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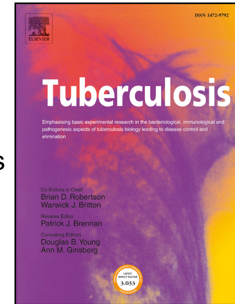

Analytical evaluation of QuantiFERON- Plus and QuantiFERON- Gold In-tube assays in subjects with or without tuberculosis

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1 **Analytical evaluation of QuantiFERON- Plus and QuantiFERON- Gold In-tube assays in**
2 **subjects with or without tuberculosis**
3
4 **Short title: evaluation of QuantiFERON- Plus assays**
5
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21
22 **keywords:**
23 tuberculosis; latency; diagnosis; quantiferon; quantiferon plus
24
25 **abbreviation:**
26 - BCG: Bacillus Calmette–Guérin
27 - Mtb: *Mycobacterium tuberculosis*
28 - QFT-GIT: Quantiferon-Gold in Tube
29 - QFT-Plus: Quantiferon- Plus
30 - TB: tuberculosis
31 - LTBI: latent tuberculosis infection
32 - IFN: interferon
33 - IQR: interquartile range

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Summary

The QuantiFERON-TB Gold Plus (QFT-Plus) represents the new QuantiFERON-TB Gold In-tube (QFT-GIT) to identify latent tuberculosis infection (LTBI). The main differences is the addition of a new tube containing shorter peptides stimulating CD8 T-cells. Aim of this study is to evaluate the accuracy of QFT-Plus compared with QFT-GIT in a cross sectional study of individuals with or without tuberculosis (TB).

We enrolled 179 participants: 19 healthy donors, 58 LTBI, 33 cured TB and 69 active TB. QFT-Plus and QFT-GIT were performed.

The two tests showed a substantial agreement. Moreover we found a similar sensitivity in active TB and same specificity in healthy donors. A higher proportion of the LTBI subjects responded to both TB1 and TB2 compared to those with active TB (97% vs 81%). Moreover, a selective response to TB2 was associated with active TB (9%) and with a severe TB disease, suggesting that TB2 stimulation induces a CD8 T-cell response in absence of a CD4-response.

In conclusion, QFT-Plus and QFT-GIT assays showed a substantial agreement and similar accuracy for active TB detection. Interestingly, a higher proportion of the LTBI subjects responded concomitantly to TB1 and TB2 compared to those with active TB, whereas a selective TB2 response associated with active TB.

1. Introduction

Tuberculosis (TB), being responsible for 10.4 million cases and 1.4 million deaths annually, represents a major public health problem [1]. Moreover, latent TB infection (LTBI), which is estimated to affect one-fifth of the world's population, may progress to active disease in about 3-15% of the LTBI individuals during their lifetimes [2-4]. Considering that LTBI subjects are the reservoir of TB disease, diagnosing and treating LTBI is one of the main goals to control and eliminate the TB epidemic [5-10]. Tuberculin skin test (TST) and T-cell interferon-γ release assays (IGRAs) are the routine diagnostic tools to identify LTBI [6]. Two IGRAs are commercially available: the QuantiFERON_-TB Gold In-Tube (QFT-GIT) (Qiagen, Hilden, Germany) and the T-SPOT.TB (Oxford Immunotec, Abingdon, UK). IGRAs have several advantages: the results are not affected by Bacillus Calmette–Guérin (BCG)-vaccination [5-8] and by the majority of

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67 environmental mycobacteria; moreover, only one patient-visit is required. However, since these
68 assays are based on detection of a *Mycobacterium tuberculosis* (Mtb) -specific immune response,
69 they have a poor sensitivity in children where the immune system is immature and in immune-
70 compromised subjects [8,11,12] furthermore IGRAs do not discriminate between active TB and
71 LTBI [6] and poorly correlate with the presence of viable bacteria and the risk of developing active
72 disease [4,13-15].
73 Recently, QFT Gold Plus (QFT-Plus), [16-21] has been proposed as a new generation of QFT-GIT.
74 QFT-Plus contains two TB-specific antigen tubes, called TB1 and TB2, for the incubation of the
75 whole blood with Mtb antigens. The TB1 tube, contains long peptides derived from ESAT-6 and
76 CFP-10 (TB-7.7, present in QFT-GIT, has been removed), and it is designed to induce a specific
77 CD4 T cells response. TB2 contains both the same long peptides of TB1 and newly designed
78 shorter peptides to induce interferon (IFN)-γ production by both CD4 and CD8 T-cells [22] . IGRA
79 are designed to diagnose LTBI. However, there is not a gold standard for LTBI detection, therefore
80 active TB is used as a surrogate reference standard for evaluating test accuracy [6].
81
82 Compared to QFT-GIT, it has been reported that the accuracy of QFT-Plus is similar [18,23], or
83 that the sensitivity for active TB or LTBI detection is higher [16,17]. Moreover, in a low-incidence
84 setting the occurrence of conversions and reversions for the new QFT-Plus in serial testing of a
85 high-TB risk cohort [19] has been described similar to that observed for QFT-GIT [24].
86
87 Therefore, the aim of this study is to evaluate the accuracy of the QFT-Plus assay compared with
88 the QFT-GIT in a cross sectional study of individuals enrolled as healthy donors, subjects with
89 active TB disease, cured TB or LTBI. The response to QFT-Plus is selectively evaluated in terms of
90 single or combined response to TB1 and TB2.
91
92
93 **2. Material and Methods**
94
95 **2.1. Population characteristics**
96 This study was approved by the Ethical Committee of "L. Spallanzani" National Institute of
97 Infectious Diseases (INMI), approval number 72/2015. Written informed consent was required to
98 participate in the study that was conducted at INMI. We prospectively enrolled HIV-uninfected
99 patients with pulmonary and extra-pulmonary active TB, cured TB subjects and LTBI. Enrolled
100 patients were classified as ''confirmed TB'' if the diagnosis was based: i) in those with pulmonary
101 TB by a positive culture for Mtb from the sputum or broncholavage; ii) in those with

102 extrapulmonary TB by a) positive Mtb -specific RNA amplification (TRCReady M.TB, Tosoh, 103 Japan) and/or Mtb -specific NAT (Home-made PCR (IS6110) GeneXpert, Cepheid; Genotype 104 MTBDRPlus Hain Lifescience) from biological specimens or b) by histo-pathological findings 105 consistent with TB and presence of acid fast bacilli (AFB) in a tissue sample or c) by positive 106 culture for Mtb in clinical samples (pleural fluid and abscesses). Conversely, patients were 107 classified as ‘‘clinical TB’’ if the diagnosis was based on clinical and radiologic criteria (having 108 excluded other diseases) including appropriate response to standard anti-TB therapy. TB patients 109 were enrolled within 7 days of starting the specific treatment.

110 Cured TB patients were defined as those who had completed a 6-month course of treatment for 111 culture-positive (drug-susceptible) pulmonary TB and who resulted Mtb culture negative upon 112 treatment completion.

113 In the absence of clinical, microbiological and radiological signs of active TB, LTBI was defined 114 based on a positive score to QFT-GIT (Qiagen, Hilden, Germany). Finally, we enrolled 19 healthy 115 control subjects with low risk of TB infection. Demographic and epidemiological information were 116 collected at enrollment (Table 1).

117

118 **2.2. Chest X-ray evaluation**

119 All chest X-rays were evaluated blind to operators for the presence of nodules, fibrosis, infiltrates, 120 cavitation, bronchial spread, miliarity, pleural effusion and adenopathy, as previously reported [25]. 121 Cavity size in centimeters was recorded (<4 cm or >4 cm). The proportion of the affected lung was 122 analyzed by a visual estimate of the extent of parenchymal infiltrates; a proportion of 30% of 123 affected lung was used as our internal cut-off value to grade TB severity. In agreement with 124 literature data [25] and on the basis of experience, the disease was graded (by DG, FP) using a 125 sliding scale of severity as follows: 0: normal chest X-rays; 1: mild grade (nodules and or infiltrates 126 with proportion of lung affected <30%); 2: intermediate grade (infiltrates with proportion of lung 127 affected >30% and/or cavitation <4 cm in diameter); 3: high grade (an infiltrate of any percentage 128 of extension with cavitation >4 cm in diameter and/or bronchial spread and/or miliarity and/or 129 pleural effusion and/or adenopathy). All subjects underwent standard chest X-rays at the time of TB 130 diagnosis.

131

132 **2.3. QFT-GIT and QFT-Plus**

133 QFT-GIT and QFT-Plus assays were performed for each subject enrolled. For 11 patients the QFT-134 GIT value of IFN-γ production was not available because the assay was done in another hospital 135 and only the score of the test was provided. QFT-Plus kits were donated by Qiagen and used

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136 according to manufacturer's instructions [22]. Levels of IFN-γ were quantified by ELISA. The
137 results were analyzed by a QFT-Plus Analysis Software (available from www.quantiFERON.com).
138 The software performs a quality control assessment of the assay, generates a standard curve and
139 provides a test result for each subject. Test results were analyzed according to manufacturer's
140 criteria for both assays [22]. All patients resulted positive to mitogen stimulation.
141
142 **2.4. Statistical analysis**
143 Data were analyzed using SPSS software (Version 19 FORWindows, Italy SRL, Bologna, Italy).
144 The median and interquartile ranges (IQRs) were calculated for continuous measures. Chi square
145 was used for categorical variables. The Kruskall Wallis test was used for comparisons among
146 several groups and the Mann Whitney U test was used for pairwise comparisons. Test concordance
147 was assessed by k-statistics where $k \leq 0.20$ was considered 'slight', $0.20 < k \leq 0.40$ 'fair', $0.40 < k \leq$
148 0.60 'moderate', $0.60 < k \leq 0.80$ 'substantial' and $0.80 < k \leq 1.00$ 'optimal'.
149
150 **3. Results**
151 **3.1. Population characteristics**
152 We enrolled 179 participants: 19 healthy donors, 58 LTBI subjects, 33 cured TB and 69 active TB
153 patients. Among the active TB patients, 49 were microbiologically confirmed (among them two
154 patients had an extra-pulmonary form) and 20 clinically diagnosed (4 patients had an extra-
155 pulmonary form). Forty-seven percent of the enrolled subjects were from Western Europe and
156 female. The majority of TB and cured TB patients were from countries other than west Europe and
157 they were BCG-vaccinated, consequently we found significant differences for BCG vaccination and
158 origin among the different groups (Table 1).
159
160 **3.2. Concordance between QFT-GIT and QFT-Plus assays**
161 First, we evaluated the accuracy of the QFT-Plus and QFT-GIT assays. The sensitivity of QFT-Plus
162 in active TB cases, based on the response to either TB1 or TB2 ("either TB1 or TB2"), was 90%
163 (62/69) (Table 2), the specificity calculated on the low TB risk population of healthy donors was
164 100% (19/19); similarly the sensitivity of the QFT-GIT assay was 88% whereas the specificity
165 100% (table 2). The proportion of response to QFT-Plus were significantly different comparing TB
166 *vs* LTBI group (p=0.007) and LTBI *vs* cured TB (p=0.02) (table 3).
167
168 Agreement between QFT-Plus and QFT-GIT results was evaluated (Table 2). The concordance
169 among all samples evaluated was substantial (k= 0.8). In active TB, a moderate agreement (k=0.5)
170 was achieved whereas for cured TB it was substantial (k=0.7). In the LTBI group one patient scored

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171 positive by QFT-GIT resulted negative by QFT-Plus; it was not possible to evaluate the agreement
172 because the QFT-GIT score was a constant.
173
174 **3.3. Analysis of the QFT-Plus results based on the response to TB1 and TB2 tubes**
175 To analytically evaluate the response to the peptides contained in TB1 and TB2 tubes, we stratified
176 the QFT-Plus results according to the ability of subjects to respond to both TB1 and TB2 ("TB1 and
177 TB2"), only to TB1 ("only TB1") or only to TB2 ("only TB2"). We found that almost all LTBI
178 subjects (97%) responded to both "TB1 and TB2" while among TB patients and cured TB only 81%
179 and 82% respectively responded. These proportions were significantly different comparing TB *vs*
180 LTBI group (p=0.007) and LTBI *vs* cured TB (p=0.02) (Table 3).
181 Interestingly, a selective response to TB2 was found only among those with active TB (6/69, 9%).
182 Regarding the stimulation to TB1, the responders were: 81% within TB patients, 98% within LTBI
183 and 82% within cure TB. These proportions were significantly different comparing TB *vs* LTBI
184 group (p=0.002) and LTBI *vs* cured TB (p=0.005) (Table 3).
185 Regarding the cumulative response to TB2, the responders were: 90% within the TB patients, 97%
186 in LTBI and 82% in the cured TB. These proportions were significantly different comparing LTBI
187 *vs* cured TB group (p=0.02) (Table 3).
188 Interestingly, in the active TB group a higher proportion of patients responded to TB2 compared to
189 TB1 (90% *vs* 81%). The higher sensitivity of the TB2 stimulation was likely due to the CD8-
190 specific peptides contained in the TB2 tube and consequently to the presence of Mtb-specific CD8
191 T-cells [26].
192
193 **3.4. Impact of mycobacterial load and severity of TB disease on the QFT-Plus assay**
194 **results**
195 To evaluate the impact of the mycobacterial load on the immunological response to QFT-Plus, we
196 stratified the active TB patients according to the microbiological diagnosis. Interestingly we found
197 that the six patients showing a "only TB2" response had a microbiological diagnosis (Table 4).
198
199 Moreover, we investigated whether the severity of pulmonary TB disease had an impact on the
200 response to QFT-Plus. Clinical severity was estimated evaluating the lung lesions based on the
201 radiological findings. The radiological data from 63 pulmonary TB patients were analysed: patients
202 characterized by an intermediate/high radiological severity (grades 2 and 3) were combined and
203 compared with those from patients with low radiological severity (grade 1). As shown in Table 5
204 the patients with intermediate/high radiological severity TB had a similar proportion of responders
205 (91%) to either TB1 or TB2 compared to the patients with low radiological severity (88%).

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206 Interestingly, we found that the six TB patients showing "only TB2" response (Table 3) were
207 classified as: 5 with high/intermediate radiological severity and only 1 with low radiological
208 severity (Table 5).
209 These results suggest that CD8 T-cell response associates with the radiological severity of TB
210 disease (Table 5) and with the mycobacterial load (Table 4).
211

212 **3.5. Comparison of IFN-γ production using QFT-Plus and QFT-GIT**

213 We evaluated the results also by quantitative means (Figure 1). Using the QFT-Plus, we found that
214 in the TB group the median of TB1 response (1.9 IU/mL, IQR: 0.7-6.8) was significantly lower
215 than that observed in LTBI (5.6 IU/mL, IQR: 2-10) (p=0.0007). Similar results were obtained in
216 response to TB2, the median in active TB (2.5 IU/mL , IQR: 0.9-7.5) was significantly lower than
217 in LTBI (7.3 IU/ mL, IQR: 1.9-10) (p=0.003). The cured TB group showed levels of IFN-γ
218 production similar to that reported in the active TB (TB1: 1.9 UI/ml, IQR: 0.6-8.2 and TB2: 2.3
219 UI/ml, IQR: 0.5-8.2). Comparing the cured TB group with LTBI we found significant differences in
220 response to TB1 (p=0.016). These results suggest that LTBI subjects have a higher immunological
221 ability to respond to Mtb stimulation compared to those that experienced a higher Mtb load.
222

223 Regarding the QFT-IT, we found that the median production of IFN-γ in response to AgTB
224 stimulation was higher, although not significant, in the LTBI (5.6 UI/mL, IQR: 2-10) than in active
225 TB (2.6 UI/mL, IQR: 1-8) and significantly higher compared to cured TB subjects (1.6 UI/mL,
226 IQR: 0.2-7) (p=0.01).
227

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228 **4. Discussion**

229 We evaluated in a low TB endemic country such as Italy, the accuracy of QFT-Plus in comparison
230 to QFT-GIT, dissecting the response to TB1, TB2 and AgTB in a cohort of subjects with LTBI,
231 active TB, cured TB and healthy donors. The accuracy for active TB detection of QFT-Plus was
232 similar to that found for QFT-GIT. The two tests showed a substantial agreement and similar
233 sensitivity in active TB patients and same specificity in the healthy donors. Interestingly, the
234 majority of the LTBI subjects responded concomitantly to both QFT-Plus antigens TB1 and TB2
235 compared to the active TB (97% vs 81%); moreover, the response “only to TB2” was associated to
236 active TB.
237 Based on the product information [22], TB1 contains long peptides eliciting a CD4 T-cell response
238 whereas TB2, beside the same long peptides, contains additional short peptides specific for the CD8
239 T-cells. Therefore, when a selective response to TB2 is found, it is plausible to assume that the CD8
240 T-cells played a role in this antigen recognition otherwise, the response should have been observed
241 also in response to TB1. Differently, if a simultaneously response to “either TB1 or TB2”, is found,
242 the reasonable scenario is that CD4 T-cells recognize the CD4 peptides present in TB1 and TB2
243 tubes. Therefore stratifying the QFT-Plus results according to the ability of subjects to respond or
244 not simultaneously to TB1 and TB2, we found that among the LTBI subjects the majority of them
245 responded to both TB1 and TB2 stimulation (97%). Based on the assay format, it is unknown
246 whether the response to TB2 is mediated by the CD8 or CD4 T-cells. Conversely, among the active
247 TB patients showing “only TB2” response, it can reasonably be assumed that this response is
248 mediated by CD8 T-cells. By cytometry this result could have been more refined, as we recently
249 showed that the CD8 T-cells are mainly induced by TB2 and are associated to active TB (in 44%)
250 although it may be found at a lower proportion also in LTBI (in 11%) [26].
251 Several studies have described that CD8 T cells play a unique function in the recognition and
252 containment of intracellular infection with Mtb. CD8 T cells are important players to control the
253 Mtb bacterial load, emerging in the presence of replicating Mtb and declining during TB treatment
254 [15]. Interestingly, in our study we did not observe any “only TB2” response after the completion of
255 TB therapy, suggesting a loss of the CD8 T-cell response in parallel with the decrease of
256 mycobacterial load. Therefore, investigation of the TB1 and TB2 response could be a springboard
257 to find new tools to monitor the efficacy of TB therapy.
258 In line with the literature reporting a correlation between mycobacterial load and CD8 T-cell
259 response [15], we found that patients showing a selective “only TB2” response had a
260 microbiologically diagnosed TB. Interestingly, we showed that the majority of them had an
261 intermediate/high level of TB severity, supporting the concept that CD8 T-cells limit bacterial

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262 survival and at the same time produce tissue damage [27]. These data confirm recent findings
263 generated by cytometry [26]. Altogether, these results suggest that the Mtb load and consequently
264 the lung damage may influence the ability to respond to the peptides specific for the CD8 T-cells,
265 contained in the TB2 tube.
266 In those with active TB and cured TB a proportion of patients was scored as negative to QFT-GIT
267 and QFT-Plus. This can be due in those with active TB group to a higher amount of specific cells at
268 site of TB disease compared to peripheral blood [28,29]; differently, in the cured TB to a decrease
269 of Mtb load after therapy.
270 Looking at the quantitative levels of the IFN-γ produced, the LTBI subjects showed higher IFNγ
271 levels in response to either TB1, or TB2 stimulation compared to the active TB patients. These
272 results are mainly due to the absence of IFN-γ production in 7 patients with active TB.
273 We found that within the group of patients evaluated, the stimulation with either TB1, or TB2 or
274 AgTB induced similar level of IFN-γ. Therefore, the absence of TB7 peptides in the QFT-Plus did
275 not reduce the IFN-γ response. This finding is different from what recently reported in two studies
276 performed in Japan and Germany in which it was described that the QFT-GIT induced higher level
277 of IFN-γ compared to QFT-Plus in active TB patients [18,23] and the QFT-Plus induced higher
278 IFN-γ amount in LTBI subjects [23]. Regarding the German study, the data of QFT-Plus and QFT-
279 GIT are related to the total cohort of patients with LTBI and TB, making difficult the comparison
280 with our findings [18].
281 Using the QFT-Plus, we indirectly demonstrated that TB2 stimulation induces a CD8 T-cell
282 response in absence of a CD4 T-cell response in the active TB patients. This ability to selectively
283 induce a TB2 response could be potentially very useful in conditions of immune depression
284 resulting from CD4 T-cell impairments. In line with this, it has been recently published a study
285 demonstrating that human immunodeficiency virus (HIV) infection did not reduce the sensitivity of
286 the QFT-Plus for active TB detection [30]. Moreover, the comparison of QFT-Plus and QFT-IT
287 results in co-infected HIV-TB subjects, demonstrated the higher sensitivity of the QFT-Plus
288 compare to QFT-IT [30-31].
289 Future studies on patients with different stages of Mtb infection, followed overtime during TB
290 treatment could be useful to characterize distinct profile of Mtb-specific response to distinguish
291 LTBI and active TB patients and to monitor TB therapy efficacy as previously suggested with
292 different experimental settings [32-35] and as recently proposed with QFT-Plus assay [36].
293 In conclusion, this is the first report of the characterization of TB1, TB2 and AgTB response of the
294 QFT-Plus and QFT-GIT assays respectively done in healthy donors and subjects with active TB
295 disease, cured TB and LTBI. We demonstrated that the two tests have similar accuracy. Moreover,

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296 we indirectly demonstrated that TB2 stimulation induces a CD8 T-cell response in absence of a
297 CD4 T-cell response in the active TB patients.
298

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306

307 **Conflict of interest**
308 None of the authors has a conflict of interest.
309

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314

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419 **Figure Legend**

420 **Figure 1: Quantitative IFN-γ response to stimulation with QFT-Plus antigen TB1 and TB2**
421 **and QFT-GIT antigen AgTB.** Horizontal lines indicate the median production. A $p \leq 0.016$ was
422 considered significant after Bonferroni correction. The data are presented as IU/ml. Footnotes: IFN:
423 interferon; IU: international unit.

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Table 1: Demographic characteristic of enrolled patients

	Active TB	LTBI	Cured TB	Healthy donors	Total	p
N (%)	69 (38)	58 (32)	33 (18)	19 (10.5)	179	
Sex female N (%)	28(41)	30 (52)	15(45)	10(53)	84(47)	0.6 [§]
Age median (IQR)	35 (28-44)	42(31.75-57)	35 (28.5-42.5)	43 (33-48)	38 (29-47)	0.02 [#]
BCG-vaccinated N(%)	49 (71)	*23 (40)	24 (73)	1 (5)	98 (54)	≤0.0001 [#]
Origin (%)						≤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; [§]Kruskal Wallis test ; [#]Chi Square test ; *For one patient the BCG-vaccination status is not available

Table 2: Concordance of QFT-GIT and QFT-Plus results

QFT-GIT vs QFT-Plus			
Groups of subjects	**Positive within the group over total N (%)**	**k**	**#p**
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 costant; N:number; #Chi Square test.

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Table 3: QFT-Plus response among the different groups of patients with or without TB.

QFT-Plus response to:	TB status						Comparisons		
	TB (N. 69)		LTBI (N. 58)		Cured TB (N. 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
either TB1 or TB2	7 (10)	62 (90)	1 (2)	57 (98)	6 (18)	27(82)	-	-	0.005
TB1 and TB2	13 (19)	56 (81)	2 (3)	56 (97)	6 (18)	27 (82)	0.007	-	0.02
only TB1	69 (100)	0 (0)	57 (98)	1 (2)	33 (100)	0 (0)	-	-	-
only TB2	63 (91)	6 (9)	58 (100)	0 (0)	33 (100)	0 (0)	0.02	-	-
TB1	13 (19)	56 (81)	1 (2)	57 (98)	6 (18)	27 (82)	0.002	-	0.005
TB2	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; N:number

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Table 4: QFT-Plus response in active TB patients according to the microbiological results

QFT-Plus response to:	Clinical TB (N. 20)		Microbiologically confirmed TB (N. 49)	
	Negative N (%)	Positive N (%)	Negative N (%)	Positive N (%)
either TB1 or TB2	4 (20)	16 (80)	3 (6)	46 (94)
TB1 and TB2	4 (20)	16 (80)	9 (18)	40 (82)
only TB1	20 (100)	0 (0)	49 (100)	0 (0)
only TB2	20 (100)	0 (0)	43 (88)	6 (12)
TB1	4 (20)	16 (80)	9 (18)	40 (82)
TB2	4 (20)	16 (80)	3 (6)	46 (94)

Footnotes: TB: tuberculosis; TB1: tube 1, TB2: tube 2; N:number

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Table 5: QFT-Plus response in active pulmonary TB patients according to lung lesions severity

QFT-Plus response to:	Low severity TB (N. 17)		Intermediate/high severity TB (N. 46)	
	Negative response N (%)	**Positive response N (%)**	**Negative response N (%)**	**Positive response N (%)**
either TB1 or TB2	2 (12)	15 (88)	4 (9)	42 (91)
TB1 and TB2	3 (18)	14 (82)	9 (20)	37 (80)
only TB1	17 (100)	0 (0)	46 (100)	0 (0)
only TB2	16 (94)	1 (6)	41 (89)	5 (11)
TB1	3 (18)	14 (82)	9 (20)	37 (80)
TB2	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; N: number

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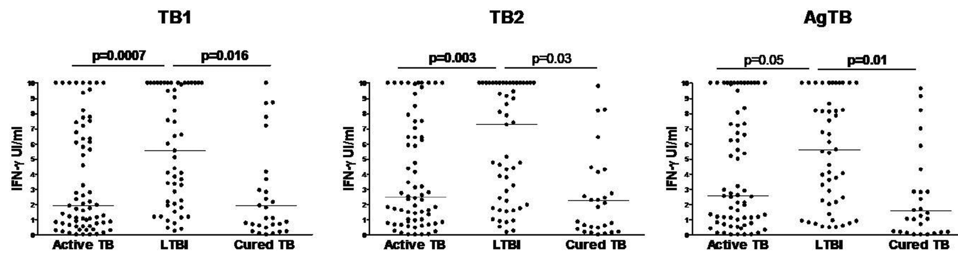

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