

## **Online Data Supplement**

### **Performance of QuantiFERON-TB Gold Plus assays in paediatric tuberculosis – a multicentre PTBNET study**

Danilo Buonsenso, Antoni Noguera-Julian, Rossana Moroni, Ángel Hernández-Bartolomé, Nora Fritschi, Laura Lancella, Laura Cursi, Aleix Soler-Garcia, Renate Krüger, Cornelia Feiterna-Sperling, Michela Sali, Andrea Lo Vecchio, Sara Maria Scarano, Alicia Hernanz-Lobo, Maria Espiau, Antoni Soriano-Arandes, Benhur Sirvan Cetin, Folke Brinkmann, Iveta Ozere, Fernando Baquero, Maria Tsolia, Tiago Milheiro Silva, Matilde Bustillo-Alonso, Andrea Martin-Nalda, Margherita Mancini, Anna Starshinova, Nicole Ritz, Svetlana Velizarova, Laura Ferreras-Antolin, Florian Götzinger, Olga Bilogortseva, Vira Chechenieva, Marc Tebruegge, Begoña Santiago-García, on behalf of the ptbnet QFT-Plus study group

**Table E1.** Underlying disorders in the 136 children with pre-existing comorbidities that were part of the study population.

<b>Comorbidities</b>	<b>No. (percentage)</b>
Respiratory disease	24 (17.6)
Cardiovascular disease	21 (15.4)
Neurological disease	20 (14.7)
Premature birth	17 (12.5)
Non-malignant hematological disease	9 (6.6)
Sickle cell disease	8 (5.9)
Malignant hematological disease	9 (6.6)
Rheumatological disease	13 (9.6)
Malnutrition	12 (8.8)
Gastrointestinal disease	10 (7.4)
Immune disorder <sup>a</sup>	57 (42.0)
Immunosuppressive treatment	20 (14.7)
Renal/urological disease	9 (6.6)
Diabetes mellitus	5 (3.7)
Solid organ tumor	4 (2.9)
Other medical conditions	32 (23.5)

<sup>a</sup> Includes 8 children with human immunodeficiency virus infection

**Table E2.** Summary of the test performance of interferon-gamma release assays and the tuberculin skin test (at  $\geq 5$  mm and  $\geq 10$  mm cut-offs) in children with microbiologically-confirmed tuberculosis (n=488) according to demographics and clinical characteristics.

	No. of patients with positive test result / No. of patients tested Test sensitivity [95%CI]					QFT-Plus vs. QFT-GIT*	TST5 vs.			TST10 vs.		
	QFT-Plus n=182	QFT-GIT n=285	T-SPOT. <i>TB</i> n=33	TST10 n=268	TST5 n=268		QFT-Plus*	QFT-GIT*	T-SPOT. <i>TB</i> *	QFT-Plus*	QFT-GIT*	T-SPOT. <i>TB</i> *
<b>Male</b>	80/94 85.1 [79.0 – 93.1]	110/133 82.7 [76.3 – 89.1]	12/15 80.0 [51.9 – 95.7]	103/132 78.0 [71.0 – 85.1]	115/132 87.1 [81.4 – 92.8]	0.63	0.66	0.31	0.44	0.18	0.33	0.86
<b>Female</b>	79/88 89.8 [83.4 – 96.1]	133/152 87.5 [82.2 – 92.8]	12/18 66.7 [44.9 – 88.4]	110/136 80.9 [74.3 – 87.5]	122/136 89.7 [84.6 – 94.8]	0.59	0.98	0.55	<b>0.006</b>	0.07	0.12	0.16
<b>Age &lt;1 year</b>	24/28 85.7 [72.8 – 98.7]	39/43 90.7 [82.0 – 99.4]	2/3 66.7 [9.0 – 99.0]	17/29 58.6 [40.7 – 76.5]	24/29 82.8 [69.0 – 96.5]	0.51	0.76	0.32	0.49	0.02	<b>0.0013</b>	0.78
<b>Age 1-5 years</b>	56/62 90.3 [83.0 – 97.7]	97/119 81.5 [74.5 – 88.5]	8/10 80.0 [55.2 – 97.0]	88/110 80.0 [72.5 – 87.5]	99/110 90.0 [84.4 – 95.6]	0.12	0.94	0.06	0.33	0.07	0.77	0.99
<b>Age 6-10 years</b>	28/32 87.5 [76.0 – 99.0]	26/30 86.7 [74.5 – 98.8]	6/7 85.7 [59.8 – 99.6]	35/45 77.8 [65.6 – 89.9]	37/45 82.2 [71.1 – 93.4]	0.92	0.53	0.60	0.82	0.27	0.33	0.63
<b>Age &gt;10 years</b>	51/60 85.0 [77.7 – 95.2]	81/93 87.1 [80.3 – 93.9]	8/13 61.5 [35.1 – 88.0]	73/84 86.9 [79.7 – 94.1]	77/84 91.7 [85.8 – 97.6]	0.71	0.21	0.32	<b>0.002</b>	0.74	0.36	<b>0.02</b>
<b>Respiratory TB</b>	111/121 91.7 [86.8 – 96.6]	157/177 88.7 [84.0 – 93.4]	15/17 88.2 [72.9 – 98.5]	141/171 82.5 [76.8 – 88.2]	153/171 89.5 [84.9 – 94.1]	0.39	0.51	0.81	0.87	0.02	0.09	0.54
<b>Miliary and/or CNS TB</b>	15/19 78.9 [60.6 – 97.3]	35/50 70.0 [57.3 – 82.7]	6/10 60.0 [29.6 – 90.4]	16/36 44.4 [28.2 – 60.7]	26/36 72.2 [57.6 – 86.9]	0.45	0.58	0.82	0.45	<b>0.014</b>	<b>0.017</b>	0.38
<b>Other disease focus †</b>	33/42 78.6 [68.4 – 92.6]	51/58 87.9 [79.5 – 96.3]	3/6 50.0 [10.0 – 90.0]	56/61 91.8 [84.9 – 98.7]	58/61 95.1 [89.7 – 98.9]	0.21	<b>0.010</b>	0.16	<b>0.0002</b>	0.05	0.48	<b>0.002</b>
<b>Pre-existing comorbidities</b>	26/31 83.9 [70.9 – 96.8]	31/44 70.5 [57.0 – 83.9]	5/11 45.5 [16.0 – 74.9]	32/43 74.4 [61.4 – 87.5]	35/43 81.4 [69.8 – 93.0]	0.18	0.78	0.23	<b>0.015</b>	0.33	0.68	0.06
<b>Immunocompromise (including HIV)</b>	13/17 76.5 [56.3 – 96.6]	16/26 61.5 [42.8 – 80.2]	4/8 50.0 [15.4 – 84.6]	15/25 60.0 [40.8 – 79.2]	17/25 68.0 [49.7 – 86.3]	0.31	0.55	0.63	0.35	0.26	0.91	0.62

\* Two-sided Wald Chi-square tests with Bonferroni correction ( $\alpha=0.017$ ) to account for multiple comparisons.

† Other disease focus comprised: lymph node, osteoarticular, genitourinary, abdominal, and pericardial TB.

CNS = central nervous system; HIV = human immunodeficiency virus; TB = tuberculosis.

**Table E3.** Summary of the test performance of interferon-gamma release assays and the tuberculin skin test (at  $\geq 10$  mm and  $\geq 5$  mm cut-offs) in children with tuberculosis according to ethnic background.

	No. of patients with positive test result / No. of patients tested Test sensitivity [95%CI]					QFT-Plus vs. QFT-GIT*	TST5 vs.			TST10 vs.		
	QFT-Plus n=358	QFT-GIT n=600	T-SPOT. TB n=58	TST10 n=636	TST5 n=636		QFT-Plus*	QFT-GIT*	T-SPOT. TB*	QFT-Plus*	QFT-GIT*	T-SPOT. TB*
<b>White / Caucasian</b>	136/157 86.6 [80.3 - 91.5]	268/315 85.1 [80.6 - 88.8]	36/42 85.7 [71.5 - 94.6]	257/291 88.3 [84.1 - 91.7]	275/291 94.5 [91.2 - 96.8]	0.65	<b>0.004</b>	<b>0.0001</b>	0.03	0.61	0.24	0.63
<b>Black / African American</b>	36/39 92.3 [79.1 - 98.4]	40/47 85.1 [71.7 - 93.8]	3/8 37.5 [8.52 - 75.5]	35/39 89.7 [75.7 - 97.1]	36/39 92.3 [79.1 - 98.4]	0.29	1.00	0.29	<b>0.0002</b>	0.69	0.52	<b>0.0006</b>
<b>Asian</b>	26/31 83.8 [66.3 - 94.5]	37/38 97.3 [86.2 - 99.9]	2/2 100.0 [15.8 - 100.0]	33/39 84.6 [69.5 - 94.1]	36/39 92.3 [79.1 - 98.4]	0.05	0.27	0.32	0.28	0.93	0.05	0.54
<b>Arab / Berber</b>	25/31 80.6 [62.5 - 92.5]	17/19 89.4 [66.8 - 98.7]	1/2 50.0 [1.26 - 98.7]	26/33 78.7 [61.1 - 91.1]	27/33 81.8 [64.5 - 93.0]	0.41	0.91	0.46	0.27	0.85	0.33	0.35
<b>Hispanic / Latin American</b>	28/34 82.3 [65.5 - 93.2]	19/23 82.6 [61.2 - 95.1]	0/1 0 [0.00 - 97.5]	25/40 62.5 [45.8 - 77.3]	32/40 80 [64.3 - 90.9]	0.98	0.79	0.79	0.06	0.06	0.09	0.21

\* Two-sided Wald Chi-square tests with Bonferroni correction ( $\alpha=0.017$ ) to account for multiple comparisons.

**Table E4.** Comparison of retrospective and prospective study arms regarding demographics, BCG vaccination status, TB confirmation status, site of disease, and test performance of interferon-gamma release assays and the tuberculin skin test (at  $\geq 10$  mm and  $\geq 5$  mm cut-offs).

	Retrospective arm (n=607)	Prospective arm (n=394)	Statistical comparison *
<b>Baseline characteristics</b>			
Age (years), median (IQR)	5.5 (2.3 - 12.4)	5.8 (2.6 - 11.6)	0.72
Gender (male) <sup>†</sup>	329 (54.2)	208 (52.9)	0.70
Ethnic background			
White / Caucasian	385 (63.4)	120 (30.5)	<b>&lt;0.001</b>
Black / African American	63 (10.4)	28 (7.1)	
Asian	49 (8.1)	21 (5.3)	
Arab / Berber	20 (3.3)	32 (8.1)	
Hispanic / Latin American	23 (3.8)	34 (8.6)	
Mixed race	4 (0.7)	16 (4.0)	
Unknown	63 (10.4)	143 (36.3)	
Pre-existing comorbidities <sup>†</sup>	81 (13.3)	55 (14.0)	0.77
Underlying immunocompromise			
HIV infection	5 (0.8)	3 (0.8)	0.88
Immunosuppressive treatment	12 (2.0)	8 (2.0)	
Other immune disorder	27 (4.6)	22 (5.8)	
BCG vaccination status			
BCG-vaccinated	138 (22.7)	97 (24.6)	0.79
BCG-unvaccinated	395 (65.1)	251 (63.7)	
Unknown	74 (12.2)	46 (11.7)	
Microbiologically-confirmed TB	316 (52.1)	172 (43.7)	<b>0.009</b>
Site of disease			
Respiratory	457 (75.3)	289 (73.4)	0.50
Miliary and/or CNS	55 (9.1)	33 (8.4)	
Other focus <sup>†</sup>	94 (15.5)	72 (18.3)	
<b>Test sensitivity</b>			
QFT-Plus	119/138 86.2 [80.0 - 92.0]	181/220 82.3 [76.6 - 87.1]	0.25
QFT-GIT	395/455 86.8 [83.3 - 89.7]	118/145 81.4 [74.1 - 87.4]	0.10
T-SPOT.TB	19/27 70.4 [49.8 - 86.2]	26/31 83.8 [66.3 - 94.5]	0.22
TST5	295/317 93.1 [89.7 - 95.6]	277/319 86.8 [82.6 - 90.3]	<b>0.009</b>
TST10	276/317 87.1 [82.8 - 90.5]	254/319 79.6 [74.8 - 83.9]	<b>0.012</b>
* P-values based on two-sided Wald Chi-square tests, except for the age comparison (Mann Whitney <i>U</i> test).			
<sup>†</sup> Other disease focus comprised: lymph node, osteoarticular, genitourinary, abdominal, and pericardial TB.			
BCG = bacille Calmette Guérin; CNS = central nervous system; HIV = human immunodeficiency virus; IQR = interquartile range; TB = tuberculosis; TST = tuberculin skin test.			

**Table E5.** Detailed breakdown of test results in patients with confirmed TB versus those with unconfirmed TB, and in patients with immunocompromise and those without known immunocompromise.

	Entire cohort n=1001	Confirmed TB n=488	Unconfirmed TB n=513	p	Immuno-compromise * n=77	No Immuno-compromise n= 924	p
<b>QFT-Plus (n=358)</b>	<b>n=358</b>	<b>n=182</b>	<b>n=176</b>		<b>n=24</b>	<b>n=334</b>	
Positive	300 (83.8)	159 (87.4)	141 (80.1)	0.06	18 (75.0)	282 (84.4)	0.22
Negative	47 (13.1)	17 (9.3)	30 (17.0)	0.03	5 (20.8)	42 (12.6)	0.22
Indeterminate	11 (3.1)	6 (3.3)	5 (2.8)	0.80	1 (4.2)	10 (3.0)	0.53
<b>QFT-GIT (n=600)</b>	<b>n=600</b>	<b>n=285</b>	<b>n=315</b>		<b>n=47</b>	<b>n=553</b>	
Positive	513 (85.5)	243 (85.3)	270 (85.7)	0.87	28 (59.6)	485 (87.7)	<0.0001
Negative	66 (11.0)	25 (8.8)	41 (13.0)	0.09	18 (38.3)	48 (8.7)	<0.0001
Indeterminate	21 (3.5)	17 (6.0)	4 (1.3)	0.003	1 (2.1)	20 (3.6)	<0.0001
<b>T-SPOT. TB (n=58)</b>	<b>n=58</b>	<b>n=33</b>	<b>n=25</b>		<b>n=11</b>	<b>n=47</b>	
Positive	45 (77.6)	24 (72.7)	21 (84.0)	0.30	5 (45.5)	40 (85.1)	0.010
Negative	8 (13.8)	6 (18.2)	2 (8.0)	0.26	5 (45.5)	3 (6.4)	0.004
Indeterminate	5 (8.6)	3 (9.1)	2 (8.0)	0.88	1 (9.1)	4 (8.5)	1.00
<b>TST ≥10 mm (n=636)</b>	<b>n=636</b>	<b>n=268</b>	<b>n=368</b>		<b>n=44</b>	<b>n=592</b>	
Positive	530 (83.3)	213 (79.5)	317 (86.1)	0.03	26 (59.1)	504 (85.1)	<0.0001
Negative	106 (16.7)	55 (20.5)	51 (13.9)	0.03	18 (40.9)	88 (14.9)	<0.0001
<b>TST ≥5mm (n=636)</b>	<b>n=636</b>	<b>n=268</b>	<b>n=368</b>		<b>n=44</b>	<b>n=592</b>	
Positive	572 (89.9)	237 (88.4)	335 (91.0)	0.28	30 (68.2)	542 (91.6)	<0.0001
Negative	64 (10.1)	31 (11.6)	33 (9.0)	0.28	14 (31.8)	50 (8.4)	<0.0001

\* Immunocompromise includes children with immune disorder (n=57) and children receiving immunosuppressive treatment (n=20).

**Table E6.** Proportion of agreement and Cohen's Kappa coefficient between categorical tuberculin skin test results ( $\geq 5$  mm and  $\geq 10$  mm cut-offs) and the categorical results of the three interferon-gamma release assays. Indeterminate assay results were excluded from this analysis.

	TST5		Agreement	Cohen's K	TST10		Agreement	Cohen's K
	Negative	Positive			Negative	Positive		
<b>QFT-Plus</b>								
Negative	16	19	87.3	0.462	20	15	83.7	0.429
Positive	9	177			21	165		
<b>QFT-GIT</b>								
Negative	16	29	87.6	0.343	19	26	84.1	0.301
Positive	17	309			33	293		
<b>T-SPOT.TB</b>								
Negative	1	2	88.6	0.271	1	2	68.6	0.025
Positive	2	30			9	23		