

# Analytical evaluation of Quantiferon Plus and Quantiferon In-tube assays in subjects with or without tuberculosis

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**Introduction:** Interferon- $\gamma$  release assays (IGRAs) are diagnostic assays used to identify subjects with latent tuberculosis infection (LTBI). The QuantiFERON-TB Gold Plus (QFT-Plus) represents the new generation of the QuantiFERON-TB Gold In-tube (QFT-GIT), the main differences is the addition of a new test tube containing shorter peptides able to stimulate CD8 T cells. The aim of this study is to evaluate the accuracy of the QFT-Plus assay compared with the QFT-GIT in a cross sectional study of healthy controls and individuals prospectively enrolled categorized as active TB, cured TB or LTBI. The response to QFT-Plus was evaluated as single or combined response to TB1 and TB2.

**Methods:** We enrolled 179 participants: 19 healthy low risk for TB exposure, 58 LTBI subjects, 33 cured TB and 69 active TB patients. QFT-Plus and QFT-GIT assays were performed at the same time.

**Table 1: Demographic characteristic**

	TB	LTBI	Cured TB	Healthy donors	Total	p
<b>N (%)</b>	69 (38)	58 (32)	33 (18)	19 (10.5)	179	
<b>Sex</b>						
female N (%)	28(41)	30 (52)	15(45)	10(53)	84(47)	0.6 <sup>§</sup>
<b>Age<sup>#</sup></b>						
median (IQR)	35 (28-44)	42 (32-57)	35 (29-43)	43 (33-48)	38 (29-47)	0.02
<b>BCG vaccinated<sup>#</sup></b>						
N (%)	49 (71)	*23 (40)	24 (73)	1 (5)	98 (54)	$\leq 0.0001$
<b>Origin<sup>#</sup> N (%)</b>						$\leq 0.0001$
West Europe	20 (29)	36 (62)	9 (27)	19(100)	84 (47)	
East Europe	26 (38)	17 (29)	13 (39)	0 (0)	55 (30.5)	
Asian	11 (16)	1 (2)	5(15)	0 (0)	19 (10.5)	
Africa	7 (10)	3 (5)	4(12)	0 (0)	14 (8)	
South America	5 (7)	1 (2)	2(6)	0 (0)	7 (4)	
Central America	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.5)	

**Footnotes:** TB: tuberculosis; LTBI: latent tuberculosis infection; BCG: Bacillus Calmette et Guérin; <sup>§</sup>Kruskal Wallis test; <sup>#</sup>Chi Square test; \*For one patient the BCG vaccination status is not available

**Table 2: Concordance of QFT-GIT and QFT-PLUS results**

Groups of subjects	QFT-GIT vs QFT-PLUS		k	#p
	Positive within the group over total n(%)			
Active TB	61/69 (88) vs 62/69 (90)		0.5	<0.0001
LTBI	58/58 (100) vs 57/58 (98)		na	na
Cured TB	24/33 (73) vs 27/33 (82)		0.7	<0.0001
Healthy donors	0/19 (0) vs 0/19 (0)		na	na
Total patients	143/179 (80) vs 146/179 (81)		0.8	<0.0001

**Footnotes:** QFT: quantiferon, IT: in tube; k= Cohen's kappa coefficient; na: not available because the QFT-IT score is a constant; #Chi Square test

**Table 4: QFT-PLUS response in active TB patients according microbiological results**

Response to:	Clinical TB (20)		Microbiologically confirmed TB (49)	
	Negative N (%)	Positive N (%)	Negative N (%)	Positive N (%)
<b>TB1 or TB2</b>	4 (20)	16 (80)	3 (6)	46 (94)
<b>TB1 and TB2</b>	4 (20)	16 (80)	9 (18)	40 (82)
<b>only TB1</b>	20 (100)	0 (0)	49 (100)	0 (0)
<b>only TB2</b>	20 (100)	0 (0)	43 (88)	6 (12)
<b>TB1</b>	4 (20)	16 (80)	9 (18)	40 (82)
<b>TB2</b>	4 (20)	16 (80)	3 (6)	46 (94)

**Footnotes:** TB : tuberculosis; TB1: tube 1, TB2: tube 2

**Table 3: QFT-PLUS response among the subjects at different TB stages**

Response to:	TB (69)		LTBI (58)		Cured TB (33)		TB vs LTBI	TB vs cured TB	LTBI vs cured TB
	Negative N (%)	Positive N (%)	Negative N (%)	Positive N (%)	Negative N (%)	Positive N (%)	#p	#p	#p
<b>TB1 or TB2</b>	7 (10)	62 (90)	1 (2)	57 (98)	6 (18)	27(82)	-	-	0.005
<b>TB1 and TB2</b>	13 (19)	56 (81)	2 (3)	56 (97)	6 (18)	27 (82)	0.007	-	0.02
<b>only TB1</b>	69 (100)	0 (0)	57 (98)	1 (2)	33 (100)	0 (0)	-	-	-
<b>only TB2</b>	63 (91)	6 (9)	58 (100)	0 (0)	33 (100)	0 (0)	0.02	-	-
<b>TB1</b>	13 (19)	56 (81)	1 (2)	57 (98)	6 (18)	27 (82)	0.002	-	0.005
<b>TB2</b>	7 (10)	62 (90)	2 (3)	56 (97)	6 (18)	27 (82)	-	-	0.02

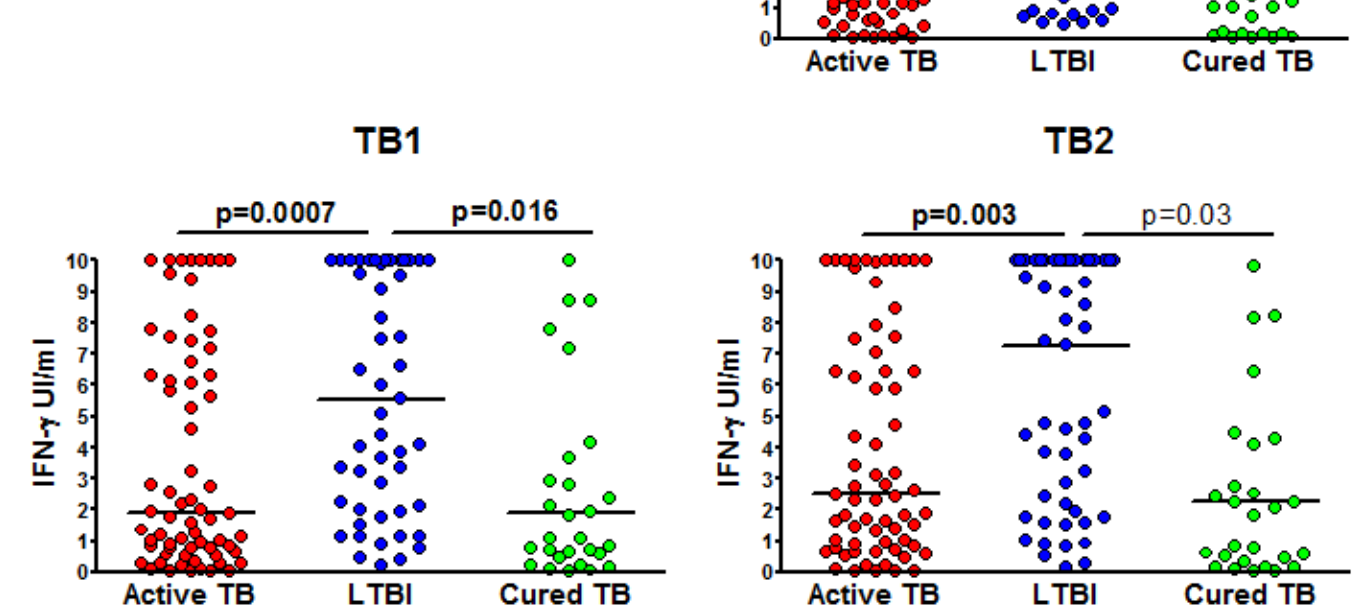
**Footnotes:** TB : tuberculosis; LTBI: latent TB infection; # Chi Square test TB1: tube 1, TB2: tube 2

**Table 5: QFT-PLUS response in active pulmonary TB patients according to lung lesion severity**

Response to:	low severity TB (17)		intermediate/high severity TB (46)	
	Negative N (%)	Positive N (%)	Negative N (%)	Positive N (%)
<b>TB1 or TB2</b>	2 (12)	15 (88)	4 (9)	42 (91)
<b>TB1 and TB2</b>	3 (18)	14 (82)	9 (20)	37 (80)
<b>only TB1</b>	17 (100)	0 (0)	46 (100)	0 (0)
<b>only TB2</b>	16 (94)	1 (6)	41 (89)	5 (11)
<b>TB1</b>	3 (18)	14 (82)	9 (20)	37 (80)
<b>TB2</b>	2 (12)	15 (88)	4 (9)	42 (91)

**Footnotes:** TB : tuberculosis; grade 0: low severity; grade 1: intermediate and high severity; TB1: tube 1, TB2: tube 2.

**Quantitative IFN- $\gamma$  response to stimulation with QFT-Plus antigen TB1 and TB2 and QFT-GIT antigen AgTB.** Horizontal lines indicate the median production. A  $p \leq 0.016$  was considered significant after Bonferroni correction.



**Results:** The two tests showed a substantial agreement and similar sensitivity, evaluated in active TB patients, and same specificity, evaluated in healthy donors. Interestingly, a higher proportion of the LTBI subjects responded concomitantly to both QFT-Plus antigens TB1 and TB2 compared to those with active TB (97% vs 81%). Moreover, in a small proportion of active TB patients (9%) we found a selective TB2 response, indirectly demonstrating that TB2 stimulation induces a CD8 T-cell response in absence of a CD4 T-cell response, as previously showed by cytometry, CD8 response correlate with disease severity.

**Conclusion:** QFT-Plus and QFT-GIT assays were showed a substantial agreement and similar accuracy for active TB detection. Interestingly, a higher proportion of the LTBI subjects responded concomitantly to both QFT-Plus antigens TB1 and TB2 compared to those with active TB.