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Variability of interferon-γ release assays in people at high risk of tuberculosis infection or progression to tuberculosis disease living in the United States

Kathryn Winglee[1,*], **Andrew N. Hill**[1], **Robert Belknap**[2], **Jason E. Stout**[3], **Tracy L. Ayers**[1]

[1)]Division of Tuberculosis Elimination, Centers for Disease Control and Prevention, Atlanta, GA, USA

[2)]Public Health Institute at Denver Health, Denver, CO, USA

[3)]Department of Medicine, Duke University School of Medicine, Durham, NC, USA

Abstract

Objectives: Interferon-γ release assays, including T-SPOT.*TB* (TSPOT) and QuantiFERON Gold In-Tube (QFT), are important diagnostic tools for tuberculosis infection, but little work has been done to study the performance of these tests in populations prioritized for tuberculosis testing in the United States, especially those other than health care personnel.

Methods: Participants were enrolled as part of a large, prospective cohort of people at high risk of tuberculosis infection or progression to tuberculosis disease. All participants were administered a tuberculin skin test, TSPOT, and QFT test. A subset of participants had their QFT (n = 919) and TSPOT (n = 885) tests repeated when they returned to get their tuberculin skin test read 2 to 3 days later (repeat study). A total of 531 participants had a TSPOT performed twice on the same sample taken at the same time (split study).

Results: The QFT repeat test interpretations were discordant (one test positive and the other negative) for 6.4% of participants (59 of 919), and the TSPOT tests were discordant for 60 of 885 participants in the repeat study (6.8%) and 41 of 531 participants in the split study (7.7%). There was a high degree of variability in the quantitative test results for both QFT and TSPOT, and discordance was not associated with both test results being near the established cut-offs. Furthermore, the proportion of discordance was similar when comparing participants in both the TSPOT repeat and TSPOT split studies.

Discussion: Both QFT and TSPOT were 6% to 8% discordant. The results should be interpreted with caution, particularly when seeing a conversion or reversion in serial testing.

[*]Corresponding author. Kathryn Winglee, Division of Tuberculosis Elimination, Centers for Disease Control and Prevention, 1600 Clifton Road NE, Mailstop US12-4, Atlanta, GA, 30329–4018, USA. nrf1@cdc.gov (K. Winglee).

Author contributions

TA and KW conceived the study. KW reviewed the literature, designed the study, conducted all analyses, and drafted the manuscript. ANH, RB, JES, and TA provided additional analytic guidance and feedback on the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2022.02.020.

Keywords

Epidemiology; Interferon-γ release assay; QuantiFERON; T-SPOT.TB; Tuberculosis

Introduction

Although the incidence of tuberculosis (TB) in the United States continues to decline, there were more than 7000 cases in 2020, of which 87.5% were not attributed to recent transmission [1]. Thus, detection of persons with latent TB infection (LTBI) is critical to eliminating TB in the United States. Currently, the commercially available options for TB infection testing in the United States are the tuberculin skin test (TST) and interferon-γ release assays (IGRAs), including QuantiFERON (QFT) and T-SPOT.*TB* (TSPOT). The latest U.S. guidelines recommend IGRA over TST in persons >5 years of age, and IGRAs are increasingly used for LTBI screening because they do not require a repeat visit and have a higher specificity in persons vaccinated with bacille Calmette-Guérin [2].

Multiple studies have reported IGRA conversion (i.e. tests change from negative to positive) and reversion (i.e. positive to negative) proportions [3–13]. These studies have been mostly in low-incidence countries and generally focused on administrative testing for health care personnel. This makes it harder to extrapolate how IGRAs would perform in populations prioritized for LTBI primary care screening in the United States [14]. Our study investigated the results of QFT and TSPOT in people living in the United States who are at a high risk of TB infection or progression to TB disease. We analyzed the results from two samples drawn 2 to 3 days apart (QFT and TSPOT) and from the same sample split in half (TSPOT only). These results help characterize the QFT and TSPOT variability and could help inform clinicians when making decisions about interpreting IGRA results.

Methods

Participants for this study were part of a larger prospective study, the Tuberculosis Epidemiologic Studies Consortium (TBESC). A detailed description of the main study has been published previously [15]. In short, 18 TBESC-affiliated clinics enrolled participants from July 2012 to April 2017 who were at increased risk for TB infection or likely to progress to TB disease if infected. Sites drew blood for QFT Gold In-Tube (Qiagen, Germantown, MD) and TSPOT (Oxford Immunotec, Marlborough, MA) testing, followed immediately by placement of a TST using the Mantoux method. QFT was processed using standard clinical protocols at each site, and TSPOT was processed at the manufacturer's central laboratory in Memphis, Tennessee, except for Hawaii, where TSPOT was processed locally by personnel trained by Oxford Immunotec staff.

This paper describes two TBESC substudies: the repeat study and the split study. In the repeat study, a second blood sample was drawn to repeat the QFT and TSPOT at the time that the participant returned for their TST reading (within 44–76 hours of skin test placement). Thus, the repeat analyses represent a QFT or TSPOT performed twice on two different blood samples taken 2 to 3 days apart. In the split study, two tubes of blood were drawn from each participant from the same venipuncture, and a TSPOT was performed on

each tube. Thus, the split analyses represent a TSPOT performed twice on the same sample taken at the same time. Only TSPOT was evaluated in this study. For the analyses of the repeat results, the first test is the test performed on the sample taken when the TST was placed, and the second test is the test performed on the sample taken when the participant returned for their TST reading. For the analyses of the split results, one test was arbitrarily chosen to be the first test.

Participants who were recruited in the larger study were sequentially asked to participate in the substudies until each site met its target for recruitment for the substudy (Fig. 1). Targets were assigned based on overall recruitment at the study site. Two sites did not participate in the repeat study, and four sites did not participate in the split study. For the repeat study, participants had to be at least 18 years old and return for the TST reading within the appropriate time. For the split study, participants were excluded if they were <15 years old or immunocompromised. Four participants living with HIV were mistakenly enrolled in the split study and were removed from the analyses. Participants were also excluded if one or both tests failed, were invalid or indeterminate, were not performed, or were performed more than 1 week after the first test.

Some participants were enrolled as close contacts of persons with pulmonary TB and had all three tests repeated on their contact investigation follow-up visit (Fig. S1). Two participants had the TSPOT split analysis performed on both the first and follow-up visits (i.e. they had two sets of TSPOT split results 6 or 7 weeks apart), but only the first set of the split test results was used. Of the 47 participants who were in both the repeat and split TSPOT study, two were contacts whose first set of results was included in the split analysis, and the repeat test was performed when they returned for follow-up testing. These two participants were excluded from the analysis of both studies. For the remaining 45 participants, there were two split tests on the first day and then a third test 2 to 3 days later. For these participants, because it was unclear which of the two split tests to use as the first of the repeat TSPOT tests, if the split tests were discordant (one positive, one negative), the participant was excluded from all TSPOT repeat analyses; however, if the split tests were concordant, participants were excluded from the quantitative TSPOT repeat analyses but included in the dichotomous TSPOT repeat analyses (because both tests had the same dichotomous result).

For TSPOT, we used the maximum of the panel A minus nil and panel B minus nil spot counts and the U.S. Food and Drug Administration–approved cut-off for positive (≥8 spots positive) and considered results ≤7 spots to be negative [16]. Supplementary analyses explored splitting out the borderline zone (5–7 spots), using the international cut-off for TSPOT (≥6 spots positive, ≤5 spots negative) [17], or analyzing the panels separately. For QFT, we used the standard manufacturer cut-offs and interpretations, with a TB antigen minus nil value of 0.35 IU/ml as the cut-off between positive and negative [18]. For both tests, if the value was above the maximum (50 for TSPOT and 10 for QFT), it was set to the maximum. Agreement between quantitative measurements was analyzed with a Bland-Altman plot [19]. All analyses were performed in R, version 4.0.2 [20].

All participants provided written informed consent, assent, or parental permission. The study was approved by CDC's institutional review board and local institutional review boards that did not defer to the CDC and was registered at clinicaltrials.gov (Identifier NCT01622140).

Results

QFT and TSPOT were repeated after 2 or 3 days for 919 or 885 participants, respectively (repeat study), and 531 participants had two TSPOT tests run on the same blood sample (split study; Fig. 1). Most participants were aged 15 to 44 years, and >85% were not born in the United States (Table 1). In addition, more than half self-reported bacille Calmette-Guérin vaccination.

In the repeat study, QFT was discordant for 6.4% of participants (59 of 919), and TSPOT was discordant for 6.8% of participants (60 of 885; Table 2). The TSPOT split study was similar, with 7.7% of participants (41 of 531) discordant. Conversion proportions were 27 of 563 (4.8%) for QFT repeat and 31 of 609 (5.1%) for TSPOT repeat. Reversion proportions were 32 of 356 (9.0%) for QFT repeat and 29 of 276 (10.5%) for TSPOT repeat. Of the 861 participants in both repeat studies, 763 (88.6%) were concordant in both. Of the remaining 98 participants, only 14 (14.3%) were discordant for both QFT and TSPOT.

We saw a high degree of variability in the quantitative test results, both in the concordant and discordant tests (Fig. 2). Among the discordant test results, even when one of the test results was near the cut-off, the other was not (Fig. 2 and S2–5). Given these large differences, modifying the cut-off points did not noticeably reduce the proportion of discordant test results (Fig. S6). Furthermore, if the first TSPOT test was borderline, a borderline was the least frequent result for the second test, but positive and negative results were similar in both the repeat and split studies (Table S1). The percentage of discordance increased to 7.0% and 8.7% for the TSPOT repeat and split studies, respectively, when using the international cut-off (Table S2).

We further explored the variability in TSPOT results, starting with analyzing whether variability would decrease if we were comparing results from one panel, as opposed to taking the maximum of panel A or panel B minus nil [16]. When comparing the TSPOT results within panels, we saw similar levels of variability compared with using the maximum result (Fig. 3 and S7). Thus, TSPOT variability was not driven by using two different panels. We also analyzed the dichotomous results of the 45 participants in both the TSPOT repeat and split studies. Six participants (13.3%) were discordant when comparing the split tests, as opposed to 5 (11.1%) when comparing the repeat test to each split test. Thus, the repeat result is not more likely to be different from the split result, indicating that the error rate within a sample is similar to the error rate between samples.

Discussion

IGRAs continue to be one of the primary tools for LTBI screening. Extensive work has been done on the reproducibility and repeatability of IGRAs in health care personnel [3–10,21], but, to our knowledge, nothing has been published specifically focusing on results for people who live in a low TB incidence country but are prioritized for LTBI testing because they are

at high risk of infection or progression to TB disease. We evaluated the performance of both QFT and TSPOT in this population. We found that both tests had a discordance of 6% to 8%. Similar numbers were reported in prior studies [3–5,8]. For TSPOT, this error was the same regardless of whether the test was performed on the same sample taken on the same day or on two different samples 2 to 3 days apart. Furthermore, our analysis showed that discordance is not due to test results near the test cut-off. Instead, there was a high degree of variability in the quantitative test results in both concordant and discordant participants.

Variability in IGRA test results can occur for a variety of reasons. One potential cause is a change in the participant's immune response to TB antigens over time due to exposure to TB or boosting from a prior TST test [22]. However, in our study, the TSPOT split tests were run on the same sample, so there is no possibility of alterations in immune response, and the variability was comparable between the TSPOT split and TSPOT repeat studies, suggesting that timing and sampling is not causing the variability for TSPOT. Furthermore, prior studies of tests performed within 2 to 3 days of administering a TST showed no evidence of boosting or changes in immune response [3,5,22,23].

Another potential source of variability is laboratory procedures. We did not attempt to control for laboratory variability because we wanted our results to reflect real-world performance. However, other studies have explored sources of variability and concluded that, except for blood volume and preincubation delay, which were standardized in our study, no variables identified had a significant effect on qualitative QFT results [23–25]. Combined, this suggests that the proportions of discordance are an inherent part of using IGRAs, perhaps due to sampling issues when dealing with rare cell populations in the blood, such as T cells that respond to TB antigens [26,27]. The rarity of these cell populations means that different samples, although taken at the same time in the same volume, may contain different numbers of reactive T-cells, possibly resulting in the observed variability.

Our study had several limitations. First, we used a convenience sample from our larger study, so participants might not be representative of the larger population of interest. Nevertheless, our variability and discordance rates are consistent with what has been seen in other studies [3–12,21]. Second, we evaluated the QFT Gold-In-Tube assay, which has been replaced by QFT-Plus (which only became available after study enrolment completed). However, several papers have shown agreement between these two tests [28,29]. Finally, due to low cell sizes, we could not assess participant characteristics associated with whether a participant was likely to have a discordant result. However, only 14.3% of participants in both repeat studies with a discordant result were discordant for both TSPOT and QFT, suggesting that patient characteristics may not explain discordance.

Despite these limitations, our findings add to the literature suggesting that IGRAs have consistent levels of variability regardless of the TB risk in the population tested [30]. This variability can result in false-positive and false-negative results [31]. Furthermore, our TSPOT error rate is similar, regardless of whether the TSPOT was performed on the same sample taken on the same day or different samples taken 2 to 3 days apart, suggesting the within- and between-sample error is similar. Participants were followed for 2 years after enrolment, and only one participant in the repeat study (both QFT and TSPOT) and a

different participant in the TSPOT split study progressed to TB disease. Both participants had concordant results, but larger sample sizes are needed to make conclusions about the relationship between discordance and progression to disease. Thus, these results suggest that clinicians should be aware of the possibility of both false positives and false negatives in IGRA results, even in populations at high risk of infection or progression to TB disease.

The U.S. Food and Drug Administration recommended a borderline region for TSPOT tests in the United States [16], and some have called for a QFT borderline region as well [6,12,24], but our findings of large variability in the quantitative results suggest that a borderline zone or increased test cut-off would not reduce the number of conversions and reversions. Discordant test results generally had large differences and were not near the cut-off. Thus, clinicians should not just use the quantitative results as a metric for whether a person is likely to convert or revert on follow-up. Instead, clinicians should interpret both QFT and TSPOT results with caution and consider this variability, particularly when seeing a conversion or reversion in serial testing in the absence of known exposure or TB risk factors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Transparency declaration

JES received a contract from the CDC to support the research and has authored several UpToDate cards, including on bone and joint TB. TA is a study principal investigator at the CDC. All other authors report no conflicts of interest. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the CDC. This work was supported by the CDC TBESC.

Abbreviations:

IGRA	interferon-γ release assay
LTBI	latent TB infection
QFT	QuantiFERON Gold In-Tube
TB	tuberculosis
TBESC	Tuberculosis Epidemiologic Studies Consortium
TSPOT	T-SPOT.*TB*
TST	tuberculin skin test

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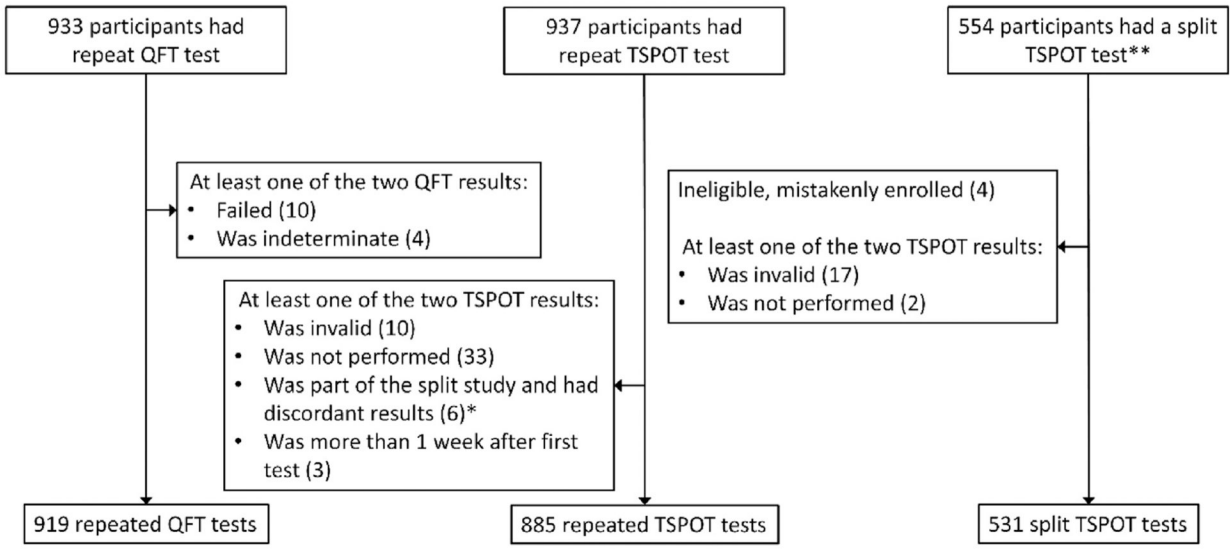

*39 additional participants were part of the split study for the first test but had concordant results. These participants were removed from analyses using the maximum number of spots.
**556 split TSPOT tests were performed. Two participants had two sets of split TSPOT results and only the first set of split results was used.

Fig. 1.

Exclusion criteria for study. Exclusion criteria are listed in the order they were applied, and the number in parentheses indicates the number of participants excluded when applying those criteria. QFT, QuantiFERON Gold In-Tube; TSPOT, T-SPOT.*TB*.

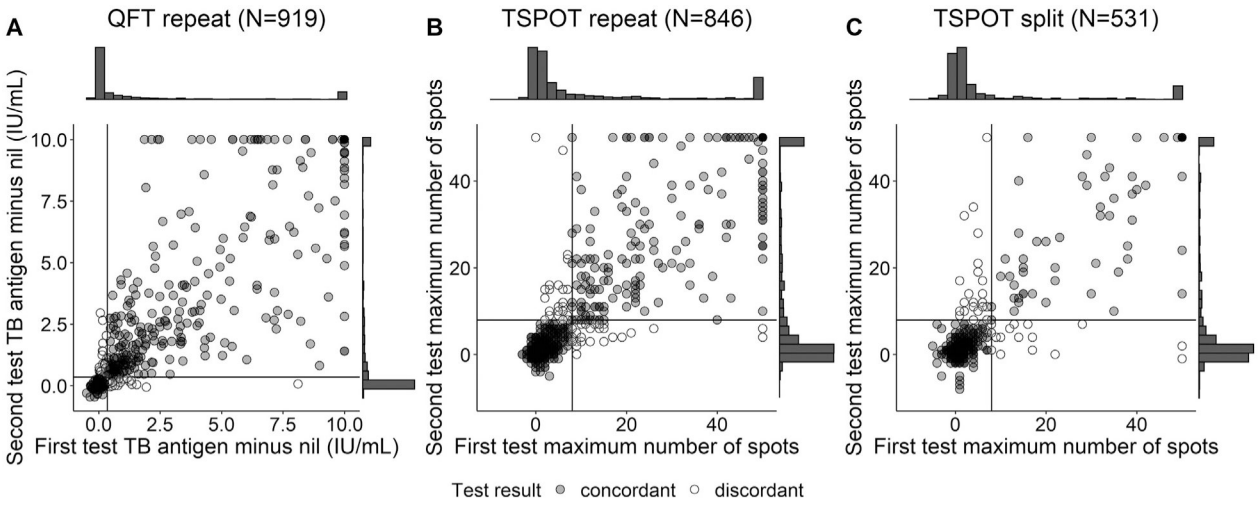

Fig. 2.

Comparison of first and second test results. Histograms indicate the distribution of test results for the first test (top; horizontal axis) and second test (right; vertical axis). Within the plot, points are coloured by whether the interpretation was concordant (grey) or discordant (white). The darker the point, the more participants with those test values. (A) Comparison of the tuberculosis (TB) antigen minus nil values used for interpretation for the QuantiFERON Gold In-Tube (QFT) repeat analysis. The black lines indicate 0.35 IU/mL. Values ≥0.35 IU/mL are interpreted as positive. All TB antigen minus nil values >10 IU/mL were set to 10 IU/mL. (B, C) Comparison of the maximum number of spots from panel A minus nil and panel B minus nil used for interpretation of T-SPOT.*TB* (TSPOT) (B) repeat or (C) split analysis. Note that 39 participants were removed from the TSPOT repeat analyses because their first test was part of the TSPOT split study and it was unclear which quantitative value to use. The black lines indicate eight spots. Values ≥8 are interpreted as positive, using the U.S. Food and Drug Administration–approved cut-off for positive [16]. All spot counts with a value of >50 were set to 50. *Repeat* means the test was performed twice on two different blood samples taken 2 to 3 days apart. *Split* means the test was performed twice on the same sample taken at the same time.

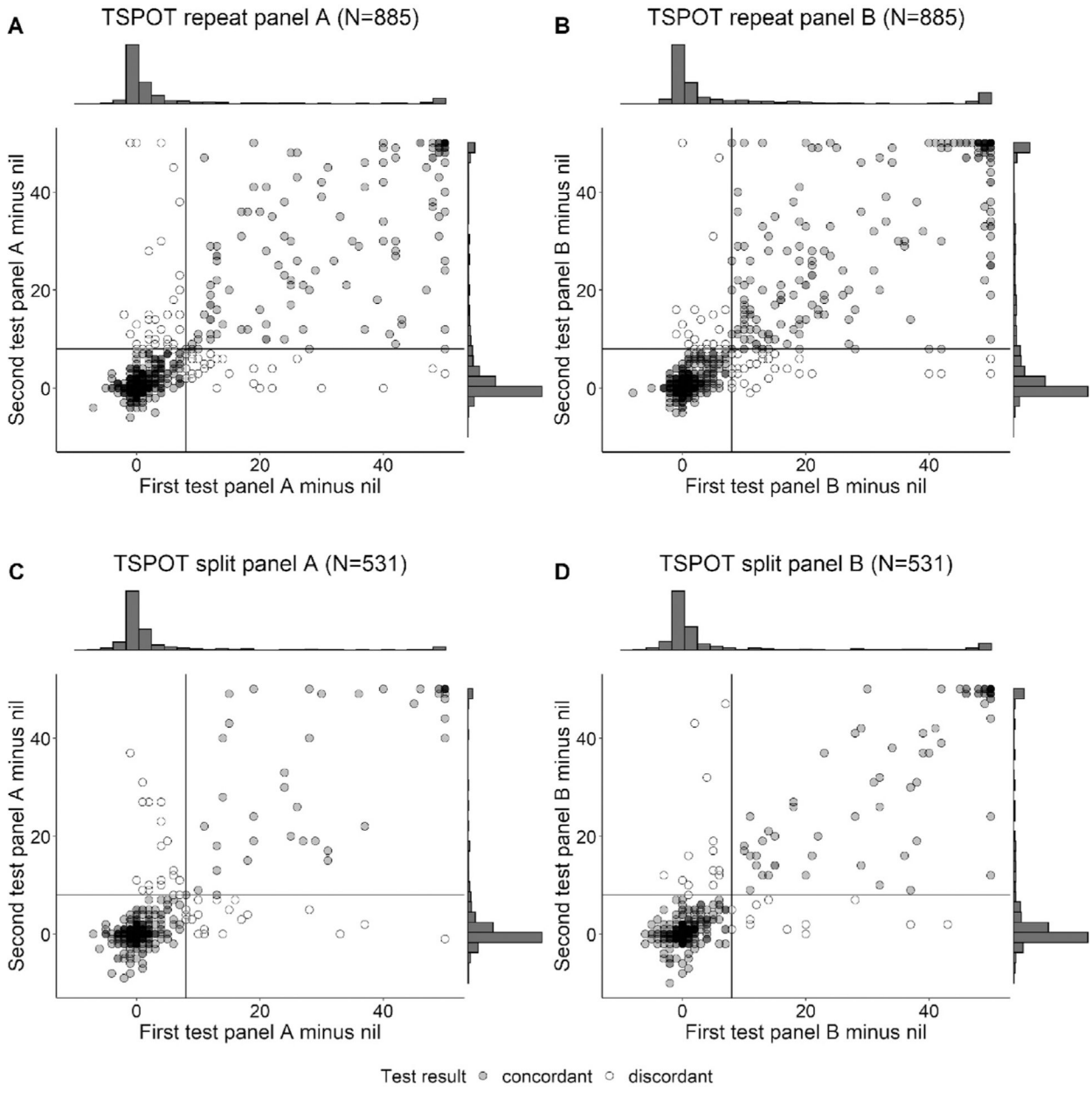

Fig. 3.

Comparison of TSPOT panel A minus nil and panel B minus nil results. Histograms indicate the distribution of test results for the first test (top; horizontal axis) and second test (right; vertical axis). Within the plot, points are coloured by whether the interpretation would have been concordant (grey) or discordant (white) if only that panel result was used. The darker the point, the more participants with those test values. The black lines indicate eight spots, the U.S. Food and Drug Administration–approved cut-off for positive [16]. Note that all spot counts with a value of >50 were set to 50. (A) Panel A minus nil results from the T-SPOT.*TB* (TSPOT) repeat study. (B) Panel B minus nil results from the TSPOT repeat study. (C) Panel A minus nil results from the TSPOT split study. (D) Panel B minus nil results from the TSPOT split study. *Repeat* means the test was performed twice on two

different blood samples taken 2 to 3 days apart. *Split* means the test was performed twice on the same sample taken at the same time.

Table 1

Participant demographics

Characteristic	All QFT repeat (*N* = 919)	QFT repeat concordant (*n* = 860)	QFT repeat discordant (*n* = 59)	All TSPOT repeat (*N* = 885)	TSPOT repeat concordant (*n* = 825)	TSPOT repeat discordant (*n* = 60)	All TSPOT split (*N* = 531)	TSPOT split concordant (*n* = 490)	TSPOT split discordant (*n* = 41)
Age group (y), *n* (%)									
15–44	622 (68)	588 (68)	34 (58)	607 (69)	572 (69)	35 (58)	376 (71)	351 (72)	25 (61)
45–64	244 (27)	224 (26)	20 (34)	233 (26)	214 (26)	19 (32)	125 (24)	114 (23)	11 (27)
≥65	53 (6)	48 (6)	5 (8)	45 (5)	39 (5)	6 (10)	30 (6)	25 (5)	5 (12)
Sex, *n* (%)									
Female	447 (49)	412 (48)	35 (59)	432 (49)	402 (49)	30 (50)	282 (53)	259 (53)	23 (56)
Male	472 (51)	448 (52)	24 (41)	453 (51)	423 (51)	30 (50)	249 (47)	231 (47)	18 (44)
Nativity, *n* (%)									
U.S.-born	109 (12)	102 (12)	7 (12)	108 (12)	106 (13)	2 (3)	74 (14)	72 (15)	2 (5)
non-U.S.-born	810 (88)	758 (88)	52 (88)	777 (88)	719 (87)	58 (97)	453 (85)	415 (85)	38 (93)
Unknown	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (1)	3 (1)	1 (2)
Race/ethnicity, *n* (%)									
Hispanic	161 (18)	157 (18)	4 (7)	154 (17)	148 (18)	6 (10)	100 (19)	95 (19)	5 (12)
Non-Hispanic, Asian/ Pacific Islander	304 (33)	277 (32)	27 (46)	286 (32)	259 (31)	27 (45)	173 (33)	157 (32)	16 (39)
Non-Hispanic, Black	180 (20)	171 (20)	9 (15)	175 (20)	167 (20)	8 (13)	81 (15)	76 (16)	5 (12)
Non-Hispanic, White	93 (10)	86 (10)	7 (12)	92 (10)	89 (11)	3 (5)	48 (9)	43 (9)	5 (12)
Multiple/other/unknown	181 (20)	169 (20)	12 (20)	178 (20)	162 (20)	16 (27)	129 (24)	119 (24)	10 (24)
Self-reported medical histories and risk factors, *n* (%)									
Bacille Calmette-Guerin vaccination	516 (56)	487 (57)	29 (49)	491 (55)	451 (55)	40 (67)	312 (59)	287 (59)	25 (61)
Human immunodeficiency virus-positive[a]	62 (7)	62 (7)	0 (0)	55 (6)	53 (6)	2 (3)	0 (0)	0 (0)	0 (0)
Diabetes	56 (6)	51 (6)	5 (8)	55 (6)	51 (6)	4 (7)	29 (5)	26 (5)	3 (7)
Chronic hepatitis or liver disease	32 (3)	31 (4)	1 (2)	30 (3)	29 (4)	1 (2)	14 (3)	14 (3)	0 (0)
Chronic kidney failure	4 (0)	4 (0)	0 (0)	3 (0)	3 (0)	0 (0)	3 (1)	2 (0)	1 (2)
Current smoker	175 (19)	167 (19)	8 (14)	162 (18)	150 (18)	12 (20)	91 (17)	85 (17)	6 (15)

Characteristic	All QFT repeat (*N* = 919)	QFT repeat concordant (*n* = 860)	QFT repeat discordant (*n* = 59)	All TSPOT repeat (*N* = 885)	TSPOT repeat concordant (*n* = 825)	TSPOT repeat discordant (*n* = 60)	All TSPOT split (*N* = 531)	TSPOT split concordant (*n* = 490)	TSPOT split discordant (*n* = 41)
Ever lived or worked in a prison or jail	85 (9)	81 (9)	4 (7)	77 (9)	75 (9)	2 (3)	48 (9)	47 (10)	1 (2)
Ever lived or worked in a homeless shelter	84 (9)	79 (9)	5 (8)	83 (9)	81 (10)	2 (3)	67 (13)	64 (13)	3 (7)
Regular alcohol use (>4 drinks/wk)[b]	24 (3)	21 (2)	3 (5)	21 (2)	20 (2)	1 (2)	19 (4)	17 (3)	2 (5)
Close contact of an infectious tuberculosis case	148 (16)	134 (16)	14 (24)	140 (16)	129 (16)	11 (18)	114 (21)	110 (22)	4 (10)
Days between tests, *n* (%)									
2 d	462 (50)	430 (50)	32 (54)	437 (49)	406 (49)	31 (52)	—	—	—
3 d	457 (50)	430 (50)	27 (46)	448 (51)	419 (51)	29 (48)	—	—	—
QFT result, *n* (%)									
Negative	563 (61)	536 (62)	27 (46)	541 (61)	530 (64)	11 (18)	382 (72)	370 (76)	12 (29)
Positive	356 (39)	324 (38)	32 (54)	338 (38)	290 (35)	48 (80)	146 (27)	117 (24)	29 (71)
Missing	0 (0)	0 (0)	0 (0)	6 (1)	5 (1)	1 (2)	3 (1)	3 (1)	0 (0)
TSPOT result, *n* (%)									
Negative	621 (68)	578 (67)	43 (73)	609 (69)	578 (70)	31 (52)	421 (79)	399 (81)	22 (54)
Positive	288 (31)	272 (32)	16 (27)	276 (31)	247 (30)	29 (48)	110 (21)	91 (19)	19 (46)
Missing	10 (1)	10 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Each column represents the number (percentage) of observations with the indicated characteristics. QFT and TSPOT results indicate the first test result (QFT and TSPOT). Self-reported medical histories and risk factors are not mutually exclusive and indicate that the participant self-reported having that factor. *Repeat* means the test was performed twice on two different blood samples taken 2 to 3 days apart. *Split* means the test was performed twice on the same sample taken at the same time. QFT, QuantiFERON Gold In-Tube; TSPOT, T-SPOT.*TB*.

[a]Participants were excluded from the TSPOT split study if they were immunocompromised.

[b]For participants age ≥ 15 years.

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Table 2

Comparison of dichotomous results

		QFT repeat		TSPOT repeat		TSPOT split	
		Second test result		Second test result		Second test result	
		−	+	−	+	−	+
First test result	−	536	27	578	31	399	22
	+	2	324	29	247	19	91

Repeat means the test was performed twice on two different blood samples taken 2 to 3 days apart. *Split* means the test was performed twice on the same sample taken at the same time. Numbers represent the number of participants with that test combination. QFT results were defined using the manufacturer's recommendations (tuberculosis antigen minus nil values ≥0.35 IU/ml are positive). TSPOT results were positive when the maximum spot values were ≥8 (based on U.S. Food and Drug Administration–approved cut-off for positive [16]). QFT, QuantiFERON Gold In-Tube; TSPOT, T-SPOT.*TB*.