Diagnostic Accuracy of Early Secretory Antigenic Target-6–Free Interferon-gamma Release Assay Compared to QuantiFERON-TB Gold In-tube

Elisa Nemes,[1] Deborah Abrahams,[1] Thomas J. Scriba,[1] Frances Ratangee,[1] Alana Keyser,[1] Lebohang Makhethe,[1] Mzwandile Erasmus,[1] Simbarashe Mabwe,[1] Nicole Bilek,[1] Virginie Rozot,[1] Hennie Geldenhuys,[1] Mark Hatherill,[1] Maria D. Lempicki,[2,a] Line Lindebo Holm,[3] Leah Bogardus,[2] Ann M. Ginsberg,[2,a] Thomas Blauenfeldt,[3] Bronwyn Smith,[4] Ruth D. Ellis,[2] Andre G Loxton,[4] Gerhard Walzl,[4] Peter Andersen,[3] and Morten Ruhwald[3]

[1]South African Tuberculosis Vaccine Initiative, Institute of Infectious Disease and Molecular Medicine & Division of Immunology, Department of Pathology, University of Cape Town; [2]AERAS, Rockville, Maryland; [3]Statens Serum Institute, Copenhagen, Denmark; and [4]South Africa Department of Science and Technology–National Research Foundation Centre of Excellence for Biomedical Tuberculosis Research, South African Medical Research Council Centre for Tuberculosis Research, Division of Molecular Biology and Human Genetics, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town

Background. Early secretory antigenic target-6 (ESAT-6) is an immunodominant Mycobacterium tuberculosis (M.tb) antigen included in novel vaccines against tuberculosis (TB) and in interferon-gamma (IFN-γ) release assays (IGRAs). Therefore, the availability of an ESAT-6–free IGRA is essential to determine M.tb infection status following vaccination with ESAT-6–containing vaccines. We aimed to qualify a recently developed ESAT-6–free IGRA and to assess its diagnostic performance in comparison to QuantiFERON-TB Gold In-tube (QFT).

Methods. Participants with different levels of M.tb exposure and TB disease were enrolled to determine the ESAT-6–free IGRA cutoff, test assay performance in independent cohorts compared to standard QFT, and perform a technical qualification of antigen-coated blood collection tubes.

Results. ESAT-6–free IGRA antigen recognition was evaluated in QFT-positive and QFT-negative South African adolescents. The ESAT-6–free IGRA cutoff was established at 0.61 IU/mL, based on receiver operating characteristic analysis in M.tb-unexposed controls and microbiologically confirmed pulmonary TB patients. In an independent cohort of healthy adolescents, levels of IFN-γ released in QFT and ESAT-6–free IGRA were highly correlated (P < .0001, r = 0.83) and yielded comparable positivity rates, 41.5% and 43.5%, respectively, with 91% concordance between the tests (kappa = 0.82; 95% confidence interval, 0.74–0.90; McNemar test P = .48). ESAT-6–free IGRA blood collection tubes had acceptable lot-to-lot variability, precision, and stability.

Conclusions. The novel ESAT-6–free IGRA had diagnostic accuracy comparable to QFT and is suitable for use in clinical trials to assess efficacy of candidate TB vaccines to prevent established M.tb infection.

Keywords. tuberculosis infection; diagnosis; IFN-γ release assay; qualification.

Early secretory antigenic target-6 (ESAT-6) is an immunodominant antigen expressed by *Mycobacterium tuberculosis* (M.tb) [1]. ESAT-6 and its heterodimeric complex protein CFP-10 are the principal components of immunodiagnostic tests for M.tb infection, including the interferon-gamma (IFN-γ) release assay (IGRA) and novel specific skin tests [2, 3]. These small, highly immunodominant antigens are encoded in the region of difference 1 of the M.tb genome, which comprises many important virulence factors as components and substrates of the ESAT-6 system

1 (ESX1) type VII secretion system [4] and are absent from bacille Calmette-Guerin (BCG), thereby, importantly, enabling distinction of M.tb infection from prior BCG vaccination [5].

ESAT-6 is also increasingly recognized as an important vaccine antigen with unique properties for infection containment and, potentially, for clearance [6–8]. As such, ESAT-6 is contained in many candidate vaccines against tuberculosis (TB), including the subunit vaccine H56:IC31, which is undergoing phase 2 clinical testing [6, 9]. Vaccine efficacy to prevent established M.tb infection, as a likely predictor of prevention of TB disease, has been proposed as an innovative and cost-effective endpoint to rationalize TB vaccine development [10]. We recently showed that BCG revaccination or vaccination with a novel subunit vaccine candidate (H4:IC31) may offer partial protection against sustained M.tb infection, measured by QuantiFERON-TB Gold In-tube (QFT) [11]. That trial provided the first proof-of-concept efficacy signal for a subunit vaccine candidate, which is very similar to H56:IC31 but does not contain ESAT-6. Such a clinical trial design, one that relies

on IGRA to measure M.tb infection, cannot currently be used to evaluate ESAT-6–containing vaccine candidates, since vaccine-induced immune responses are cross-reactive with those measured by IGRA, rendering the test false positive.

Until recently, inclusion of ESAT-6 and CFP-10 has been considered essential for adequate immunodiagnostic sensitivity. However, recent advances in antigen discovery tools and M.tb antigen recognition studies in humans point to novel diagnostic antigens that could replace ESAT-6 [12–14]. Several studies explored the diagnostic potential of new candidates either alone or in combination, and we recently established an ESAT-6–free IGRA antigen cocktail after screening of several candidates (TB7.7, EspJ, EspC, EspF, EccD1, PE35, and Rv2348) compared to ESAT-6 and CFP-10 [15].

In this study, our aim was to confirm the recognition of an ESAT-6–free IGRA antigen cocktail in adolescents from a high TB burden area, define a diagnostic algorithm for the ESAT-6–free IGRA, evaluate ESAT-6–free IGRA diagnostic performance compared to QFT, and assess the variability of ESAT-6–free IGRA blood collection tubes.

METHODS

Study Participants
All studies that involved human participants were conducted according to the principles described in the Declaration of Helsinki and were approved by relevant institutional review boards (Human Research Ethics Committee [HREC]). Written informed assent and consent were obtained from participants and their legal guardians (for minors).

We included the following 4 independent cohorts of patients to address the study aims (Supplementary Figure 1A). Cohort 1: Healthy adolescents (cohort 1a) and adults (cohort 1b) were enrolled at the South African Tuberculosis Vaccine Initiative (SATVI, South Africa, HREC reference: 126/2006) to confirm ESAT-6–free IGRA antigen recognition and perform ESAT-6–free IGRA qualification, respectively. Individuals who had received a tuberculin skin test (TST) in the last 12 months, had evidence of any acute or chronic disease or symptomatic infection (including human immunodeficiency virus [HIV] positive or TB symptoms), currently used immune-modifying drugs, had been enrolled in any experimental study, were pregnant or lactating (women), weighed <50 kg, or those with raised suspicion of anemia by recruiting officer were excluded. Cohort 2a: Healthy adults with no known exposure to M.tb were recruited at the Statens Serum Institut (SSI) in Denmark (HREC reference: H-3-2012-008). Individuals with a medical history of M.tb exposure, travel, and/or risk-seeking behavior were excluded. Cohort 2b: Adult patients with pulmonary TB disease were recruited by the Immunology Research Group (IRG) at Stellenbosch University, South Africa (HREC reference: N13/05/064). TB disease was microbiologically confirmed by

sputum liquid culture and/or GeneXpert and/or smear microscopy. Patients with HIV infection, multidrug-resistant TB, or those who started TB treatment more than 3 days before enrollment were excluded. Cohort 3: A subset of healthy adolescents screened for enrollment in a prevention-of-M.tb-infection trial at SATVI (HREC reference: 471/2013; clinicaltrials.gov identifier: NCT02075203) [11] were included in this study to evaluate ESAT-6–free IGRA performance compared to QFT.

Inclusion and exclusion criteria for cohort 3 have been described in detail elsewhere [16]. QFT results from cohort 2a, 2b, and 3 have been published in a different form [16].

Assessment of Preselected Antigens
Venous blood was collected in lithium heparin–containing vacutainers, and 1 mL per tube was dispensed in stimulation tubes (Sarstedt, Numbrecht, Germany) that contained (a) no antigen, or pools of overlapping 15–20 mer peptides representing: (b) full length CFP-10, (c) a fragment of EspC (aa54-103), (d) a fragment of EspF (aa9-44), (e) a fragment of Rv2348 (aa56-108), or (f) ESAT-6–free IGRA antigen cocktail (b+c+d+e, at a concentration of 5 μg per peptide/mL blood, as described [15]). Phytohemagglutinin (Dartford, Remel, United Kingdom) was used as the positive control (5 μg/mL), and a set of QFT tubes (Nil, TB antigen, mitogen; Qiagen, Hilden, Germany) was run in parallel as the reference. Within 2 hours of phlebotomy, stimulated blood was incubated at 37°C for 18–22 hours and then centrifuged. Plasma was harvested and cryopreserved at –80°C until batch analysis.

QFT and ESAT-6–Free IGRA Assays
Blood was collected directly in QFT tubes and in tubes that contained lyophilized ESAT-6–free IGRA peptides, manufactured at SSI, as described [15], to define a cutoff for the ESAT-6–free IGRA assay, determine assay performance in adolescents, and assess tube variability. Blood was processed following a standard operating procedure that was stricter than that recommended by the manufacturer in order to reduce known sources of assay variability and improve reproducibility, as described [16]. Briefly, 1 mL of blood was collected and mixed by 10 manual inversions and 5 minutes on a tube rotator and incubated at 37°C within 2 hours of phlebotomy, for 16–20 hours.

To determine the ESAT-6–free IGRA assay cutoff, blood was collected and processed at SSI (M.tb unexposed controls, cohort 2a) and IRG (TB patients, cohort 2b). Cryopreserved plasma was analyzed by QFT IFN-γ enzyme-linked immunosorbent assay (ELISA) at SATVI.

To assess the performance of ESAT-6–free IGRA in South African adolescents, samples were collected and processed at SATVI. Blood was simultaneously collected and processed for the QFT and ESAT-6–free IGRA. As results from QFT analysis were part of the screening procedures for real-time enrollment

in a clinical trial [11], fresh plasmas harvested from QFT tubes were analyzed by QFT IFN-γ ELISA, while plasmas harvested from ESAT-6–free IGRA tubes were cryopreserved and analyzed in batches. We have previously shown that QFT results obtained from fresh vs frozen plasma are comparable [16].

ESAT-6–Free IGRA Tube Technical Qualification
Blood was collected from participants enrolled in cohort 1b directly in QFT and ESAT-6–free IGRA tubes (Supplementary Figure 1B) and processed, as described [16]. Identical internal quality control samples were included in each plate to assess interassay variability.

IFN-γ ELISA
IFNγ was quantified by QFT IFN-γ ELISA (Qiagen, Hilden, Germany). All samples from the same participant were included in the same plate, and each sample was analyzed in triplicate or duplicate wells by a blinded operator. Results from replicate wells were averaged unless a technical error that affected 1 of the wells was recorded.

Statistical Analyses
Statistical analyses were performed using SAS 9.2 (SAS Institute) and GraphPad Prism (V7, GraphPad Software Inc.). IFN-γ values were compared using nonparametric methods across study groups (Mann-Whitney test) or assays (Wilcoxon matched-pairs signed rank test or Spearman correlation). Diagnostic accuracy and assay cutoff were determined by receiver operating characteristic analysis. Assay sensitivity and specificity for a given cutoff are reported with Clopper-Pearson 95% confidence intervals (CIs). Agreement and the marginal homogeneity between 2 assays were calculated using Cohen's kappa and the McNemar test, respectively. Assay variability and bias were calculated using Bland-Altman analysis.

RESULTS

Recognition of ESAT-6–Free IGRA M.tb Antigens by South African Adolescents
Since M.tb antigens included in the ESAT-6–free IGRA were originally selected in participants from low and intermediate TB burden settings [15], we first confirmed their recognition in adolescents from a high TB burden area who represent a target population for prevention of M.tb infection trials (cohort 1a).

The concentration of IFN-γ released in response to the ESAT-6–free IGRA antigen cocktail was significantly higher in participants with positive QFT status (QFT+; n = 35) compared to participants with negative QFT status (QFT–; n = 25; Supplementary Figure 2A), and the magnitude of the IFN-γ levels correlated with those induced by QFT (Supplementary Figure 2B). The ESAT-6–free IGRA response was mostly driven by recognition of CFP-10 and EspC (Figure 1).

Definition of the ESAT-6–Free IGRA Cutoff
The ESAT-6–free IGRA cutoff was determined by comparing M.tb-unexposed participants enrolled in a low TB endemic setting (cohort 2a, n = 50) to microbiologically confirmed TB patients recruited in South Africa (cohort 2b, n = 51; Supplementary Table 1). There was no significant difference in IFN-γ levels following whole blood incubation in QFT or ESAT-6–free IGRA Nil tubes (Figure 2A). Therefore, background IFN-γ values measured in Nil QFT tubes were subtracted from those measured in both QFT TB Ag and ESAT-6–free IGRA TB Ag tubes in all subsequent experiments. In TB patients, the IFN-γ concentrations in the QFT assays (median, 6.79; interquartile range [IQR], 1.70–10.85 IU/mL) were higher than those measured by ESAT-6–free IGRA (median 4.27; IQR, 1.59–9.51 IU/mL; P = .005), while equivalent and extremely low IFN-γ concentrations were measured in M.tb-unexposed individuals (Figure 2B). ESAT-6–free IGRA yielded diagnostic accuracy that was comparable to that of QFT for classifying TB-diseased patients vs M.tb-unexposed individuals (Figure 2C). An ESAT-6–free IGRA assay cutoff (0.61 IU/mL) was selected at the IFN-γ concentration that yielded no more than a 5% lower specificity than QFT, as defined in a preset statistical analysis plan. This cutoff resulted in a sensitivity of 82% (Supplementary Figure 3).

Validation of ESAT-6–Free IGRA Performance in South African Adolescents
Diagnostic accuracy of ESAT-6–free IGRA for M.tb infection was benchmarked against QFT in an independent cohort of 200 healthy adolescents from South Africa (cohort 3).
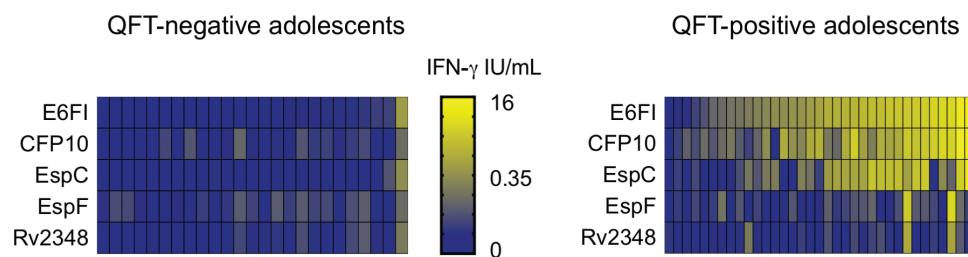

Figure 1. Early secretory antigenic target-6–free IFN-γ release assay antigen recognition by South African adolescents. Heat map of log10(IFN-γ) showing recognition patterns of all antigens in each QFT-negative (left) and QFT-positive (right) participant. In the absence of a cutoff to define recognition of each E6FI antigen, log10(IFN-γ) are presented on a continuous quantitative scale. Abbreviations: IFN-γ, interferon-gamma; QFT, QuantiFERON-TB Gold In-tube.
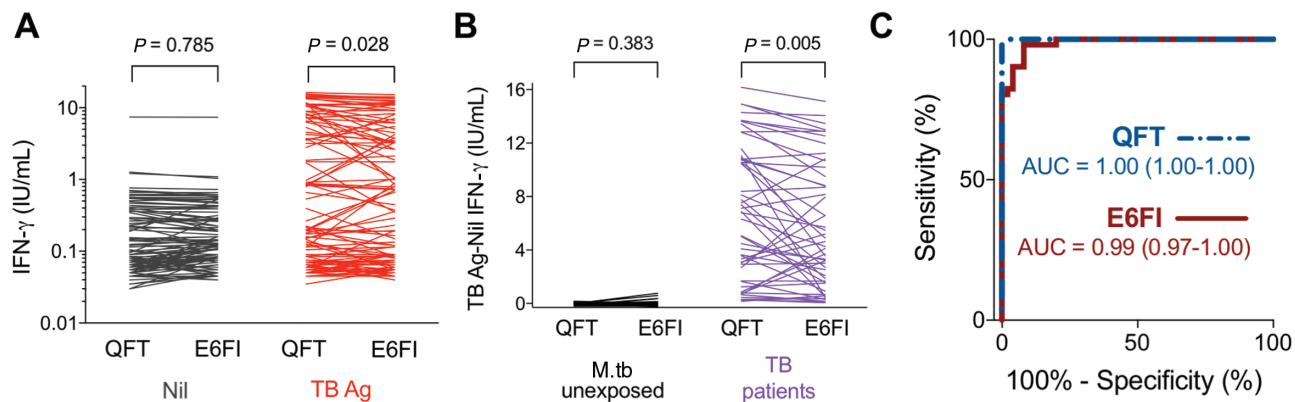
Figure 2. ESAT-6–free IGRA diagnostic accuracy in M.tb-unexposed controls and TB patients. Blood was collected from M.tb-unexposed controls (n = 50, cohort 2a) and microbiologically confirmed TB patients (n = 51, cohort 2b) in QFT and ESAT-6 free IGRA (E6FI) tubes and stimulated for 16–20 hours at 37°C; the IFN-γ concentration was measured by QFT enzyme-linked immunosorbent assay. *A*, Paired analysis (Wilcoxon matched-pairs signed rank test) of IFN-γ values measured upon blood stimulation in QFT and E6FI Nil or TB Ag tubes in all participants. *B*, Paired analysis (Wilcoxon matched-pairs signed rank test) of IFN-γ values measured by QFT and E6FI (TB Ag – Nil) in M.tb-unexposed controls and TB patients. *C*, Receiver operating characteristic analysis of diagnostic accuracy of QFT (dashed line) and E6FI (solid line) assays. Area under the curve and 95% confidence interval are shown. Abbreviations: Ag, antigen; ESAT-6, early secretory antigenic target-6; IFN-γ, interferon-gamma; IGRA, interferon-gamma release assay; M.tb, *Mycobacterium* tuberculosis; QFT, QuantiFERON-TB Gold In-tube; TB, tuberculosis.

Median participant age was 14 years (range, 12–18 years), 52% of participants were females, 46% of adolescents self-identified as black African, and 54% were from a mixed ethnic background (Cape mixed ancestry). All participants had received childhood BCG vaccination; none had received a TST within 3 months of recruitment, had previous TB disease nor were known to be exposed to patients with active TB at the time of blood collection; 4% were smokers. Figure 3A shows the distribution of IFN-γ values in adolescents who tested QFT+ (n = 83) or QFT– (n = 117). ESAT-6–free IGRA yielded slightly higher IFN-γ concentrations (median, 6.16; IQR, 2.97–10.91 IU/mL) compared to QFT (median, 5.70; IQR, 1.51–9.93

IU/mL; *P* = .015) in participants who tested QFT+, while no overall difference was observed for those who tested QFT– (Figure 3B). IFN-γ values obtained by ESAT-6–free IGRA and QFT were highly correlated (r = 0.828, *P* < .0001; Figure 3C). Overall, 91% of ESAT-6–free IGRA and QFT results were concordant (kappa = 0.82; 95% CI, 0.74–0.90; McNemar test *P* = .48; Table 1).

An uncertainty zone to interpret QFT results close to the assay cutoff, falling between 0.2 and 0.7 IU/mL, has been proposed [16–19]. In this adolescent cohort, 5 of 18 (28%) QFT and ESAT-6–free IGRA discordant results fell in the QFT uncertainty zone (Supplementary Figure 4).
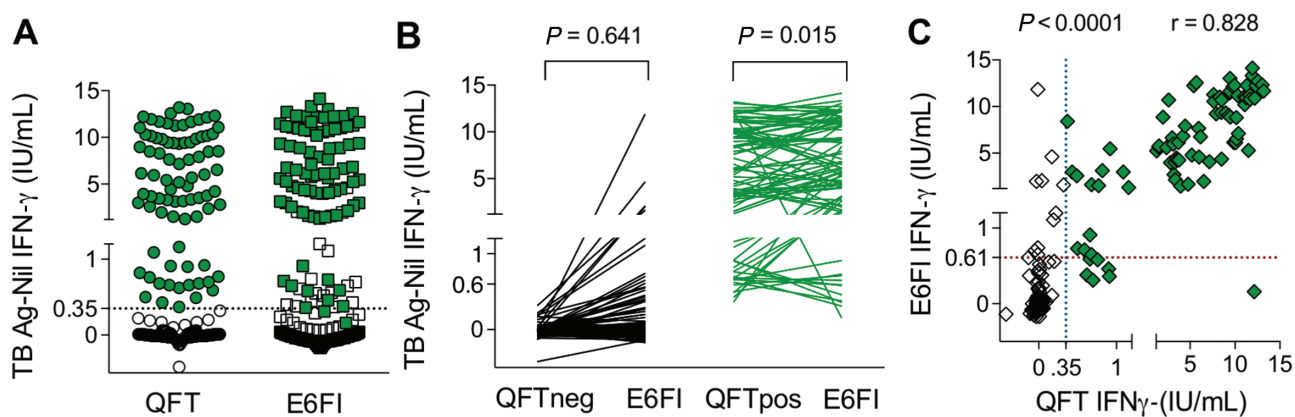

Figure 3. ESAT-6–free IGRA diagnostic accuracy in healthy South African adolescents. Blood was collected from QFT-negative (n = 117, open symbols) and QFT-positive (n = 83, filled symbols) healthy South African adolescents (cohort 3) in QFT and ESAT-6–free IGRA (E6FI) tubes and stimulated for 16–20 hours at 37°C; the IFN-γ concentration was measured by QFT enzyme-linked immunosorbent assay. *A*, Distribution of IFN-γ values measured by QFT and E6FI in all participants. The dotted line denotes QFT cutoff. *B*, Paired analysis (Wilcoxon matched-pairs signed rank test) of IFN-γ values measured by QFT and E6FI (TB Ag – Nil) in QFT-negative and QFT-positive adolescents. *C*, Correlation between IFN-γ values obtained by QFT and E6FI in all participants was assessed using the Spearman test. The dotted lines denote assay cutoffs: 0.35 IU/mL for QFT (vertical line) and 0.61 IU/mL for E6FI (horizontal line). Abbreviations: Ag, antigen; ESAT-6, early secretory antigenic target; IFN-γ, interferon-gamma; IGRA, interferon-gamma release assay; QFT, QuantiFERON-TB Gold In-tube; TB, tuberculosis.

Table 1. Concordance of ESAT-6–Free IGRA and Quantiferon-TB Gold in Healthy South African Adolescents

		ESAT-6–Free IGRA		
		Negative (n)	Positive (n)	Total (N)
Quantiferon-TB Gold	Negative (n)	106	11	117
	Positive (n)	7	76	83
Total (n)		113	87	200

Abbreviations: ESAT-6, early secretory antigenic target; IGRA, interferon-gamma release assay.

ESAT-6 Free IGRA Variability

To complete the qualification of the ESAT-6–free IGRA assay, we assessed lot-to-lot variation, precision, and stability of ESAT-6–free IGRA blood collection tubes (Supplementary Figure 1B). Experiments were conducted concurrently on blood collected from 12 healthy donors (3 who tested QFT– and 9 who tested QFT+; cohort 1b).

No significant difference was observed in IFN-γ values obtained by QFT, ESAT-6–free IGRA lot 1, and ESAT-6–free IGRA lot 2 ($P > .5$ for all comparisons; Figure 4A). Qualitatively, 1 discordant result was observed when QFT was compared to ESAT-6–free IGRA lot 1 and when ESAT-6–free IGRA lot 1 was compared to ESAT-6–free IGRA lot 2, while 2 discordant results were observed when QFT was compared to ESAT-6–free IGRA lot 2 (Figure 4A). Bland-Altman analysis of ESAT-6–free IGRA lot 1 and lot 2 tubes did not show lot-associated systematic bias (bias = –10%; 95% limits of agreement, –123% to 101%), although a high percent difference between lots was observed in some instances (Supplementary Figure 5A), resulting in a concordant correlation coefficient (ρc) = 0.89 (95% CI, 0.68–0.97; Supplementary Figure 5B).

Analysis of blood collected in 3 ESAT-6–free IGRA tubes from lot 2 yielded consistent quantitative and qualitative results, with 1 exception (Figure 4B). Variability observed across replicate ESAT-6–free IGRA assays appeared substantially higher than that due to IFN-γ ELISA intra- and interassay variability (Supplementary Figure 5C).

Storage of ESAT-6–free IGRA blood collection tubes for 2 weeks at 4, 20, 37, or 50°C prior to long-term storage at 4°C did not affect quantitative and qualitative results (Figure 4C) nor did it introduce a systematic bias (Supplementary Figure 5D), although high variability was observed in some samples (Supplementary Figure 5E).

DISCUSSION

Our aim in this study was to qualify the recently developed ESAT-6–free IGRA, a diagnostic tool for the detection of M.tb infection in individuals vaccinated with ESAT-6–containing TB vaccines. We demonstrate that the 3 antigens substituting ESAT-6 are specific and recognized in healthy individuals with a QFT-positive test. Using a cross-validation design, we defined a cutoff for positive ESAT-6–free IGRA, which is essential to the development of a diagnostic algorithm for test interpretation, and demonstrated that the ESAT-6–free IGRA had diagnostic performance that was comparable to that of QFT in South African adolescents. Also, we explored the variability, precision, and stability of the ESAT-6–free IGRA test and demonstrated that the novel test is sufficiently robust for its intended use in clinical research.

The strength of this analysis was the cross-validation design; this was achieved by determining the cutoff in a population with stringently defined M.tb-unexposed controls and TB cases and validating it in a separate cohort, representative of
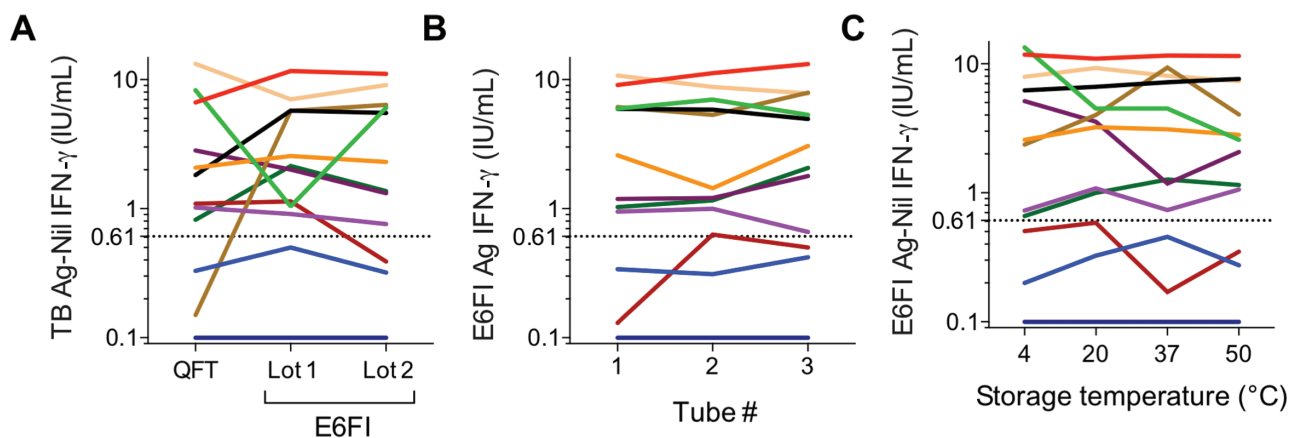

Figure 4. ESAT-6–free IGRA tube variability. Blood was collected from healthy donors (n = 12, cohort 1b) and analyzed as described in Supplementary Figure 1B. IFN-γ values were measured by QFT enzyme-linked immunosorbent assay upon blood stimulation in (*A*) QFT and 2 different lots of ESAT-6–free IGRA (E6FI) tubes (lot-to-lot variability), (*B*) 3 E6FI tubes from lot 2 (precision), and (*C*) 4 E6FI tubes stored at the indicated temperatures for 2 weeks prior to long-term storage at 4°C (stability). Descriptive statistics are shown in Supplementary Figure 5. In all figures, the dotted line denotes the E6FI assay cutoff. Abbreviations: Ag, antigen; ESAT-6, early secretory antigenic target; IFN-γ, interferon-gamma; IGRA, interferon-gamma release assay; QFT, QuantiFERON-TB Gold In-tube; TB, tuberculosis.

the target population for the test. In this study, QFT performed as expected both in terms of magnitude of IFN-γ release in samples from TB patients and controls [20] and in terms of positivity rates in the validation cohort. The validation cohort was recruited in a region with extremely high exposure to TB, resulting in about 40% prevalence of QFT+ individuals among adolescents aged 14 years, which is consistent with previous observations in the same community [21].

We observed 9% discordance between ESAT-6–free IGRA and QFT. This finding was expected, as the antigens used in the 2 tests differ and are differentially recognized in different persons [15] (Figure 1). Our findings confirm that EspC is an immunodominant and M.tb-specific antigen and the key support to CFP-10 in the ESAT-6–free IGRA antigen cocktail [13, 15]. Our initial study identified EspF and Rv2348 as important supplement antigens that augment the level of IFN-γ release and extend diagnostic coverage, especially in individuals with latent infection [15]. However, in the population included in this study, these antigens were less frequently recognized. Of note, one third of discordant results fell in the QFT uncertainty zone (IFN-γ values between 0.2 and 0.7 IU/mL) [16]. Our results suggest that the ESAT-6–free IGRA is subject to biological and analytical variability that is similar to that of QFT [16, 22]; therefore, an uncertainty zone should also be established for the ESAT-6–free IGRA.

The development of the ESAT-6–free IGRA arose from the need to detect M.tb infection after vaccination with ESAT-6–containing vaccines. ESAT-6 seems to possess unique properties that drive protective immune responses and has become a central antigen in several vaccine candidates that are in development [23]. However, clinical efficacy trials are required before even the most advanced and promising candidates will be ready for implementation in the field. A robust diagnostic assay to detect M.tb infection after vaccination is critical for the evaluation of the efficacy in preventing M.tb infection in these trials [10], which we showed is feasible in countries with high TB burden and can provide proof of concept to inform further clinical development of vaccine candidates [11].

In TB patients, the magnitude of IFN-γ release was significantly lower in the ESAT-6–free IGRA compared to QFT. While this might have affected the assay cutoff determination, it did not translate into a lower diagnostic sensitivity, determined as positivity rate in the validation cohort.

Regardless, these observations, along with tube-associated variability, suggest a need for improvement of the blood collection tubes. This was addressed through the development of an improved freeze-drying procedure that enhanced peptide solubility. Tubes manufactured through this improved process induced IFN-γ levels equivalent to QFT in individuals who tested QFT+ and lower levels of IFN-γ in controls and will be used in future efficacy studies (Kidola and Ruhwald, manuscript in preparation).

Another limitation in the study was the reliance on active TB cases and uninfected controls to determine the cutoff for a positive test. Although this approach has typically been used to define the IGRA cutoff [20, 24], ideally, endpoints should have been derived from ESAT-6–free IGRA-positive participants who develop active TB in a prospective trial. However, the scale and time required for this endeavor would vastly outweigh the potential benefit to be gained. Further, in the absence of an alternative gold standard to measure M.tb exposure, it is not possible to identify M.tb-unexposed controls in the same high endemic population.

A challenge that limited the generalizability of the blood collection tube variability and stability analysis is that we did not include a direct experimental comparison to QFT. This would allow better referencing to state-of-art assay; however, when compared to the literature [22], the ESAT-6–free IGRA tubes appear robust and field applicable.

In conclusion, we demonstrated that the novel ESAT-6–free IGRA yields a diagnostic accuracy for M.tb infection that is comparable to that of QFT in South African adolescents. The ESAT-6–free IGRA is thus suitable to measure M.tb infection in clinical trials of novel TB vaccine candidates that contain ESAT-6, targeting young, healthy adults.

Further evaluation of the assay needs to be carried out to assess its diagnostic potential among specific subpopulations such as immunocompromised patients and children.

Supplementary Data

Notes

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