensitized by infection with mycobacteria. Unlike the skin test, where assessment is based on the detection of inflammation and swelling, QuantiFERON®-TB is a relatively non-invasive laboratory test performed in two stages that tests the reactivity of lymphocytes by assessing the production of IFN- $\gamma$ . First, small aliquots of heparinized whole blood are incubated with human (from *M. tuberculosis*) and avian (from *M. avium*) PPDs, and Mitogen and Nil control antigens. After overnight incubation, plasma is removed and the amount of IFN- $\gamma$  quantified by enzyme immunoassay (EIA).

A positive QuantiFERON®-TB test indicates the presence of lymphocytes that react to PPD, which is one of the characteristics of active or latent infection with *M. tuberculosis* complex.

### 3) What is its intended use?

The QuantiFERON®-TB test is intended as an aid in the diagnosis of tuberculosis infection. It must be interpreted with all other clinical and historical patient data to determine the risk of TB infection.

### 4) What are the merits of QuantiFERON®-TB?

A major benefit of using QuantiFERON®-TB is that only one patient visit is required. Unlike the tuberculin skin test (TST, or Mantoux), which requires that patients return at 48 to 72 hr for a reading, the QuantiFERON®-TB requires one patient contact for the purpose of drawing the blood. Therefore the TB risk of the individual and the population can be determined with just one patient contact.

QuantiFERON®-TB is a controlled laboratory assay, not affected by subjective interpretation as with the TST. It incorporates a measure for assessing sensitivity stimulated by Mycobacteria other than tuberculosis (MOTT) and interpretation is not complicated by the "booster phenomenon" that is associated with repeat TSTs.

### 5) What controls are incorporated into the QuantiFERON®-TB test?

A positive control mitogen preparation is used to non-specifically stimulate an IFN- $\gamma$  response. This control acts to validate interpretation of negative PPD responses by demonstrating that assay conditions permitted the detection of production of IFN- $\gamma$  in response to a non specific stimulus; a mitogen. Only patients capable of generating detectable amounts of IFN- $\gamma$  are sufficiently immunocompetent for analysis using this test.

The avian PPD is another control; it aids in discrimination between individuals with cell-mediated immune responses to atypical mycobacteria (i.e. *Mycobacterium avium* complex; MAC) and those infected with MTB. The relative levels of IFN- $\gamma$  detected in the four plasma samples indicate whether an individual is infected with *M. tuberculosis* complex organisms.

## 6) How are the results reported, and why?

IFN- $\gamma$  responses are determined for the Nil, Mitogen, Human PPD and *M. avium* PPD stimulated blood samples. Under most circumstances the Nil Control will not generate any IFN- $\gamma$ . If IFN- $\gamma$  is detected, it is considered background and is subtracted from the other results for that subject

The IFN- $\gamma$  results are used in the calculations below to determine the **%Human Response** (the response to tuberculin) and the **% Avian Difference** (the response to *M. avium* PPD). The %Human Response and %Avian Difference calculations are used to standardize the variability observed between individual PPD and mitogen responses. Responses above specific threshold values indicate significant reactivity, likely to have been elicited from mycobacterial infection.

### Thresholds for a valid test

- The Mitogen Control generally elicits the greatest IFN- $\gamma$  response for any given individual. 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. In these rare cases the test may be repeated to confirm results and/or relevant clinical information sought to determine any possible cause of immunosuppression.
- An individual response to the Human PPD and Nil must be such that the (Human PPD - Nil) response is 1.5 IU/mL for that individual to be deemed as likely to be infected with *M. tuberculosis*, irrespective of %Human Response.

The IFN- $\gamma$  values obtained are used to calculate % Human Response and % Avian Difference values for each patient as indicated below:

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

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

Where: N=IFN- $\gamma$  (IU/mL) for Nil Control well

H=IFN- $\gamma$  (IU/mL) for Human PPD well

A=IFN- $\gamma$  (IU/mL) for Avian PPD well

M=IFN- $\gamma$  (IU/mL) for Mitogen Control well

## 7) How are the results interpreted?

Two different cut-offs are used for the %Human Response value and are based on the individual's risk factors for *M. tuberculosis* exposure.

**People with identified TB exposure risks:** A lower QuantiFERON<sup>®</sup>-TB test cut-off is used for individuals with identified risk factor(s) for tuberculosis infection (see American Thoracic Society and CDC Guidelines, 2000; refer Table 1). **In subjects at risk of tuberculosis infection a 15% Human Response is used as the diagnostic cut-off indicative of tuberculosis infection.**

**Low-Risk groups:** Although testing in individuals at very low risk of tuberculosis is not advised as a general procedure, it is recognized that in certain employment screening and other situations this may occur. **In subjects with no known risk factors for tuberculosis infection a 30% Human Response is used as the diagnostic cut-off indicative of tuberculosis infection.**

INTERPRETATION	PATIENT STATUS	M - N <sup>1</sup> (IU/mL)	H - N <sup>2</sup> (IU/mL)	% Human Response <sup>3</sup>		% Avian <sup>4</sup> Difference
POSITIVE	MTB infection indicated	1.5	1.5	15% TB Risk	≥ 30% No Risk	≤ 10%
NEGATIVE	MTB infection NOT indicated	1.5	All other response profiles			

1. **M-N must be 1.5 IU/mL** for a valid QuantiFERON-TB result. *Results are deemed **INDETERMINATE** if  $M - N < 1.5$  IU/mL.*
2. **H-N must be 1.5 IU/mL** for a patient to be considered POSITIVE for MTB infection. *If  $H - N < 1.5$  IU/mL the individual is deemed negative for MTB infection regardless of their % Human Response and % Avian Difference results.*
3. A 15% Human Response cut-off is used for individuals with identified TB exposure risk and a 30% cut-off for people with no identified risk factors.
4. A control for sensitization by Mycobacteria other than tuberculosis (MOTT) is also included in QuantiFERON-TB. MOTT is recognised as a confounding factor for the TST and a cause of false-positive reactions (Heubner et al, 1993). If the response to *M. avium* PPD is more than 10% above that to tuberculin (**>10% Avian Difference**) then the subject is more likely to be MOTT sensitised than TB infected. Infection with non-tuberculous mycobacteria such as MAC cannot be discounted.

**TABLE 1. Guidelines for determining a positive QuantiFERON<sup>®</sup>-TB response**

≥15% Human Response	≥30% Human Response
HIV-positive persons*	Persons with no risk factors for TB
Recent contacts of TB case	
Injection drug users	
Fibrotic changes on chest radiograph consistent with old TB	
Patients with organ transplants and other immunosuppressed patients* (receiving the equivalent of > 15 mg/d Prednisone for > 1 mo)	
Recent arrivals (< 5 yr) from high-prevalence countries	
Residents and employees** of high-risk congregate settings: Prisons and jails, nursing homes and other health care facilities, residential facilities for AIDS patients, and homeless shelters	
Mycobacteriology laboratory personnel	
Persons with clinical conditions that make them high-risk:*	
Silicosis, diabetes mellitus, chronic renal failure, some hematologic disorders (e.g., leukemias and lymphomas), other specific malignancies (e.g., carcinoma of the head or neck and lung), weight loss of > 10% of ideal body weight, gastrectomy, jejunioileal bypass	
Children < 4 yr of age or infants, children, and adolescents exposed to adults in high-risk categories*	

\* For people in these population groups the QuantiFERON<sup>®</sup>-TB test has not been evaluated and is not recommended

\*\* For persons who are otherwise at low risk and are tested at entry into employment, a %Human Response ≥ 30% is considered positive.

## 8) In whom can QuantiFERON<sup>®</sup>-TB be used?

QuantiFERON<sup>®</sup>-TB can be used in individuals being tested for TB infection, with the limitations outlined below.

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

The performance of the QuantiFERON®-TB test has not been evaluated in the following populations and is not recommended for use in these population groups:

1. Individuals who are immunocompromised such as those with HIV infection, AIDS, and transplant recipients.
2. Persons with other clinical conditions that may compromise the immune system: diabetes, silicosis, chronic renal failure, hematologic disorders (e.g., leukemias and lymphomas), and other specific malignancies (e.g., carcinoma of the head or neck and lung).
3. Individuals who are immunosuppressed such as those taking immunosuppressive drugs (eg. corticosteroids, methotrexate, Immuran, chemotherapy).
4. Individuals under the age of 17 years.
5. Pregnant women.

However, the clinical risk to the subject of unrecognized tuberculosis should be assessed. The prescribing physician should consider if the potential benefits of using the diagnostic test outweigh the possible risks associated with a false positive or false negative result, particularly in certain high-risk populations. (e.g. in pregnant women the close post-partum contact between a mother with active disease and an infant leaves the infant in grave danger of tuberculosis and complications such as tuberculous meningitis).

Care should be taken when interpreting QuantiFERON®-TB results in individuals who have received a tuberculin skin test (TST or Mantoux) in the previous 12 months due to possible “booster effects”. As always, a positive result should be interpreted in light of all available historical and clinical information before therapy is administered.

## 9) Example results and interpretation.

The Diagnostic standards and Classification of Tuberculosis define the 5 risk categories for TB. The following test results would be expected in a typical case.

	Mitogen –Nil (IU/mL)	Human PPD – Nil (IU/mL)	%Human Response	% Avian Difference	QuantiFERON-TB Result
ATS class 0	> 1.5	0 to 20	< 30%	-300 to +300%	Negative
ATS class 1	> 1.5	0 to 20	< 15%	-300 to +300%	Negative
ATS class 2	> 1.5	> 1.5	> 15%	< 10%	TB infection likely
ATS class 3	> 1.5	> 1.5	> 15%	< 10%	TB infection likely
ATS class 4	> 1.5	> 1.5	> 15%	< 10%	TB infection likely
MOTT sensitized	>1.5	> 1.5	> 15%	> 10%	Negative (MOTT)
Immunocompromised	<1.5	NA	NA	NA	Indeterminate

NA: Not Applicable

## 10) Results of Clinical trials

### a) Individuals with no identified risk factors for TB exposure:

QuantiFERON®-TB (30% Human Response cut-off), Tuberculin Skin Test (15mm cut-off)

		QuantiFERON®-TB	
		+	-
TST	+	0 (0%)	2 (2%)
	-	2 (2%)	94 (94%)

Study conducted by the US CDC (*Mazurek et al 2001*)

		QuantiFERON®-TB	
		+	-
	+	4 (0.3%)	18 (1.2%)
	-	28 (1.9%)	1413 (96.6%)

Study conducted by Walter Reed Army Institute of Research

Overall results show a 98% specificity in this group. Agreement with the skin test for positive results was not high, reflecting the fact that true tuberculosis infection is unlikely in this group.

**b) Individuals with defined risk factors for LTBI:**

QuantiFERON-TB (15% Human Response cut-off), Tuberculin Skin Test (10mm cut-off)

		QuantiFERON®-TB	
		+	-
TST	+	145 (15.4%)	83 (8.8%)
	-	72 (7.6%)	644 (68.2%)

Study conducted by US CDC (*Mazurek et al 2001*)

Mazurek et al (2001) analyzed the results for individuals with discordant results (positive in only one of the test systems). People with a history of BCG vaccination were more likely to be TST positive and QuantiFERON® negative, as were those over 60 years old. Males were more likely to be QuantiFERON®-TB positive and skin test negative. Thirteen of the 83 TST positive/QuantiFERON®-TB negative subjects were classified as QuantiFERON®-TB negative due to predominant reactivity to *M. avium* PPD.

**c) Sensitivity in active culture confirmed tuberculosis**

QuantiFERON®-TB at 15% Human Response cut-off detected 44/54 (81.5%) of active culture confirmed cases of tuberculosis (*Mazurek et al, 2001*).

**References**

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