Eur Respir J 2013; 41: 909–916
DOI: 10.1183/09031936.00194311

Evaluation of QuantiFERON microtube, using 0.9 mL blood, for diagnosing tuberculosis infection

Michala V. Rose*, Godfather Kimaro#, Inge Kroidl¶,+, Michael Hoelscher+, Ib C. Bygbjerg*, Sayoki M. Mfinanga# and Pernille Ravn§

ABSTRACT: The performance of QuantiFERON microtube (QFT-MT), using 0.9 mL blood, and QuantiFERON-TB Gold in-tube test (QFT-IT) (3 mL blood), for diagnosing tuberculosis (TB) was compared in children and adults in an endemic setting.

In 152 children with suspected TB and 87 adults with confirmed TB, QFT-IT was compared with two QFT-MT concentrations (QFT-MT A and B). Proportions of positive and indeterminate results, interferon (IFN)-γ responses, interassay agreement and sensitivity were assessed.

We found similar proportions of indeterminate results, levels of IFN-γ and comparable sensitivity. The interassay agreement was moderate in all children (QFT-IT *versus* QFT-MT A: 85%, k=0.44 and QFT-IT *versus* QFT-MT B: 88%, k=0.50) and adults (QFT-IT *versus* QFT-MT A: 88%, k=0.50 and QFT-IT *versus* QFT-MT B: 89%, k=0.49). Sensitivity was low (QFT-IT 23%, QFT-MT A 18% and B 19%) in children with confirmed or highly probable TB compared with adults (83%, 86% and 88%, respectively).

The QFT-MT test can be reliably performed using less than one-third of the blood volume used in QFT-IT. The reduced volume may be useful for research and future diagnosis of paediatric TB. The poor sensitivity and high indeterminate rate of both IFN-γ release assays in severely ill children, with immature or impaired immunity in an endemic setting, warrants further investigations.

KEYWORDS: Children, interferon-γ release assay, QuantiFERON-TB Gold in-tube, tuberculin skin test, tuberculosis

It is estimated that children account for as much as 10–20% of the total tuberculosis (TB) burden in a TB endemic area, such as Tanzania, and tools for diagnosing active TB in children are limited both with regard to performance [1] and access to tools, such as chest radiography, tuberculin skin test (TST), microscopy and culture, in most TB endemic areas [2, 3]; new diagnostic tools, especially for paediatric TB, are urgently needed [4].

Interferon (IFN)-γ release assays (IGRAs) are increasingly being used worldwide as an alternative to the TST for the diagnosis of infection with *Mycobacterium tuberculosis* [5]. The tests detect infection with *M. tuberculosis* and their main use is in diagnosing latent TB infection. However, IGRAs are used by clinicians as an additional tool in the diagnosis of active TB [6, 7]; an IGRA can be a useful diagnostic tool in young children, as a positive IGRA in very young children reflects recent exposure and thereby a high risk of active TB.

There are two commercially available IGRAs, QuantiFERON-TB Gold in-tube (QFT-IT; Cellestis Ltd, Chadstone, Australia) and T-SPOT-TB (T-SPOT; Oxford Immunotec, Abingdon, UK). QFT-IT measures the levels of IFN-γ released in whole blood in response to stimulation with the *M. tuberculosis*-specific early secreted antigen (ESAT)-6, culture filtrate protein (CFP)-10 and TB7.7 antigen, whilst T-SPOT uses ELISPOT methodology to identify the number of purified lymphocytes that respond to ESAT-6 and CFP-10. QFT-IT involves the collection of 3 mL of venous blood (1 mL into each of three tubes) and T-SPOT generally requires 8 mL of blood. Wide use of these tests may be limited by the volume of blood required, especially in infants and young children [8, 9], and clinicians may be discouraged to use the tests [10].

In an attempt to address this issue, Cellestis has developed a prototype version of QFT-IT that requires a total of only 0.9 mL blood (QuantiFERON-microtube (QFT-MT)). The principle

AFFILIATIONS
*Dept of International Health, Immunology and Microbiology, University of Copenhagen, Copenhagen, and
§Dept for Infectious Diseases, University Hospital Odense, Odense, Denmark.
#Muhimbili Medical Research Centre, National Institute for Medical Research, Dar es Salaam, and
¶NIMR-Mbeya Medical Research Programme, Mbeya, Tanzania.
+Dept of Infectious Diseases and Tropical Medicine, Klinikum of the University of Munich, Munich, Germany.

CORRESPONDENCE
P. Ravn
Dept of Infectious Diseases
University Hospital Hillerød
Dyrehavevej 29
DK-3400 Hillerød
Denmark
E-mail: peravn@gmail.com

Received:
Nov 07 2011
Accepted after revision:
July 11 2012
First published online:
Aug 09 2012

European Respiratory Journal
Print ISSN 0903-1936
Online ISSN 1399-3003

of QFT-MT is the same as of QFT-IT. Whole blood from the patient is incubated in a tube containing the *M. tuberculosis*-specific antigens, and thereafter levels of IFN-γ are measured by ELISA technique. The performance and feasibility of QFT-MT has not been evaluated in a clinical setting.

The aim of this study was to determine if QFT-MT is an adequate alternative to QFT-IT by comparing proportions of positive, negative and indeterminate results, levels of IFN-γ, interassay agreement and sensitivity for diagnosing active TB in children and in adults in a region where TB is endemic. In addition, the practical implementation of QFT-MT will be assessed.

MATERIALS AND METHODS

Study setting and population

The study participants were recruited prospectively from Muheza designated district hospital (Tanga, Tanzania) from December 2008 to May 2010. The hospital has a catchment area of 277 000 people and the district had a TB notification rate of 348 per 100 000 population in 2009 [11, 12]. Children aged <15 years with signs and symptoms suspect of active TB (table 1) were included as part of a larger hospital-based study on the performance of QFT-IT for diagnosing active TB in children [13]. Adults aged ⩾15 years, with sputum smear-positive TB, confirmed by either fluorescence microscopy or culture, were included from the hospital TB clinic according to the criteria in table 1. As a substudy of [13], 152 children and 87 adults who fulfilled the inclusion criteria and had paired QFT-IT and QFT-MT results were consecutively enrolled. All study participants provided a sample for microbiological examination and HIV and TST tests was performed.

A standardised questionnaire was used to record demographic and clinical details of the participants. Children were assessed at 2- and 6-month follow-ups for confirmation of diagnosis and classification into diagnostic groups.

INF-γ release assay

Venous blood was collected in a syringe and immediately dispensed into the QFT-IT and QFT-MT tubes according to the manufacturer's instructions. 1 mL was dispensed into each of the QFT-IT tubes and 300 μL into each of the QFT-MT tubes. The tubes were taken to the laboratory within 4 h and incubated at 37°C for 16–24 h. Both IGRAs included pre-coated tubes: a nil tube containing saline as a negative control; a mitogen tube containing phytohaemaglutinin as a positive control; and a TB-antigen tube containing the peptides ESAT-6, CFP-10 and TB7.7. The QFT-MT prototype was tested with two different concentrations of ESAT-6, CFP-10 and TB7.7 in the tubes designated TB-antigen (Ag) A and TB-Ag B, containing 1 $\mu g \cdot mL^{-1}$ and 3 $\mu g \cdot mL^{-1}$, respectively, of each of the peptides, with test results reported as either QFT-MT A or B. The concentration in the QFT-IT was 1 $\mu g \cdot mL^{-1}$. For 10 patients (seven children and three adults) the QFT-MT prototype was tested only with the TB-Ag A tubes due to shortage of TB-Ag B tubes.

Immediately after incubation the samples were centrifuged and the supernatants stored at -70°C until IFN-γ was measured using the QFT-IT ELISA at NIMR-Mbeya Medical Research Centre, Mbeya, Tanzania. The QFT-IT, QFT-MT A and B results were reported as positive, negative or indeterminate according to the manufacturer's instructions, with QFT-MT A and B interpreted using the same criteria. In addition, the quantitative results were recorded.

We used a microcentrifuge (Eppendorf 5418) for centrifugation of QFT-MT tubes for 3 min at 10 000 rpm prior to harvesting the plasma samples. The laboratory technician experienced no problem in removing the plasma without disrupting the pellet, even though the microtubes did not contain the separation gel used in the QFT-IT tubes. The microcentrifuge broke down just prior to the conclusion of data collection. Thus 33 samples (22 children and 11 adults) were centrifuged using the standard laboratory centrifuge (Vulcon Tech CS6C), which resulted in equally good separation of the plasma from the pellet.

Tuberculin skin testing

Two units of purified protein derivate RT23 from Staten Serum Institute (Copenhagen, Denmark), were administered intradermally using the Mantoux technique recommended by the manufacturer. The transverse diameter of the induration was recorded in millimetres after 48–72 h by specifically trained staff. An induration of ⩾10 mm was considered positive, unless HIV infected, in which case an induration of ⩾5 mm was considered positive.

TB diagnosis

TB diagnosis was performed as described in [13]. In brief a sputum sample was collected from the adults and either sputum or gastric wash samples were collected on three consecutive mornings for the children and sent for confirmatory microscopy and culture to the Central TB Reference Laboratory (CTRL) (Dar es Salaam, Tanzania). Auramine staining was used for fluorescence microscopy and Löwenstein–Jensen solid media for culture. Para-nitrobenzoic acid, which inhibits *M. tuberculosis* but not nontuberculous mycobacteria, was added in positive samples to exclude nontuberculous mycobacteria.

Each child was assigned to one of four groups ''confirmed'', ''highly probable'', ''possible TB'', and ''not TB''. Based on microbiological data, chest radiography results, clinical examination conducted by the study team and follow-up data (table 2), in line with the recent expert consensus agreement on the classification of TB in children [17]. Only children with complete follow-up data were included. Only adults diagnosed with TB based on a positive microscopy by Ziehl–Neelsen staining, as well as confirmation by either positive fluorescence microscopy or positive culture, were included.

Data management and statistical analysis

Data were double-entered into a data entry database using MS-Access (Microsoft Corp., Alexandria, VA, USA) including error, range and consistency check programs. Statistical analyses were performed using STATA (release 10; StataCorp, College Station, TX, USA). Two sample tests for proportions were used to compare proportions of positive, negative and indeterminate results. Wilcoxon's matched-pairs sign test was used to compare median levels of IFN-γ , after logarithmic transformation of data. κ statistics were used to assess the concordance between QFT-IT and QFT-MT. The κ values were interpreted according to the Landis scale [18]. For data analysis, children with ''microbiologically confirmed TB'' and ''highly probable TB'' were classified into one group. HIV

TABLE 1 Inclusion criteria for the study

Children
Age <15 years
Reported ill for ⩾2 weeks, paired QFT-IT and QFT-MT results, follow-up data and one or more of the following:
Fever ⩾2 weeks
Cough ⩾2 weeks
Reported weight loss or failure to gain weight ⩾2 weeks
Exposure to definite or probable case of TB in last 2 years
Admitted to hospital or contact to health facility ⩾2 times in last 3 months
Z-score ⩽ -2
Adults
Adults ⩾15 years with smear-positive TB by Ziel–Neelsen staining, confirmed by fluorescence microscopy or culture and paired QFT-IT and QFT-MT results

QFT-IT: QuantiFERON-TB Gold in-tube; QFT-MT: QuantiFERON-microtube; TB: tuberculosis.

status, age <2 years and malnutrition status, as determined by z-score ⩽ -2 or body mass index (BMI) <18.5 kg·m^{-2}, were preselected for the risk factor analysis for a positive or indeterminate IGRA result, using logistic regression analysis. A p-value of <0.05 was considered significant.

Ethics

The study protocol was approved by the Tanzanian Medical Research Coordinating Committee (NIMR/HQ/R.8a/Vol IX/584) and was evaluated by the Danish Central Ethical Committee without any objections. Participants with a positive HIV test were accompanied to the HIV clinic for referral. The standards for reporting diagnostic accuracy studies (STARD) were followed for reporting results (www.stard-statement.org).

RESULTS

Study populations

A total of 152 children with suspect signs and symptoms for TB and 87 adults with confirmed TB were eligible (table 3). Two children were positive by *M. tuberculosis* culture and 25 children fulfilled the criteria for highly probable TB, thus 27 were classified with ''confirmed or highly probable TB'', 59 were classified with ''possible TB'' and 66 with ''not TB''. Amongst the 87 adults with TB, diagnosis was confirmed in 68 by both fluorescence microscopy and positive culture, in 15 by microscopy alone and in four by culture alone.

QFT-IT and QFT-MT results

Overall, there were no differences in the results of the three tests (QFT-IT, QFT-MT A and QFT-MT B). Among 152 children, we found 20 (13%) positive, 93 (61%) negative and 39 (26%) indeterminate QFT-IT results (fig. 1a). The QFT-MT A and QFT-MT B results were similar, with 17 (11%) and 17 (12%) positive, 100 (66%) and 93 (64%) negative and 35 (23%) and 35 (24%) indeterminate results, respectively. Comparing the proportions of positive, negative and indeterminate responders between QFT-IT, QFT-MT A and QFT-MT B, there were no significant differences between any of the tests in children or adults, calculated using the two-sample proportion test.

TABLE 2 Diagnostic classifications

Children
Confirmed or highly probable TB#
Microbiologically confirmed TB:
Clinical specimens positive for *Mycobacterium tuberculosis* on solid culture or acid-fast bacilli on microscopy
or
Highly probable TB:
Chest radiograph highly suggestive of active tuberculosis and good clinical response
or
Good clinical response and one of following:
Cervical lymphadenopathy with sinus formation
Abdominal mass or ascites
Spinal gibbus
Clinical picture of meningitis associated with CSF changes consistent with TB meningitis
Possible TB
Children who did not have confirmed or highly probable TB, but in whom active TB could not be excluded. Includes both children who were and were not put on anti-TB treatment
Not TB
One of following:
Spontaneous symptom resolution defined as well-being without TB treatment
Alternative diagnosis confirmed
Adults
Confirmed TB
Microbiologically confirmed TB by either positive culture or fluorescence microscopy

Tuberculosis (TB) classifications in accordance with previous paediatric active TB studies (LIEBESCHUETZ *et al.* [14], MARAIS *et al.* [15], BAMFORD *et al.* [16]) and in line with a recent expert panel consensus of clinical case definitions for research in children with suspected active TB [14]. #: confirmed or highly probable TB; for data analysis the combined group of microbiologically confirmed TB and highly probable TB was combined into one group ''Confirmed or highly probable TB''.

Seven children (one with confirmed TB) and three adults had only QFT-MT A results but no QFT-MT B results.

The proportions of positive, negative and indeterminate results in children with confirmed or highly probable TB and adults are shown in figures 1b and c. Among 87 adults with ''confirmed TB'', 69 (79%) had positive, 14 (16%) negative and four (5%) indeterminate QFT-IT results, whilst for QFT-MT A and B there were 68 (78%) and 67 (80%) positive, 11 (13%) and nine (11%) negative and eight (9%) and eight (10%) indeterminate results, respectively.

Interassay agreement

The strength of the agreement between QFT-IT and both QFT-MT A and B in children and adults was moderate (table 4).

Median IFN-γ levels

There was a higher median level of IFN-γ in QFT-IT tubes coated with *M. tuberculosis*-specific antigens compared with

TABLE 3 Characteristics of study populations

	Children				All adults
	Total	Confirmed or highly probable TB	Possible TB	Not TB	
Subjects	152	27	59	66	87
Age years	4.2±3.6	4.5±3.6	4.1±3.7	4.3±3.5	39.5±15.1
Age group years					
<2	54 (35.5)	6 (22.2)	21 (35.6)	27 (40.9)	
2–5	46 (30.3)	13 (48.2)	20 (33.9)	13 (19.7)	
>5	52 (34.2)	8 (29.6)	18 (30.5)	26 (39.4)	
Male	95 (62.5)	21 (77.8)	35 (59.3)	39 (59.1)	69 (79.3)
HIV-positive	54 (35.5)	14 (51.9)	27 (45.8)	13 (9.7)	25 (28.7)
z-score ⩽ -2	78 (57.8)	11 (42.3)	34 (68.0)	26 (44.1)	
BMI <18.5 kg·m^{-2}					52 (63.1)
History contact#	45 (29.8)	9 (33.3)	19 (32.2)	17 (25.8)	
BCG vaccination	145 (95.4)	24 (88.9)	58 (98.3)	63 (95.5)	
BCG scar	136 (89.5)	23 (85.2)	50 (84.8)	63 (95.5)	67 (77.0)

Data are presented as n, mean±SD or n (%), unless otherwise indicated. TB: tuberculosis; BMI: body mass index; BCG: bacille Calmette–Guerin. #: reported contact with a patient with active TB within the last 2 years.

the QFT-MT in all 152 children ($p \leqslant 0.03$) (table 5) and in the 59 children with possible TB, the median IFN-γ antigen response was lower in the QFT-MT A tubes compared with the QFT-IT ($p=0.04$) (table 5). There was no difference in IFN-γ levels in the TB antigen-coated tubes between QFT-MT A and B. In all children, irrespective of diagnostic classification grouping, there were no differences in the median levels of IFN-γ in either the nil or mitogen tubes (data not shown).

In adults, the only difference between the three tests was a significantly higher median IFN-γ level after antigen stimulation in the QFT-MT B compared with the A tube ($p<0.01$).

Sensitivity of IGRAs and TST for the diagnosis of active TB

After excluding indeterminate results, sensitivity of the IGRAs was surprisingly low in children with confirmed or highly probable TB and possible TB, but there was no significant difference between the three tests (table 6). An equally low sensitivity of TST was found in 146 children with available TST test, two (8%) out of 25 TST-positive in children with confirmed or highly probable TB and four (8%) out of 51 with possible TB (data not shown). In the 87 adults with confirmed TB, the sensitivity of the IGRAs ranged from 83% to 88% (table 6), with no differences in sensitivity between the tests. The sensitivity of the TST in 71 adults with a valid TST result was 87% (62 out of 71) (data not shown).

Risk factor analysis

We found low sensitivity and high indeterminate rates in children compared to adults. Neither HIV infection, age <2 years nor malnutrition were significantly associated with QFT-IT, QFT-MT A or QFT MT B positive or indeterminate results in this substudy (data not shown). In the larger cohort study including 211 children, age <2 years was associated with increased odds of an indeterminate result [13].

In adults, after adjusting for age and BMI, HIV infection was negatively associated with a positive QFT-IT (adjusted OR 0.21, 95% CI (0.06–0.71); $p=0.01$) and a positive QFT-MT A (0.23 (0.06–0.89); $p=0.03$), but not QFT-MT B (0.41 (0.10–1.78); $p=0.24$) (data not shown).

DISCUSSION

QFT-IT and QFT-MT A and B demonstrated commensurate performance. TB-suspect children had higher median levels of IFN-γ in the QFT-IT antigen-coated tubes than both the QFT-MT tubes, but there were similar proportions of positive, negative and indeterminate results and the sensitivity did not differ. The tests mostly had similar quantitative levels of IFN-γ, except that the QFT-MT tube with the higher antigen concentration (QFT-MT B), seemed to perform slightly better in adults than the QFT-MT A tube, with respect to median antigen-specific IFN-γ, but again the overall test performance was similar.

The interassay agreement between the two tests was moderate, which reflects considerable interassay variability. Variations in IFN-γ levels around the cut-off for a positive result may have contributed to the variability, especially in HIV-infected and young children who are likely to have lower levels of IFN-γ in response to specific antigens. A study from New York [19] has suggested that a lower cut-off value for a positive QFT-IT may be relevant in these children, but this would also result in increased interassay variability.

Like the QFT-IT, the QFT-MT A and B appeared highly limited in their performance, in this high-burden setting, in identifying the children with active TB with low sensitivities ranging from 18–23% and high indeterminate rates up to 26%. In adults with TB, the QFT-MT format performed as well as the QFT-IT test with high sensitivity and low indeterminate rates. To our knowledge, this is the first published study on the

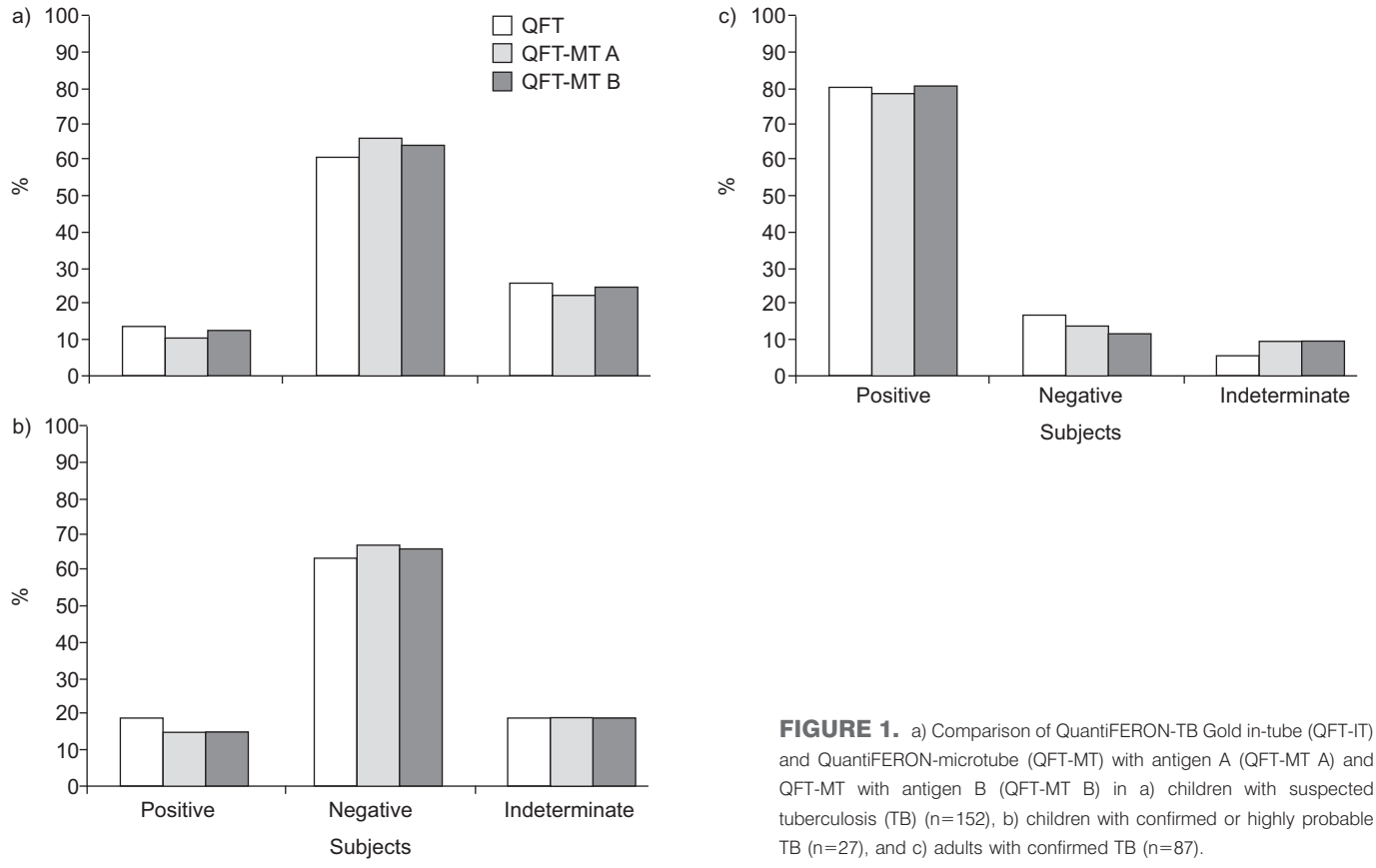

FIGURE 1. a) Comparison of QuantiFERON-TB Gold in-tube (QFT-IT) and QuantiFERON-microtube (QFT-MT) with antigen A (QFT-MT A) and QFT-MT with antigen B (QFT-MT B) in a) children with suspected tuberculosis (TB) (n=152), b) children with confirmed or highly probable TB (n=27), and c) adults with confirmed TB (n=87).

performance of QFT-MT. Our results are promising in that QFT-MT (1 and 3 μg·mL^{-1}) has commensurate performance to QFT-IT (1 μg·mL^{-1}) both in children and adults suggesting that it may be possible to reduce the volume of blood drawn without losing accuracy for the diagnosis of active TB and probably also for latent TB infection.

IGRAs are arguably the most accurate diagnostic tests for latent TB and address two of the major limitations of the TST, low specificity and logistic challenges, with tests having to be read 2–3 days after application. Despite the improvement of IGRAs over the TST, there are major obstacles in implementing the IGRAs, especially in low-resource settings, with the need for a local incubator, a nearby laboratory or freezing capacity for storage, transportation and skilled technicians for the ELISA. Simpler, safer and more flexible solutions are needed in both high- and low-resource regions. In children and infants, the volume of blood may restrict the use of the QFT-IT, especially in situations where the parent/guardian is reluctant to have blood drawn from the child or the physician is hesitant to draw blood. Reducing the amount of blood may facilitate large-scale and individual testing. Many improvements to the IGRAs are anticipated, *i.e.* test systems using other biomarkers, such as IFN-γ-induced protein (IP)-10, which can be stored on filter paper, point of care tests and a ''lab on a chip'' system [20]. Our results may pave the way for development of improved assays based on even smaller amounts of blood.

We found low sensitivity and a high indeterminate rate of QFT-IT, as in the larger cohort study evaluating the use of QFT-IT in the diagnosis of childhood TB in Tanzania [13], as well as of QFT-MT A and B, in children.

TABLE 4 Agreement between QuantiFERON-TB Gold in-tube (QFT-IT), QuantiFERON-microtube with antigen A (QFT-MT A) and with antigen B (QFT-MT B) test

	QFT-IT and QFT-MT A			QFT-IT and QFT-MT B			QFT-IT-MT A and B		
	%	κ	SE	%	κ	SE	%	κ	SE
All children	84.8	0.44	0.10	87.8	0.50	0.10	87.9	0.48	0.10
Adults with confirmed TB	88.2	0.50	0.11	89.0	0.49	0.12	90.8	0.58	0.11

Data are shown excluding indeterminate results.

TABLE 5 Comparison of median interferon (IFN)-γ levels in the tuberculosis (TB) antigen tubes for QuantiFERON-TB Gold in-tube (QFT-IT), QuantiFERON-microtube with antigen A (QFT-MT A) and with antigen B (QFT-MT B)

	Subjects n	IFN-γ U·mL^{-1}			p-value#	p-value¶	p-value+
		QFT-IT	QFT-MT A	QFT-MT B			
Children							
All TB suspect	152	0.19 (0.12–0.53)	0.17 (0.09–0.32)	0.17 (0.10–0.42)	<0.01	0.03	0.22
Confirmed or highly probable TB	27	0.17 (0.11–0.53)	0.17 (0.10–0.30)	0.19 (0.10–0.42)	0.67	0.73	0.53
Possible TB	59	0.23 (0.11–0.56)	0.13 (0.07–0.33)	0.15 (0.09–0.53)	0.04	0.62	0.06
Not TB	66	0.19 (0.12–0.49)	0.19 (0.11–0.32)	0.17 (0.10–0.36)	0.90	0.34	0.77
Adults with confirmed TB	87	3.24 (0.95–6.58)	2.89 (0.89–7.73)	3.71 (1.12–8.99)	0.69	0.29	<0.01

Data are presented as median (interquartile range), unless otherwise stated. Median levels of IFN-γ measured in the TB antigen tube in QFT-IT, QFT-MT A and B with Wilcoxon's matched-pairs sign test were used to test differences in median IFN-γ levels between: #: QFT-IT and QFT-MT A; ¶: QFT-IT and QFT-MT B; and +: QFT-MT A and QFT-MT B. $p<0.05$ was considered significant.

The sensitivity in this population was lower than reported in some studies [21] and the indeterminate rate as high as reported in other studies [22, 23]. High indeterminate rates and low sensitivity have been attributed to host factors, such as young age [22, 24, 25], HIV infection with decreasing CD4 count [26–28], malnutrition and helminth infection [29–31]. Poor sensitivity may also be explained by misclassification or overdiagnosis of TB in these very sick children or by poor technical technique.

Risk factor analysis has demonstrated that the low sensitivity and high indeterminate rate could not be explained by any one single factor but rather by a combination of factors leading to impaired T-cell response, such as malnutrition, severity of disease, HIV infection, young age and immature immunity [13].

The low sensitivity suggests that the use of both IGRAs as well as TST cannot be recommended for the diagnosis of active TB in this setting and World Health Organization has recently stated that neither IGRA nor TST should be used for the diagnosis of active TB disease in low- and middle-income countries at all [32]. In high-income, low-TB-burden countries, however, IGRAs play an important role in diagnosis of latent TB and in the diagnostic work-up for active TB in children in conjunction with microbiological methods. In this setting, QFT-MT may be a useful, lower-blood volume alternative.

Another explanation for the poor performance in children could be inadequate technique or laboratory errors. But in contrast to children, all the IGRAs performed well in adults with confirmed TB, and since the QFT-IT and QFT-MT samples of both children and adults were taken and processed concurrently, at the same hospital using the same laboratory staff, equipment and standard operating procedures, we find it highly unlikely that the poor performance in children was due to technical errors.

The microtube format holds some, but few limitations. The amount of plasma is limited and there may be little scope for re-running samples, if needed for confirmation of results or evaluation of intra-test variability. In our study, none of the QFT-MT samples were re-run, but we would expect variation

TABLE 6 Sensitivity of QuantiFERON®-TB Gold in-tube (QFT-IT), QuantiFERON-microtube with antigen A (QFT-MT A) and with antigen B (QFT-MT B) in children and adults with active tubeculosis (TB)

	QFT-IT positive			QFT-MT A positive				QFT-MT B positive			
	n/total tested	%	95% CI	n/total tested	%	95% CI	p-value#	n/total tested	%	95% CI	p-value¶
Children											
Confirmed or highly probable TB	5/22	22.7	4.5–41.0	4/22	18.2	1.4–34.9	0.71	4/21	19.1	1.5–36.6	0.77
Possible TB	9/42	21.4	8.7–34.1	8/41	19.5	7.1–31.9	0.83	11/38	29.0	14.2–43.7	0.43
Not TB	6/49	12.2	2.9–21.6	5/54	9.3	1.4–17.1	0.63	2/51	3.9	-1.5–9.4	0.13
Adults											
Confirmed TB	69/83	83.1	74.9–91.4	68/79	86.1	78.3–93.9	0.60	67/76	88.2	80.7–95.6	0.36

Indeterminate results were excluded. A two-sample proportion test used to test difference between sensitivity of #QFT-IT and QFT-MT A, and ¶QFT-IT and QFT-MT B.

similar to the QFT-IT. This issue is a matter of concern and there is an ongoing debate about how to interpret reversions, conversions and borderline positive and negative results [33], which must be considered if the QFT-MT format should be taken forward. One practical issue reported was that the space for writing identification details on the tubes was very small, otherwise the study team found it easy to use the QFT-MT format.

There were no problems in filling the tubes using a syringe through the cap, and a black line clearly marks the 300 μL mark. Although a microcentrifuge is recommended by the manufacturer for separating plasma in the QFT-MT tubes, we had to use the standard laboratory centrifuge for a small number of the samples due to breakdown of the microcentrifuge. This was found to be equally suited to separating supernatant from the pellet. Therefore, it should be possible to process the QFT-MT tubes with exactly the same equipment as QFT-IT.

Conclusion

Overall, the performance of the QFT-MT test using 0.9 mL of blood was equal to the QFT-IT test using 3 mL of blood. There were no differences between the proportions of positive, negative and indeterminate results and there was good interassay agreement. The performance of the QFT-MT test at 1 $\mu g \cdot mL^{-1}$, and 3 $\mu g \cdot mL^{-1}$ was commensurate with a trend toward higher IFN-γ levels in the 3-$\mu g \cdot mL^{-1}$ tube in adults. Technically, the QFT-MT requires little or no adaptation and no significant practical difficulties were reported.

The mictrotube format has potential as a low blood volume test and these promising preliminary results should stimulate further large-scale investigations, while the high indeterminate rate and poor sensitivity of both IGRAs in severely ill children with immature or impaired immunity in a high burden setting warrants further investigations.

To our knowledge this is the first published study on the performance of QFT-MT and the perspective may be to further reduce the volume of blood, *i.e.* in a point of care test.

SUPPORT STATEMENT

The research was funded by grants to the IMPACT-TB project, as part of a PhD thesis. Funding was also provided by Reinholdt Jorck's Fund, Cluster in International Health, the University of Copenhagen (211-0357/07-3012), Justesens Fund, the Danish Research Council (09-064923), the KNCV (U.07.0674) and Danish International Development Assistance (74-08-UHH DANIDA). Cellestis accepted the study protocol and provided the QuaniFERON microtubes, ELISA reagents and microcentrifuge but Cellestis were not otherwise involved in the design, analysis of results or writing of the manuscript.

STATEMENT OF INTEREST

None declared.

ACKNOWLEDGEMENTS

The authors would like to thank patients and parents/guardians, the staff and administration at the Muheza Designated District Hospital (Muheza, Tanzania) for facilitating the research. The also thank B. Amos (Teule Hospital) and laboratory staff at NIMR-Mbeya Medical Research Programme for invaluable help with laboratory issues, the Regional and District TB coordinators, L. Kijazi (Bombo Hospital, Tanga Region, Tanzania) and M. Fadhili (Muheza Designated District Hospital, Muheza District, Tanga, Tanzania) for assistance and supportive supervision and the Joint Malaria Programme for administrative support.

REFERENCES

1 Marais BJ, Gie RP, Schaaf HS, *et al.* Childhood pulmonary tuberculosis: old wisdom and new challenges. *Am J Respir Crit Care Med* 2006; 173: 1078–1090.
2 Davies PD, Pai M. The diagnosis and misdiagnosis of tuberculosis. *Int J Tuberc Lung Dis* 2008; 12: 1226–1234.
3 Pai M, Zwerling A, Menzies D. Systematic review: T-cell-based assays for the diagnosis of latent tuberculosis infection: an update. *Ann Intern Med* 2008; 149: 177–184.
4 Marais BJ, Schaaf HS. Childhood tuberculosis: an emerging and previously neglected problem. *Infect Dis Clin North Am* 2010; 24: 727–749.
5 Pai M, Castro K, Mori T, *et al.* Guidelines on IGRAs: concordant or discordant. *Int J Tuberc Lung Dis* 2010; 14: Suppl. 1, S64–S70.
6 Diel R, Goletti D, Ferrara G, *et al.* Interferon-γ release assays for the diagnosis of latent *Mycobacterium tuberculosis* infection: a systematic review and meta-analysis. *Eur Respir J* 2011; 37: 88–99.
7 Sester M, Sotgiu G, Lange C, *et al.* Interferon-γ release assays for the diagnosis of active tuberculosis: a systematic review and meta-analysis. *Eur Respir J* 2011; 37: 100–111.
8 Lewinsohn DA, Zalwango S, Stein CM, *et al.* Whole blood interferon-γ responses to mycobacterium tuberculosis antigens in young household contacts of persons with tuberculosis in Uganda. *PLoS One* 2008; 3: e3407.
9 Mazurek M, Jereb J, Vernon A, *et al.* Updated guidelines for using interferon γ release assays to detect *Mycobacterium tuberculosis* infection - United States, 2010. *MMWR Recomm Rep* 2010; 59: 1–25.
10 National Institute for Health and Clinical Excellence. NICE Clinical Guideline for Tuberculosis. 2011. www.nice.org.uk/guidance/CG117 Date last accessed: February 4, 2013.
11 Tanzania National Bureau of Statistics. 2002 Population and Housing Census. Tanzania National Bureau of Statistics, Dar es Salaam, Tanzania. 2010. www.nbs.go.tz/takwimu/references/2002popcensus.pdf
12 Kijazi LJ. Annual Implementation Report 2007. Tuberculosis and Leprosy. TB/HIV. Tanga Region. 2008.
13 Rose MV, Kimaro G, Nissen TN, *et al.* QuantiFERON®-TB Gold in-tube performance for diagnosing active tuberculosis in children and adults in a high burden setting. *PLoS One* 2012; 7: e37851.
14 Liebeschuetz S, Bamber S, Ewer K, *et al.* Diagnosis of tuberculosis in South African children with a T-cell-based assay: a prospective cohort study. *Lancet* 2004; 364: 2196–2203.
15 Marais BJ, Gie RP, Hesseling AC, *et al.* A refined symptom based approach to diagnose pulmonary tuberculosis in children. *Pediatrics* 2006; 118: e1350–e1359.
16 Bamford AR, Crook AM, Clark JE, *et al.* Comparison of interferon-γ release assays and tuberculin skin test in predicting active tuberculosis (TB) in children in the UK: a Paediatric TB Network study. *Arch Dis Child* 2010; 95: 180–186.
17 Graham SM, Ahmed T, Amanullah F, *et al.* Evaluation of tuberculosis diagnostics in children: 1. proposed clinical case definitions for classification of intrathoracic tuberculosis disease. Consensus from an expert panel. *J Infect Dis* 2012; 205: Suppl. 2, S199–S208.
18 Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics* 1977; 33: 159–174.
19 Lighter J, Rigaud M, Eduardo R, *et al.* Latent tuberculosis diagnosis in children by using the QuantiFERON-TB Gold in-tube test. *Pediatrics* 2009; 123: 30–37.
20 Ruhwald M, Aabye MG, Ravn P. IP-10 release assays in the diagnosis of tuberculosis infection: current status and future directions. *Expert Rev Mol Diagn* 2012; 12: 175–187.
21 Mandalakas AM, Detjen AK, Hesseling AC, *et al.* Interferon-γ release assays and childhood tuberculosis: systematic review and meta-analysis. *Int J Tuberc Lung Dis* 2011; 15: 1018–1032.
22 Dogra S, Narang P, Mendiratta DK, *et al.* Comparison of a whole blood interferon-γ assay with tuberculin skin testing for the

detection of tuberculosis infection in hospitalized children in rural India. *J Infect* 2007; 54: 267–276.
23 Haustein T, Ridout DA, Hartley JC, *et al.* The likelihood of an indeterminate test result from a whole-blood interferon-γ release assay for the diagnosis of *Mycobacterium tuberculosis* infection in children correlates with age and immune status. *Pediatr Infect Dis J* 2009; 28: 669–673.
24 Tsiouris SJ, Austin J, Toro P, *et al.* Results of a tuberculosis-specific IFN-γ assay in children at high risk for tuberculosis infection. *Int J Tuberc Lung Dis* 2006; 10: 939–941.
25 Bergamini BM, Losi M, Vaienti F, *et al.* Performance of commercial blood tests for the diagnosis of latent tuberculosis infection in children and adolescents. *Pediatrics* 2009; 123: e419–e424.
26 Aabye MG, Ravn P, PrayGod G, *et al.* The impact of HIV infection and CD4 cell count on the performance of an interferon-γ release assay in patients with pulmonary tuberculosis. *PLoS One* 2009; 4: e4220.
27 Raby E, Moyo M, Devendra A, *et al.* The effects of HIV on the sensitivity of a whole blood IFN-γ release assay in Zambian adults with active tuberculosis. *PLoS One* 2008; 3: e2489.
28 Hoffmann M, Ravn P. The use of interferon-γ release assays in HIV positive individuals. *Eur Infect Dis* 2010; 4: 23–29.
29 Thomas TA, Mondal D, Noor Z, *et al.* Malnutrition and helminth infection affect performance of an interferon γ-release assay. *Pediatrics* 2010; 126: e1522–e1529.
30 Pai M, Lewinsohn DM. Interferon-γ assays for tuberculosis: is anergy the Achilles' heel? *Am J Respir Crit Care Med* 2005; 172: 519–521.
31 Cegielski JP, McMurray DN. The relationship between malnutrition and tuberculosis: evidence from studies in humans and experimental animals. *Int J Tuberc Lung Dis* 2004; 8: 286–298.
32 World Health Organization. Use of Tuberculosis Interferon-gamma Release Assays (IGRA) in Low- and Middle-income Countries. Geneva, WHO Press, 2011. Policy statement. Available from http://whqlibdoc.who.int/publications/2011/9789241502672_eng.pdf Date last accessed: January 29, 2013.
33 van Zyl-Smit RN, Zwerling A, Dheda K, *et al.* Within-subject variability of interferon-g assay results for tuberculosis and boosting effect of tuberculin skin testing: a systematic review. *PLoS One* 2009; 4: e8517.