

# QUANTIFERON –TB PERFORMANCE CHARACTERISTICS (AS PER FDA PANEL SUBMISSION)

## I. ANALYTICAL SENSITIVITY

### *Background*

The QuantiFERON-TB test measures the amount of IFN- $\gamma$  generated by whole blood incubation after stimulation with antigens. A Mitogen positive control antigen is incorporated in the test to identify individuals who are capable of generating detectable levels of IFN- $\gamma$ . Therefore it was important to determine the minimum detectable amount (analytical sensitivity) of IFN- $\gamma$  measured in the QuantiFERON-TB EIA.

Desem and Jones (1998) investigated the linear range of the QuantiFERON-TB test by testing various concentrations of recombinant human IFN- $\gamma$  (0 - 300 IU/mL) on two occasions by two operators using two batches of reagents. They reported the linear range of the test as between 0.5 IU/mL (approximately 20 pg/mL) and 150 IU/mL (approximately 5 ng/mL). As a result of these findings, the original QuantiFERON-TB kit Package Insert released in Australia stated that if an individual response to Mitogen-Nil was less than 0.5 IU/mL the assay results for that individual were invalid.

### *Objective*

To estimate the upper limit of “Zero”, based on linear regression against optical density, using repeated dilutions of several samples.

### *Method*

To confirm the results described above, the analytical sensitivity of the QuantiFERON-TB EIA was determined by titration of a standard preparation of recombinant human IFN- $\gamma$  in the EIA. Data from five batch lots of plates that were used to manufacture QuantiFERON-TB kits were compared. The comparison was made using 36 replicates per lot, tested at concentrations of 75, 37.5, 18.75, 9.38, 4.69 and 2.34 units, and a true zero cell. Optical densities at each concentration were read.

Replicates were plotted using concentration against optical density. Because these plots do not show clearly the differences between results at the lower concentrations, the logarithm of optical density was plotted against dilution number. The zero concentration was plotted using “dilution = 8” even though this was not a true dilution but a true zero concentration.

### *Results*

Figure 5.4 below is a simple plot of Optical Density (OD) against concentration (units PPD), for each Lot number.

Figure 5.4 Optical Density (OD) against concentration (units PPD), for different Lots

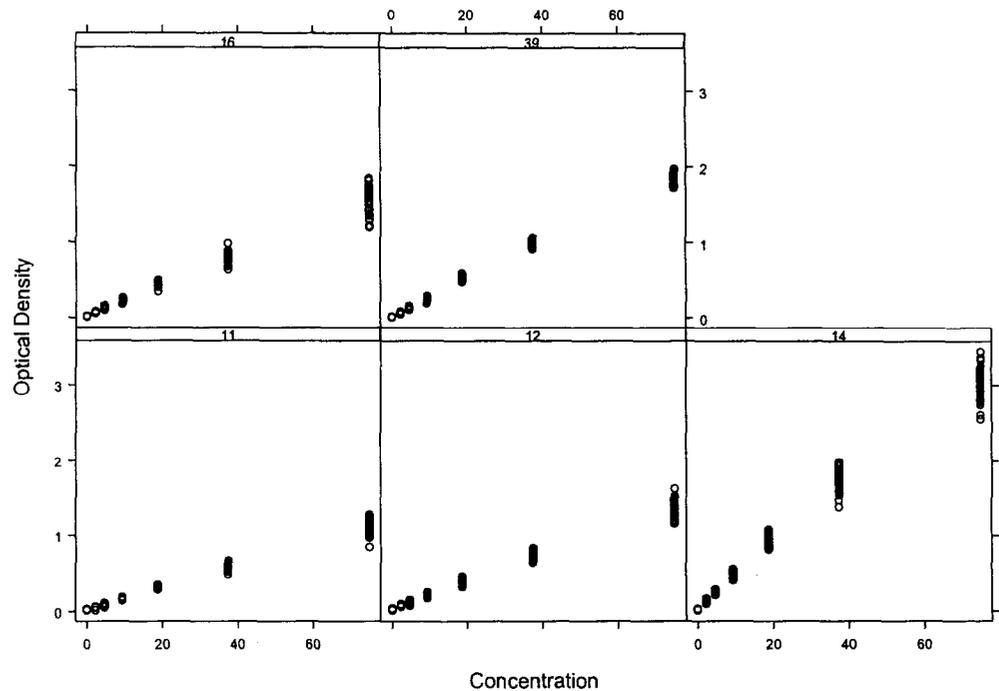


Figure 5.5 Log [Optical Density (OD)] against dilution for different Lots

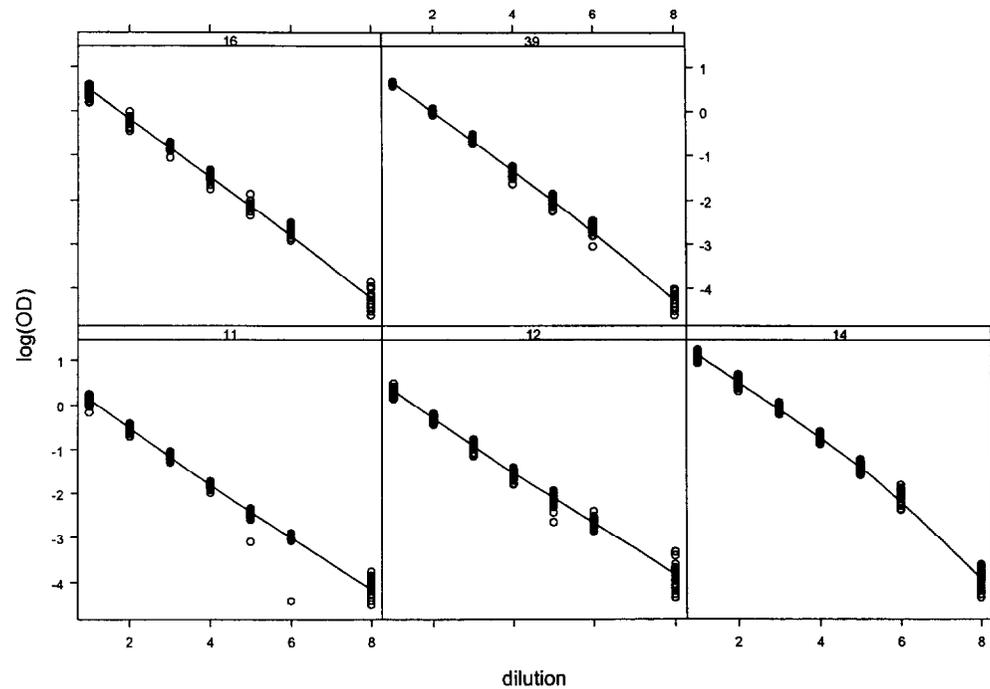


Figure 5.5 shows that the “zero” dilution (i.e. dilution = 8) unambiguously identifies OD values. The separation between OD from diluted samples and from the zero cells is clear, although one replicate, considered as an outlier, in Batch 11 did show an unusually low OD value, within the same range as the ODs from the zero cells.

### Conclusion

A log(OD) roughly between  $-4$  and  $-3$  can be expected to differentiate between the lowest real dilution (concentration = 2.34) and the zero concentration. Concentrations 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 do not show a detectable response and are deemed as having an indeterminate result for the QuantiFERON-TB test. Similarly, 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 to have responded detectably to human PPD. A % Human PPD Response can only be calculated for individuals giving Mitogen – *Nil* and /Human PPD – *Nil* values greater than or equal to 1.5 IU/mL. Individuals with Mitogen – *Nil* values greater than or equal to 1.5IU/mL, but Human PPD – *Nil* values less than 1.5IU/mL are deemed not infected with *M. tuberculosis*.

## LINEAR RANGE

### *Background*

The QuantiFERON-TB test relies on calculating the concentration of IFN- $\gamma$  in plasma samples from the standard curve generated on each QuantiFERON-TB EIA plate. For this calculation to be accurate, the standard curve should be linear. The correlation coefficient of the standard curve is representative of the linearity of that curve. The acceptance criteria for a valid QuantiFERON-TB EIA, as detailed in the kit Package Insert, includes a criterion that the correlation coefficient for the standard curve must be greater than 0.98. Studies were performed to confirm the linearity of the standard curve calculated from the standards provided with the QuantiFERON-TB kit.

Desem and Jones (1998) investigated the linear range of the QuantiFERON-TB test by testing various concentrations of recombinant human IFN- $\gamma$  (0 - 300 IU/mL) on two occasions by two operators using two batches of reagents. They reported the linear range of the test as between 0.5 IU/mL (approximately 20 pg/mL) and 150 IU/mL (approximately 5 ng/mL). The linearity of the human IFN- $\gamma$  EIA was investigated by examining the correlation coefficient of the standard curves generated from 167 tests performed over a 14-month period. All correlation coefficients were greater than 0.980 with a mean of 0.996 and a coefficient of variation of 0.3%.

### *Objective*

Studies were performed to define the linearity of the standard curve calculated from the standards provided with the QuantiFERON-TB kit and thereby define the linear range of the test.

### *Method and Results*

The linearity and reproducibility of the QuantiFERON-TB EIA are routinely confirmed during in-process manufacturing testing to determine the potency of the QuantiFERON-TB kit standards. Results from three of these tests, run on different days with different operators and different QuantiFERON-TB kit lots, are shown in Tables 5.6 to 5.8. Twenty five replicates of each High, Medium, Low and Zero kit standard were tested on each occasion and their respective potencies (IU/mL) were determined against serial dilutions of the NIH, a reference human recombinant IFN- $\gamma$  standard. The range of the NIH standard was 200 IU/mL to 3.125 IU/mL. The dilution series was 200 IU/mL, 100 IU/mL, 50 IU/mL, 25 IU/mL, 12.5 IU/mL, 6.25 IU/mL and 3.125 IU/mL. A 0 IU/mL standard was also included to check for background. All correlation coefficients for the NIH standard curves were greater than 0.980 with a mean of 0.996 and a coefficient of variation of 0.38%.

The mean, standard deviation (SD) and coefficient of variation (% CV) of the 25 replicates of the kit standards were recorded. Correlation coefficients for the standard curves calculated from all individual 75 replicate sets of the QuantiFERON-TB kit standards were greater than 0.99, demonstrating excellent linearity. The % CV for the absorbance values of the High, Medium and Low standards demonstrated excellent reproducibility and were consistently less than 10% (refer to Table 5.6 to 5.8). The mean for the replicate absorbances for the Zero

standards was consistently within 0.040 units of their respective maximum and minimum absorbance values. Tables 5.6 to 5.8 show the % CV's for the potencies (IU/mL) of the High, Medium and Low standards were also less than 10%, again demonstrating good linearity, and inter- and intra-assay reproducibility.

**Mean, Standard Deviation and Coefficient of Variation**

*Table 5.6 QuantiFERON® Kit Standards (Mean OD<sub>450/620nm</sub> and Mean IU/mL) Test 1*

<i>Test 1</i>	<i>High</i>	<i>Medium</i>	<i>Low</i>	<i>Zero</i>	<i>Correlation Coefficient</i>
<b>Mean OD</b>	3.164	1.803	0.359	0.027	0.9998
SD	0.076	0.057	0.009	0.002	0.0003
% CV	2.403	3.188	2.636	7.844	0.0303
Max	3.322	1.952	0.380	0.030	1.0000
Min	3.020	1.712	0.344	0.022	0.9986
<b>Mean IU/mL*</b>	167.378	93.202	16.858	-2.387	
SD	4.143	3.131	0.548	0.121	
% CV	2.475	3.360	3.250	-5.050	
Max	176.013	101.348	18.057	-2.190	
Min	159.554	88.269	15.974	-2.650	

The correlation coefficient was calculated for each of the 25 replicates of the QuantiFERON-TB kit standards. \*IU/mL values were calculated from a curve generated from the NIH IFN-γ standard.

*Table 5.7 QuantiFERON® Kit Standards (Mean OD<sub>450/620nm</sub> and Mean IU/mL) Test 2*

<i>Test 2</i>	<i>High</i>	<i>Medium</i>	<i>Low</i>	<i>Zero</i>	<i>Correlation Coefficient</i>
<b>Mean OD</b>	2.778	1.688	0.416	0.027	0.9983
SD	0.050	0.057	0.016	0.004	0.0005
%CV	1.786	3.370	3.816	15.116	0.0487
Max	2.863	1.781	0.434	0.039	0.9993
Min	2.683	1.572	0.366	0.022	0.9972
<b>Mean IU/mL*</b>	151.797	88.630	13.215	-6.228	
SD	2.875	3.296	0.794	0.204	
% CV	1.894	3.718	6.007	-3.281	
Max	156.710	94.025	14.127	-5.630	
Min	146.282	81.917	10.725	-6.480	

The correlation coefficient was calculated for each of the 25 replicates of the QuantiFERON-TB kit standards. \*IU/mL values were calculated from a curve generated from the NIH IFN-γ standard.

Table 5.8 QuantiFERON® Kit Standards (Mean OD<sub>450/620nm</sub> and Mean IU/mL) Test 3

<i>Test 3</i>	<i>High</i>	<i>Medium</i>	<i>Low</i>	<i>Zero</i>	<i>Correlation Coefficient</i>
<b>Mean OD</b>	2.305	1.216	0.281	0.014	0.9997
SD	0.065	0.041	0.017	0.001	0.0003
%CV	2.836	3.397	6.133	7.645	0.0340
Max	2.443	1.266	0.311	0.015	1.0000
Min	2.191	1.113	0.252	0.012	0.9992
<b>Mean IU/mL*</b>	159.113	82.512	17.379	-0.681	
SD	4.595	2.903	1.165	0.071	
%CV	2.888	3.518	6.701	-10.442	
Max	168.798	86.055	19.431	-0.597	
Min	151.082	75.300	15.440	-0.800	

The correlation coefficient was calculated for each of the 25 replicates of the QuantiFERON-TB kit standards.  
\*IU/ml values were calculated from a curve generated from the NIH IFN-γ standard

### *Conclusion*

The QuantiFERON-TB's linear range for detecting the concentration of IFN-γ is between zero and 200 IU/mL (Correlation coefficient > 0.99, n = 75 x 4).

## **PROZONE OR HIGH-DOSE HOOK EFFECT STUDIES**

### *Background*

A prozone or high dose hook effect is known to occur with some sandwich EIAs when a sample contains excessively high concentrations of the analyte to be detected. The simultaneous saturation of both the capture and detecting antibodies, due to the excessive analyte concentration, prevents the formation of the capture antibody/analyte-detecting antibody complex, causing a decrease in the detection signal.

### *Objective*

To identify the detection range of the test and to determine if there is a high dose hook effect associated with it, a number of IFN-γ spiked plasma samples were examined.

### *Method*

Pooled normal human plasma samples were spiked with various concentrations of recombinant human IFN-γ ranging from 0 to 100,000 IU/mL. Testing of the spiked plasma samples was performed on two occasions by two operators and with two batches of reagents.

## *Results*

The test detected concentrations as high as 100,000 IU/mL of recombinant IFN- $\gamma$  (highest tested), although concentrations greater than 150 IU/mL were not quantifiable without sample dilution (Desem and Jones 1998). Mitogen or PPD responses greater than 100,000 IU/mL in the targeted population have not been encountered. Data from all clinical studies performed to date (approximately 5000 individuals) demonstrated no responses to either PPD or mitogen greater than 550 IU/mL.

## *Conclusion*

No prozone or hook effect has been detected with the *QuantiFERON-TB* EIA for concentrations of IFN- $\gamma$  up to 100,000 IU/mL. Samples with concentrations of IFN- $\gamma$  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 test due to a prozone or high-dose hook effect.

## **RELATIVE SPECIFICITY AND SENSITIVITY**

### *Background*

As there is currently no 'gold standard' test for tuberculosis infection there can be no absolute indication as to an individual's true status of infection. Hence, the absolute specificity and sensitivity of the *QuantiFERON-TB* diagnostic test cannot be determined.

### *Objective*

An estimate of the specificity and sensitivity of the test was made based on 501 individuals, defined as either having or not having evidence of infection based on classification for tuberculosis infection status according to the American Thoracic Society's (ATS) guidelines (American Thoracic Society and Centers for Disease Control. Diagnostic Standards and Classification of Tuberculosis in Adults and Children).

### *Method*

The specificity estimate was determined from 417 TST negative individuals with no history or evidence of exposure to tuberculosis (Class 0) whilst sensitivity was estimated from 182 individuals classified according to the ATS guidelines as being infected with TB (Class 2).

### *Results*

The specificity and sensitivity of the test, using the labeled 15% cut-off, is 97.6% (95% CI, 96.1 to 99.0%) and 89.6% (95% CI, 85.1 to 94.0%) respectively (Streton et al 1998).

*Table 5.9 Low and high risk population data used for specificity and sensitivity estimation using the 15% Human PPD Response cut-off*

<i>Disease Class</i>	<i>Number of Individuals</i>		<i>Median Age (range)</i>	<i>Number Positive</i>	<i>Total Number</i>
	<i>M</i>	<i>F</i>			
Class 0. Low risk No tuberculosis infection/no exposure	272	145	23 (15 – 92)	10 (2.4%)	417
Class 2. High risk Tuberculosis infection/no disease	107	75	39 (11 – 87)	163 (89.6%)	182

### *Conclusion*

The specificity of the test was estimated to be 97.6% (95% CI, 96.1 to 99.0%) and the sensitivity of the test was estimated to be 89.6% (95% CI, 85.1 to 94.0%).

## **REPRODUCIBILITY**

### *Background*

An important aspect of any diagnostic test is its performance characteristics and in particular the precision of the test. The precision is a measure of the degree of reproducibility of the test under normal operating circumstances. Reproducibility of the EIA is critical and was established through a number of studies. The reproducibility between day and/or test sites and within runs of the test was also assessed in separate studies.

### *Reproducibility of the IFN- $\gamma$ 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- $\gamma$  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- $\gamma$  (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 (Data in Appendix 12), 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 mitogen plasma samples. As would be expected, the relative differences were greatest at the lowest levels of IFN- $\gamma$ , 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. The statistical analysis is shown in **Appendix 15**.(not attached)

Within each batch, release criteria of the QuantiFERON-TB test require that the CV of the high, medium and low standards must each be less than 10% when testing 8 replicates of each standard. As further confirmation of low intra-tray variation, for an EIA run to be valid in use, within each EIA run the CV on replicate standards must be <15%. In the CDC study, data was generated from more than 200 EIA runs, of which 7 (<3.5%) were invalid for any reason, and 6 of these were from one site. This indicates that EIA reproducibility within test runs is high in practice.

To test reproducibility of results in the IFN- $\gamma$  EIA across different manufacturing batches plasma samples generated from 24 donors were tested. The % Human Response and % Avian Response remained consistent across batches, although higher deviations between batches occurred at very low levels of % Human Response, and as such these were of no diagnostic significance. Overall agreement across the two batches was very high, and although there was a change in test result for one subject whose %Human Response altered from 15.9 to 14.5%, this was not due to any significant change in QuantiFERON-TB reproducibility. The data is presented in Table 5.10.

Table 5.10 – Comparison of %Human and %Avian Response across batches

Donor	% Human Response IU		% Avian Response IU		Diagnosis	
	Batch #9	Batch #16	Batch #9	Batch #16	Batch #9	Batch #16
1	11.880	12.284	3.780	4.462	Negative	Negative
2	15.683	16.542	8.279	6.767	Mtb	Mtb
3	17.515	16.691	7.974	7.831	Mtb	Mtb
4	0.910	1.062	0.852	0.968	Negative	Negative
5	1.384	1.546	1.712	2.250	Negative	Negative
6	2.336	1.909	2.028	2.188	Negative	Negative
7	<b>0.508</b>	<b>0.265</b>	<b>0.000</b>	<b>0.151</b>	Negative	Negative
8	2.350	1.769	1.060	1.284	Negative	Negative
9	15.906	14.494	12.839	12.636	Mtb	Negative
10	50.547	48.370	31.882	31.934	Mtb	Mtb
11	<b>-0.505</b>	<b>-0.346</b>	<b>-2.276</b>	<b>-0.337</b>	Negative	Negative
12	39.213	34.526	20.621	20.298	Mtb	Mtb
13	19.518	17.5	14.165	13.827	Mtb	Mtb
14	31.534	28.053	19.849	21.385	Mtb	Mtb
15	34.577	33.383	22.228	21.115	Mtb	Mtb

16	1.880	-1.341	1.880	-1.521	Negative	Negative
17	4.548	4.328	1.247	1.674	Negative	Negative
18	4.963	4.908	2.560	3.067	Negative	Negative
19	9.806	9.316	2.868	3.447	Negative	Negative
20	29.449	29.226	7.707	11.451	Mtb	Mtb
21	82.211	85.214	16.648	14.938	Mtb	Mtb
22	1.759	1.175	3.078	-0.234	Negative	Negative
23	2.795	4.204	5.320	9.915	Negative	Negative
24	56.427	50.851	11.811	12.291	Mtb	Mtb

## *Reproducibility of Antigen Stimulation*

### *Objective*

To determine the level of reproducibility in the antigen stimulation and incubation phase of the test.

### *Method*

Incubation to incubation reproducibility for Human PPD and Mitogen stimulated blood was determined using data from donor blood samples. Two different Human tuberculin PPD batches, manufactured by CSL Ltd, and the Mitogen control antigen were evaluated for reproducibility using blood from 100 donors and Avian PPD reproducibility was tested using 23 donor blood samples. Each Human tuberculin PPD and the Mitogen were tested in triplicate and 6 replicates of Avian PPD were tested (see Appendix 5.I to 5.IV). Reproducibility was determined by Intraclass correlation coefficient (ICC).

### *Results*

Table 5.11 shows the results obtained.

*Table 5.11 Reproducibility of Stimulation Antigens*

<i>Antigen</i>	<i>ICC</i>
Human PPD Lot X	0.956 (95% CI 0.94 to 0.97)
Human PPD Lot Y	0.972 (95% CI 0.96 to 0.98)
Avian PPD	0.969 (95% CI 0.95 to 0.99)
Mitogen	0.949 (95% CI 0.933 to 0.966)

For the QuantiFERON-TB Nil stimulation antigen, the ICC statistic is not appropriate due to the low values routinely observed. To demonstrate the reproducibility for the Nil antigen,

incubations were performed with blood from 65 individuals. Blood from 41 of these individuals was incubated in duplicate and blood from 24 of these individuals was incubated in triplicate. In all cases none of the EIA ODs for an individual differed from the mean for that individual by more than 0.04 OD units.

### ***Conclusion***

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

The two batches of Human PPD demonstrated strong agreement with one another, with a kappa chance adjusted agreement statistic of 92% and a correlation of 0.97. Refer to the report titled 'Equivalence testing of human tuberculin purified protein derivative (PPD) preparations for use with the QuantiFERON-TB diagnostic test' 018-equiv.rpt in Appendix 5.V.

## ***Reproducibility Between Testing Sites***

### ***Objective***

A measure of the reproducibility and robustness of a diagnostic test is its ability to generate equivalent results at two or more different testing sites and with different operators. The objective of this study was to determine the level of agreement between two test sites.

### ***Method***

The site-to-site reproducibility of the QuantiFERON-TB test was evaluated between two testing sites with 50 replicate blood samples. The samples were distributed between the two testing sites and tested, using the same batch of kits, without knowledge of the donors TB status. Results from the two testing sites were collated and the reproducibility (agreement) of the test between sites was evaluated using a kappa chance adjusted agreement statistic.

### ***Results***

Results from the two testing sites were collated (Table 5.12) and the reproducibility (agreement) of the test between sites was determined to be greater than 98% with a kappa chance adjusted agreement statistic of 90% and an ICC of 0.948 (refer to Table 5.13). There was no significant site-to-site variability observed.

#### ***1. Conclusion***

The reproducibility (agreement) of the test between sites is greater than 98% with a kappa chance adjusted agreement statistic of 90% and an ICC of 0.948.

Table 5.12 % Human PPD Responses Generated with Duplicate Blood Samples Tested at Two Different Sites

<i>Sample Number</i>	<i>Site 1 % Human PPD Response</i>	<i>Site 2 % Human PPD Response</i>	<i>Sample Number</i>	<i>Site 1 % Human PPD Response</i>	<i>Site 2 % Human PPD Response</i>
1	89.28	106.34	26	12.81	10.09
2	48.28	46.95	27	12.87	10.04
3	140.68	126.56	28	223.16	210.72
4	140.25	140.63	29	203.86	183.80
5	193.79	205.78	30	196.62	186.14
6	127.76	122.88	31	223.16	215.20
7	125.9	132.53	32	203.86	188.15
8	189.11	163.67	33	196.62	191.83
9	178.22	176.67	34	169.23	140
10	182.08	207.80	35	144.23	131.43
11	123.58	133.75	36	111.54	113.96
12	11.07	9.86	37	93.43	97.87
13	156.93	203.19	38	128.2	135.78
14	90.35	119.46	39	67.94	80.90
15	100	155.15	40	77.78	139.18
16	14.31	17.87	41	35.93	36.24
17	240.02	239.41	42	37.22	100.65
18	47.44	31.06	43	46.11	62.75
19	235.99	230.67	44	26.71	45.71
20	147.02	207.73	45	30.28	67.12
21	245.72	413.06	46	185.39	142.72
22	27.02	22.69	47	145.2	127.18
23	11.25	11.31	48	39.78	47.06
24	10.98	12.72	49	15.9	29.99
25	30.53	25.95	50	16.54	34.62

Table 5.13 Comparison of QuantiFERON-TB test results generated from 50 duplicate blood samples tested at two different sites

		<i>Site 1</i>		<i>% Agree</i>	<i>Kappa</i>	<i>ICC (95% CI)</i>
		<i>Positive</i>	<i>Negative</i>			
<i>Site 2</i>	<i>Positive</i>	44	1	0.980	0.898	0.948 (0.92-0.976)
	<i>Negative</i>	0	5			

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

### ***Objective***

An individual's response to an antigen may vary over time due to variations in the different blood samples drawn, which may include fluctuations in the strength of the individual's immune response. A study was conducted to determine the level of variation expected in an individual's immune response over time.

### ***Method***

To investigate the longitudinal reproducibility of the QuantiFERON-TB test, blood from 36 individuals was tested every 2 weeks over six consecutive visits.

### ***Results***

Figure 5.6 on the following page shows the results of the 36 individuals tested over a period of 10 weeks. The results for the 36 individuals were consistent for all tests performed except for four (4) individuals who moved from positive to negative or vice versa at only one of the testing times and three (3) individuals results varied on two occasions. These discordant interpretations all resulted from minor changes in the individual's % Human PPD Response. Statistical analysis of results from this study demonstrated good correlation for the within-subject variability (one individual's response over time), which was determined by ICC to be 84%. This level of variation over time (ICC = 0.84) is larger than the level of variation seen between sites (ICC = 0.94). Identifying the cause of this level of variation between different blood samples from an individual over time was not in the scope of this study, however variation in an individual's immune response may explain some of the variation over that seen between sites.

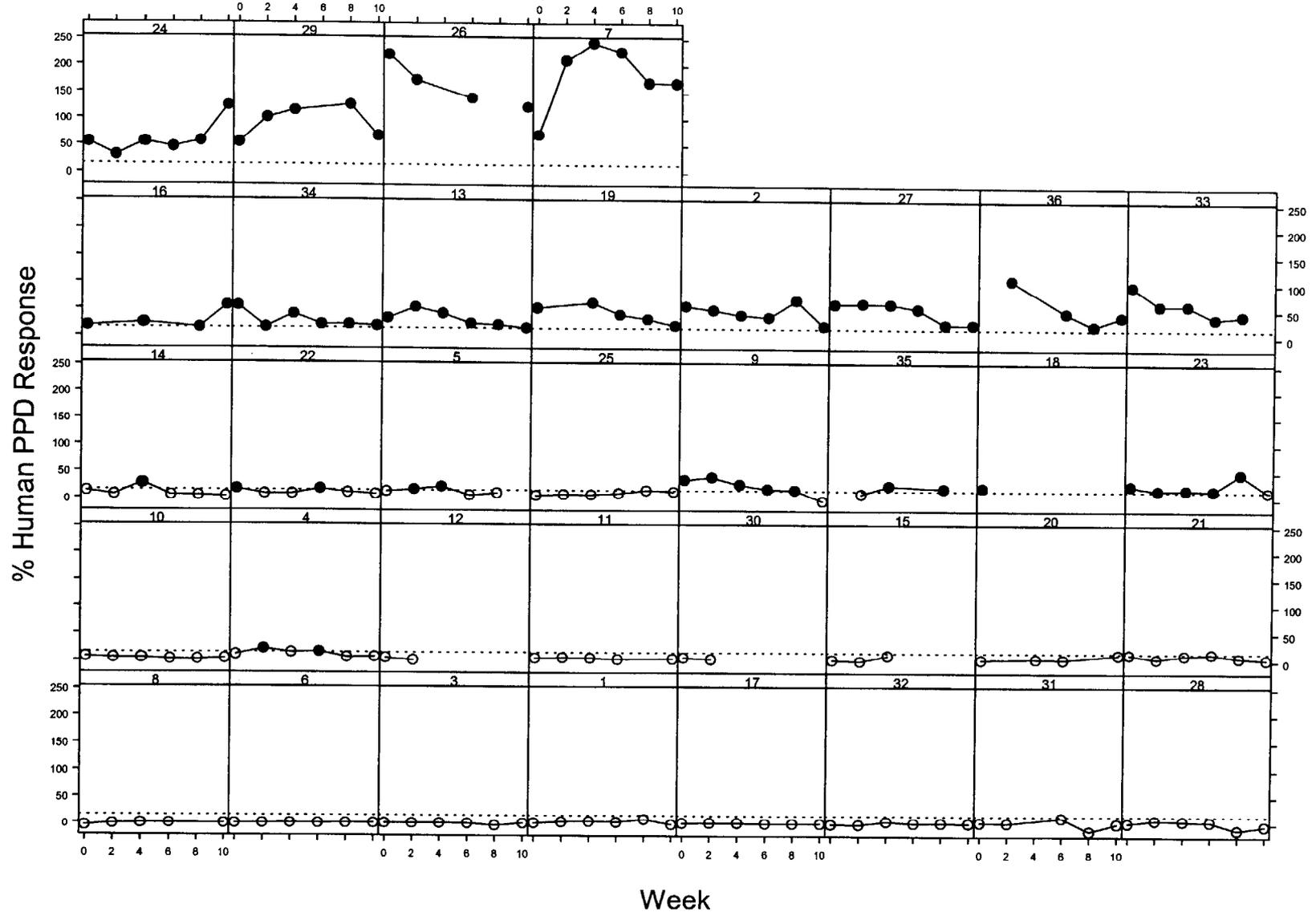
### ***Conclusion***

Individual results over a period of ten weeks are consistent.

Variation in the % Human PPD within an individual has been studied and found to have an ICC of 84%. This level of variation in an individual's blood sample over time may lead to an individual giving discordant results when the QuantiFERON-TB test is used on individuals whose % Human PPD Response is close to the 15% cut-off. However the chance of an individual's result being affected by these variations is minimal due to the low frequency of responses about the 15% Human PPD cut-off.

Figure 5.6

Longitudinal plot of % Human PPD Response study of 36 individuals over 10 weeks





## TECHNICAL NOTE

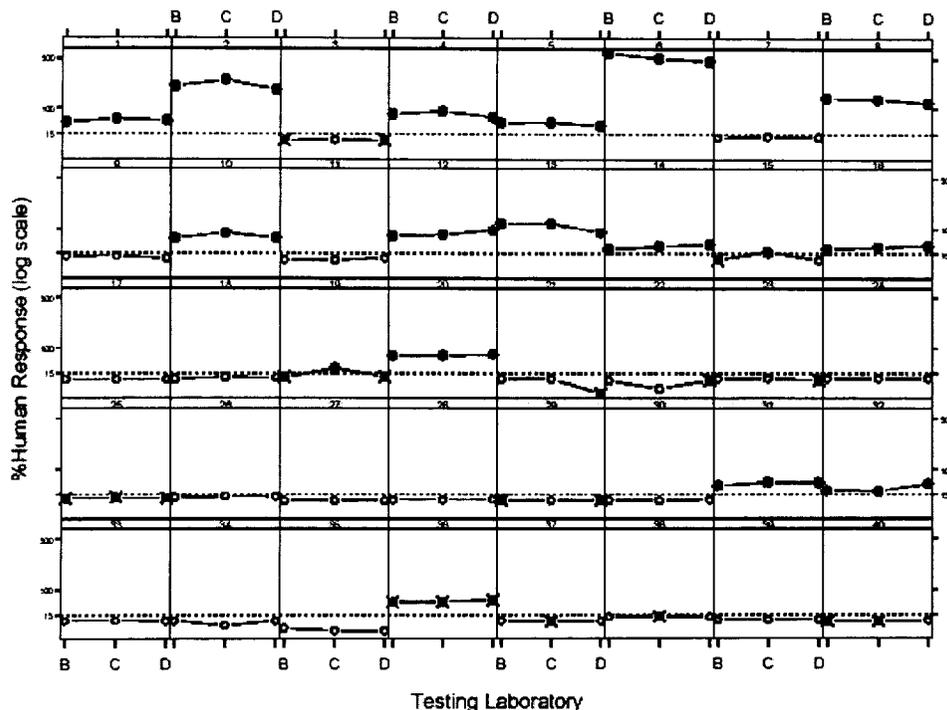
### Reproducibility of QuantiFERON®-TB Between Laboratory Sites (February 2002)

QuantiFERON®-TB assay results from three independent laboratories from a Quality Assurance Panel of 40 samples were analyzed for reproducibility between laboratories. The plasma samples were from individuals with a range of responses in the QuantiFERON®-TB test and were assayed at the three sites with different operators, as per the manufacturers protocol.

Qualitative results showed complete agreement for 38 (95%) of the 40 samples tested. For the two samples with qualitative disagreement, high results for %Avian difference were seen.

Quantitative results for %Human response showed excellent reproducibility, with an intraclass correlation coefficient of ICC = 0.988 (95% CI: 0.980 to 0.993)

Figure: %Human Response by Laboratory for each of 40 individuals; assays interpreted **positive** for *Mycobacterium tuberculosis* are shown with closed circles, those **negative** with open circles. Assays with %Avian Difference >10% are indicated by crosses.



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