

# Improved Diagnostic Evaluation of Suspected Tuberculosis

Davinder P.S. Dosanjh, DPhil; Timothy S.C. Hinks, MD; John A. Innes, MD; Jonathan J. Deeks, PhD; Geoffrey Pasvol, DPhil; Sarah Hackforth, RGN; Hansa Varia, RGN; Kerry A. Millington, DPhil; Rubamalar Gunatheesan, MD; Valerie Guyot-Revol, PhD; and Ajit Lalvani, DM

**Background:** The role of new T-cell–based blood tests for tuberculosis in the diagnosis of active tuberculosis is unclear.

**Objective:** To compare the performance of 2 interferon- $\gamma$  assays and tuberculin skin testing in adults with suspected tuberculosis.

**Design:** Prospective study conducted in routine practice.

**Setting:** 2 urban hospitals in the United Kingdom.

**Patients:** 389 adults, predominantly of South Asian and black ethnicity, with moderate to high clinical suspicion of active tuberculosis.

**Intervention:** Tuberculin skin testing, the enzyme-linked immunosorbent assay (ELISpot) incorporating early secretory antigenic target-6 and culture filtrate protein-10 (standard ELISpot), and ELISpot incorporating a novel antigen, Rv3879c (ELISpot<sup>PLUS</sup>) were performed during diagnostic assessment by independent persons who were blinded to results of the other test.

**Measurements:** Sensitivity, specificity, predictive values, and likelihood ratios.

**Results:** 194 patients had a final diagnosis of active tuberculosis, of which 79% were culture-confirmed. Sensitivity for culture-

confirmed and highly probable tuberculosis was 89% (95% CI, 84% to 93%) with ELISpot<sup>PLUS</sup>, 85% (CI, 79% to 90%) with standard ELISpot, 79% (CI, 72% to 85%) with 15-mm threshold tuberculin skin testing, and 83% (CI, 77% to 89%) with stratified thresholds of 15 and 10 mm in vaccinated and unvaccinated patients, respectively. The ELISpot<sup>PLUS</sup> assay was more sensitive than tuberculin skin testing with 15-mm cutoff points ( $P = 0.01$ ) but not with stratified cutoff points ( $P = 0.10$ ). The ELISpot<sup>PLUS</sup> assay had 4% higher diagnostic sensitivity than standard ELISpot ( $P = 0.02$ ). Combined sensitivity of ELISpot<sup>PLUS</sup> and tuberculin skin testing was 99% (CI, 95% to 100%), conferring a negative likelihood ratio of 0.02 (CI, 0 to 0.06) when both test results were negative.

**Limitations:** Local standards for tuberculin skin testing differed from others used internationally. The study sample included few immunosuppressed patients.

**Conclusion:** The ELISpot<sup>PLUS</sup> assay is more sensitive than standard ELISpot and, when used in combination with tuberculin skin testing, enables rapid exclusion of active infection in patients with moderate to high pretest probability of tuberculosis.

*Ann Intern Med.* 2008;148:325-336.

www.annals.org

For author affiliations, see end of text.

Improved diagnosis of tuberculosis is necessary to contain and reverse the rising global burden of this disease (1). The poor speed and sensitivity of existing diagnostic tools (2–4) cause delays in diagnosis and treatment of active tuberculosis, and diagnosis of extrapulmonary and paucibacillary forms is often especially challenging. Given that *Mycobacterium tuberculosis* infection is a prerequisite for active tuberculosis, reliable determination of infection status could accelerate diagnostic assessment by enabling rapid exclusion of tuberculosis. Recently developed T-cell–based interferon- $\gamma$  release assays for tuberculosis may overcome some of the limitations of the tuberculin skin test (5–10). These immunoassays detect interferon- $\gamma$  secreted by T cells in response to antigens encoded in the region of difference 1 of *M. tuberculosis* complex, a genomic segment absent from bacille Calmette–Guérin and most environmental mycobacteria (11). Test results are therefore not confounded by previous bacille Calmette–Guérin vaccination, conferring higher specificity than the tuberculin skin test (7–10). Moreover, results are available the next day and are unaffected by the boosting phenomenon (12).

The 2 types of T-cell–based interferon- $\gamma$  release assay are whole-blood enzyme-linked immunosorbent assay (ELISA) and enzyme-linked immunosorbent assay (ELISpot). The whole-blood ELISA is available commercially as QuantiFERON-TB Gold and an “in-tube” variant, QuantiFERON-TB Gold In-tube (Cellestis, Carnegie, Australia) (13, 14). The ELISpot, developed by Lalvani and

colleagues (15, 16), is available commercially as T-SPOT.TB (Oxford Immunotec, Abingdon, United Kingdom) (15–18). Recent U.S. and United Kingdom national guidelines (19, 20) recommend T-cell–based interferon- $\gamma$  release assays for diagnosis of latent tuberculosis, but their clinical utility in evaluation of patients with suspected active tuberculosis is poorly defined.

We compared the performance characteristics of 2 assays and tuberculin skin testing individually and in combination for the diagnostic work-up of patients with suspected active tuberculosis. The standard ELISpot assay uses early secretory antigenic target-6 and culture filtrate protein-10, the same peptides as the 2 region of difference 1–encoded antigens included in T-SPOT.TB. The ELISpot<sup>PLUS</sup> assay incorporates a novel region of difference 1–encoded anti-

See also:

#### Print

Editors' Notes . . . . . 326  
Editorial comment . . . . . 398

#### Web-Only

Appendix Figures  
Conversion of graphics into slides  
Audio summary

**Context**

Can new T-cell–based blood tests rule out active tuberculosis?

**Contribution**

In this study involving 389 adults with moderate to high suspicion of active tuberculosis, the sensitivity of a new T-cell–based enzyme-linked immunoassay (ELISpot<sup>PLUS</sup>) was higher than that of 15-mm threshold tuberculin skin testing (89% vs. 79%) and similar to that of 10-mm threshold skin testing (83%) in unvaccinated patients for identifying culture-confirmed and highly probable tuberculosis. Patients with negative results on both ELISpot<sup>PLUS</sup> and skin tests had a very low likelihood of tuberculosis (negative likelihood ratio, 0.02).

**Implication**

The ELISpot<sup>PLUS</sup> assay, particularly when used in combination with tuberculin skin testing, can help exclude a diagnosis of active tuberculosis.

—The Editors

gen, Rv3879c, alongside early secretory antigenic target-6 and culture filtrate protein-10 (21).

**METHODS****Participants**

One day each week from 12 July 2002 to 29 June 2005, we prospectively enrolled adults who presented to Heartlands Hospital, Birmingham, and Northwick Park Hospital, London, United Kingdom, with suspected tuberculosis. We invited patients 16 years of age or older to participate and provide written informed consent if their attending physician considered tuberculosis to be part of the differential diagnosis. We used no exclusion criteria. The Birmingham Heartlands Hospital, Harrow, and Oxford Clinical Research Ethics Committees granted ethical approval. Because the background prevalence of HIV is low in this population (22), we tested patients for HIV antibodies during diagnostic work-up only on the basis of clinical suspicion. We identified only 20 patients who were HIV antibody–positive.

**Tuberculin Skin Testing**

Tuberculin skin testing was performed as part of routine care, and experienced nurses read the results by using the Mantoux method with 0.1 mL (10 tuberculin units) of purified protein derivative (PPD)–Siebert (Evans Vaccines, Liverpool, United Kingdom). Intradermal inoculation was confirmed by the cutaneous appearance of *peau d'orange*. Induration was measured after 72 hours with a ruler and recorded in millimeters. Because PPD for Mantoux testing was unavailable for 10 months during the study period, 104 patients were tested by using the Heaf method, with the standard multiple puncture 6-needle disposable-head

Heaf gun (Bignall Surgical Instruments, Littlehampton, United Kingdom) and concentrated PPD (100 000 tuberculin units/mL; Evans Vaccines). Heaf tests were read 1 week later, as recommended, and graded from 0 to 4. The nurses who performed and read skin tests were blinded to ELISpot results. We considered induration of 15 mm or greater on the Mantoux test or grade 3 or 4 on the Heaf test (which is considered equivalent to the 15-mm threshold [23, 24]) to be a positive result. We assessed bacille Calmette–Guérin vaccination status by history and, where present, by scar. We also assessed tuberculin skin test performance by using stratified cutoff points of 15 mm and 10 mm (grade 2 to 4 on the Heaf test) in vaccinated and unvaccinated patients, respectively (“stratified 10-mm threshold”) (23, 24).

**The ELISpot Assay**

Before or within 1 week of initiating therapy, we obtained a sample of 30 mL of heparinized blood and used  $7.5 \times 10^6$  peripheral blood mononuclear cells for ELISpot assays. We seeded precoated interferon- $\gamma$  ELISpot plates (Mabtech AB, Stockholm, Sweden) with  $2.5 \times 10^5$  peripheral blood mononuclear cells per well (17). Duplicate wells contained no antigen (negative control) or phytohemagglutinin (positive control) (ICN Biomedical, Aurora, Ohio) at 5  $\mu\text{g/mL}$ . A further 13 pairs of duplicate wells each contained 1 of 13 peptide pools, which incorporated 5 to 7 overlapping 15-mer peptides. The assay on which T-SPOT.TB is based, defined as standard ELISpot in this study, comprises 35 such peptides assembled into 6 pools and spanning the length of early secretory antigenic target-6 and culture filtrate protein-10. Forty-five peptides from selected regions of Rv3873, Rv3878, and Rv3879c (Research Genetics, Huntsville, Alabama) were assembled into 7 pools (21). The ELISpot<sup>PLUS</sup> assay was defined as the 35 early secretory antigenic target-6 and culture filtrate protein-10 peptides, with 17 peptides from Rv3879c, assembled into 3 additional pools. The final concentration of each peptide was 10  $\mu\text{g/mL}$ . After overnight incubation at 37 °C in 5% carbon dioxide, the plates were developed with pre-conjugated detector antibody (Mabtech AB) followed by chromogenic substrate (Moss, Pasadena, Maryland) (17). Spot-forming cells were counted by using an automated ELISpot reader (AID-GmbH, Strassberg, Germany). We predefined settings for the intensity and size of a counted spot and used the same settings throughout. We transferred mean readings from duplicate wells to a spreadsheet electronically and scored them as positive or negative by using a customized software program, ELISTAT (AID-GmbH). We scored responses as positive if test wells contained a mean of at least 5 spot-forming cells more than the mean of the negative control wells and were at least twice the mean of the negative control wells. This predefined cutoff point is the standard threshold used with our assay format in 9 studies including a total of 2506 participants (15–17, 25–30). Operators performing and

reading the assays were blinded to tuberculin skin test results and personal identifiers. The ELISpot results were checked and countersigned before data entry by a scientist who did not perform the assays.

### Diagnosis

Attending physicians performed diagnostic work-up as part of routine clinical practice, which was directed on a case-by-case basis by the patient's clinical presentation. Two research nurses collected clinical, radiologic, and microbiological data on standardized forms at recruitment, during follow-up, and at final case notes review. Two physicians who were blinded to all ELISpot data assigned each patient to 1 of 4 predefined diagnostic categories (Table 1), which are similar to those used in other studies (25). Category 4 inevitably included some patients with inactive or latent tuberculosis. To compensate for heterogeneity, we further classified patients in this category into 4 predefined subgroups, analogous to American Thoracic Society classes 4, 2, 1, and 0 (31), in decreasing likelihood of latent tuberculosis (Table 1). We stratified by radiologic evidence or history of previous tuberculosis, risk factors for latent infection, and skin test results.

### Statistical Analysis

We calculated the sensitivity, specificity, likelihood ratios, and predictive values for each test and for sequences of 2 tests used in combination. Because the primary clinical

utility of immune-based testing is to rule out tuberculosis, our predefined analytical plan focused primarily on sensitivities, negative likelihood ratios, and negative predictive values. We compared proportions by using Pearson chi-square and Fisher exact tests where sample sizes differed substantially (because of missing results for 1 of the tests). We compared the data from the ELISpot and ELISpot<sup>PLUS</sup> tests by using the McNemar chi-square test, treating the data as paired and dropping the 6 individuals who did not have an ELISpot<sup>PLUS</sup> result. We assumed that missing data were missing completely at random for our analyses, but we provide sufficient information for other analyses to be performed with different assumptions about reasons for missing data. Two-tailed *P* values are reported.

We calculated primary analyses of sensitivity, specificity, likelihood ratios, and predictive values by using categories 1 (culture-confirmed), 2 (highly probable), and 4 (active tuberculosis excluded) (Table 1). Category 3 (clinically indeterminate) cases were not included in the calculation of test performances. To investigate whether the inclusion of category 2 cases affected sensitivity, we calculated performance by using only categories 1 and 4, and to investigate whether the inclusion of categories 4A, 4B, and 4C (which included patients with latent and inactive tuberculosis) affected specificity, we calculated performance by using only categories 1, 2, and 4D (no risk factors for tuberculosis exposure).

Table 1. Categorization of the Study Population\*

Diagnostic Category	Criteria	Patients (n = 387), n
1: Culture-confirmed tuberculosis	Microbiological culture of <i>Mycobacterium tuberculosis</i> and Suggestive clinical and radiologic findings	154
2: Highly probable tuberculosis	Clinical and radiologic features highly suggestive of tuberculosis and unlikely to be caused by other disease and A decision to treat made by a clinician and Appropriate response to therapy and Histology supportive if available†	40
3: Clinically indeterminate	A final diagnosis of tuberculosis was neither highly probable nor reliably excluded	39
4: Active tuberculosis excluded	All microbiological samples smear and culture negative and A definite alternative diagnosis identified	
Subclassification		
4A: Inactive tuberculosis	Previous episode or stable chest radiograph changes and TST positive‡ (if done) and Bacteriologically negative (if done) and No clinical evidence of active disease	19
4B: ≥1 risk factors for tuberculosis exposure§, TST positive	TST positive and Bacteriologically negative (if done) and No clinical evidence of active disease	18
4C: ≥1 risk factors for tuberculosis exposure§, TST negative	History of tuberculosis exposure and TST negative (if done)	92
4D: No risk factors for tuberculosis exposure§, TST negative	No history of tuberculosis exposure and TST negative (if done)	25

\* TST = tuberculin skin test.

† Histologic findings were available for 16 of 40 patients and supportive in all 16 patients (13 had granulomas, 7 had epithelioid cells, 6 had caseation, and 1 had necrosis with acute inflammation).

‡ Threshold values for positivity were induration ≥15 mm on the Mantoux test or grade 3–4 on the Heaf test.

§ Recent exposure to a patient with active tuberculosis, or born in a country or belonging to an ethnic group with a high prevalence of tuberculosis (incidence >100 per 100 000 persons [22]).

We assessed the performance of combinations of the 2 tests by using logistic regression models. For this analysis, we necessarily restricted the data set to patients with results from both tests. Logistic regression models can be constructed in the same form as Bayesian updating (posttest odds = pretest odds × likelihood ratio) by including the log of the pretest odds of prevalence (a constant term of known value) as an offset in the model. The linear predictor then estimates log likelihood ratios rather than log odds ratios, but bootstrap methods are required to obtain valid CIs (32). We used model parameterizations from Knottnerus (33) to compute likelihood ratios for the additional diagnostic value of each test in a testing sequence, whereas we followed the recommendations of Albert (34) to obtain

estimates of likelihood ratios for combinations of tests. We computed nonparametric, bias-adjusted CIs for parameter estimates from 1000 bootstrap samples.

We computed predictors of false-negative test results by using chi-square or Fisher exact tests, using the Bonferroni correction for multiple comparisons. We performed our analyses by using GraphPad Prism 4 (GraphPad Software, San Diego, California) and Stata, version 9.0 (Stata, College Station, Texas).

**Role of the Funding Source**

The Wellcome Trust had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

**Table 2. Participant Characteristics**

Characteristic	Culture-Confirmed Tuberculosis	Highly Probable Tuberculosis	Clinically Indeterminate	Active Tuberculosis Excluded	Total
<b>Total, n (%)</b>	154 (39.6)	40 (10.3)	39 (10.0)	154 (39.6)	389 (100)*
<b>Median age (range), y</b>	31.5 (16–85)	30 (17–74)	34 (17–88)	47 (16–95)	36 (16–95)
<b>Men, n (%)</b>	94 (61.0)	20 (50.0)	25 (64.1)	95 (61.7)	235 (60.4)
<b>Ethnic origin, n (%)</b>					
Indian subcontinent†	90 (58.4)	26 (65.0)	23 (59.0)	83 (53.9)	222 (57.1)
Black‡	34 (22.1)	11 (27.5)	11 (28.2)	28 (18.2)	86 (22.1)
White	22 (14.3)	1 (2.5)	2 (5.1)	65 (22.7)	60 (15.4)
Chinese	1 (0.6)	0 (0.0)	1 (2.6)	0 (0.0)	2 (0.5)
Other	7 (4.5)	2 (5.0)	2 (5.1)	8 (5.2)	19 (4.9)
<b>Bacille Calmette–Guérin vaccination status, n (%)</b>					
Vaccinated	93 (60.4)	19 (47.5)	24 (61.5)	68 (44.2)	204 (52.4)
Unknown	20 (13.0)	7 (17.5)	6 (15.4)	39 (25.3)	74 (19.0)
<b>Preexisting conditions and illnesses, n (%)</b>					
None	110 (71.4)	35 (87.5)	21 (53.8)	76 (49.4)	244 (62.7)
Previous tuberculosis	12 (7.8)	4 (10.0)	9 (23.1)	20 (13.0)	45 (11.6)
Diabetes mellitus	13 (8.4)	0 (0.0)	1 (2.6)	17 (11.0)	31 (8.0)
Asthma	3 (1.9)	0 (0.0)	1 (2.6)	16 (10.4)	20 (5.1)
HIV infection	8 (5.2)	0 (0.0)	4 (10.3)	8 (5.2)	20 (5.1)
Alcoholism	0 (0.0)	0 (0.0)	1 (2.6)	5 (3.2)	6 (1.5)
Ischemic heart disease	2 (1.3)	0 (0.0)	0 (0.0)	4 (2.6)	6 (1.5)
Sarcoidosis	4 (2.6)	0 (0.0)	0 (0.0)	2 (1.3)	6 (1.5)
Hypertension	0 (0.0)	0 (0.0)	1 (2.6)	5 (3.2)	6 (1.5)
Iron deficiency anemia	3 (1.9)	1 (2.5)	0 (0.0)	1 (0.6)	5 (1.3)
Chronic liver disease	1 (0.6)	0 (0.0)	0 (0.0)	3 (1.9)	4 (1.0)
Chronic renal failure	1 (0.6)	0 (0.0)	0 (0.0)	2 (1.3)	3 (0.8)
Epilepsy	3 (1.9)	0 (0.0)	0 (0.0)	0 (0.0)	3 (0.8)
Hypothyroidism	1 (0.6)	0 (0.0)	0 (0.0)	2 (1.3)	3 (0.8)
Intravenous drug use	1 (0.6)	0 (0.0)	0 (0.0)	2 (1.3)	3 (0.8)
Carcinoma	1 (0.6)	0 (0.0)	0 (0.0)	2 (1.3)	3 (0.8)
Congestive cardiac failure	1 (0.6)	0 (0.0)	0 (0.0)	1 (0.6)	2 (0.5)
Pneumonia	1 (0.6)	0 (0.0)	0 (0.0)	1 (0.6)	2 (0.5)
Rheumatoid arthritis	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.3)	2 (0.5)
Other§	6 (3.9)	0 (0.0)	1 (2.6)	20 (13.0)	27 (6.9)

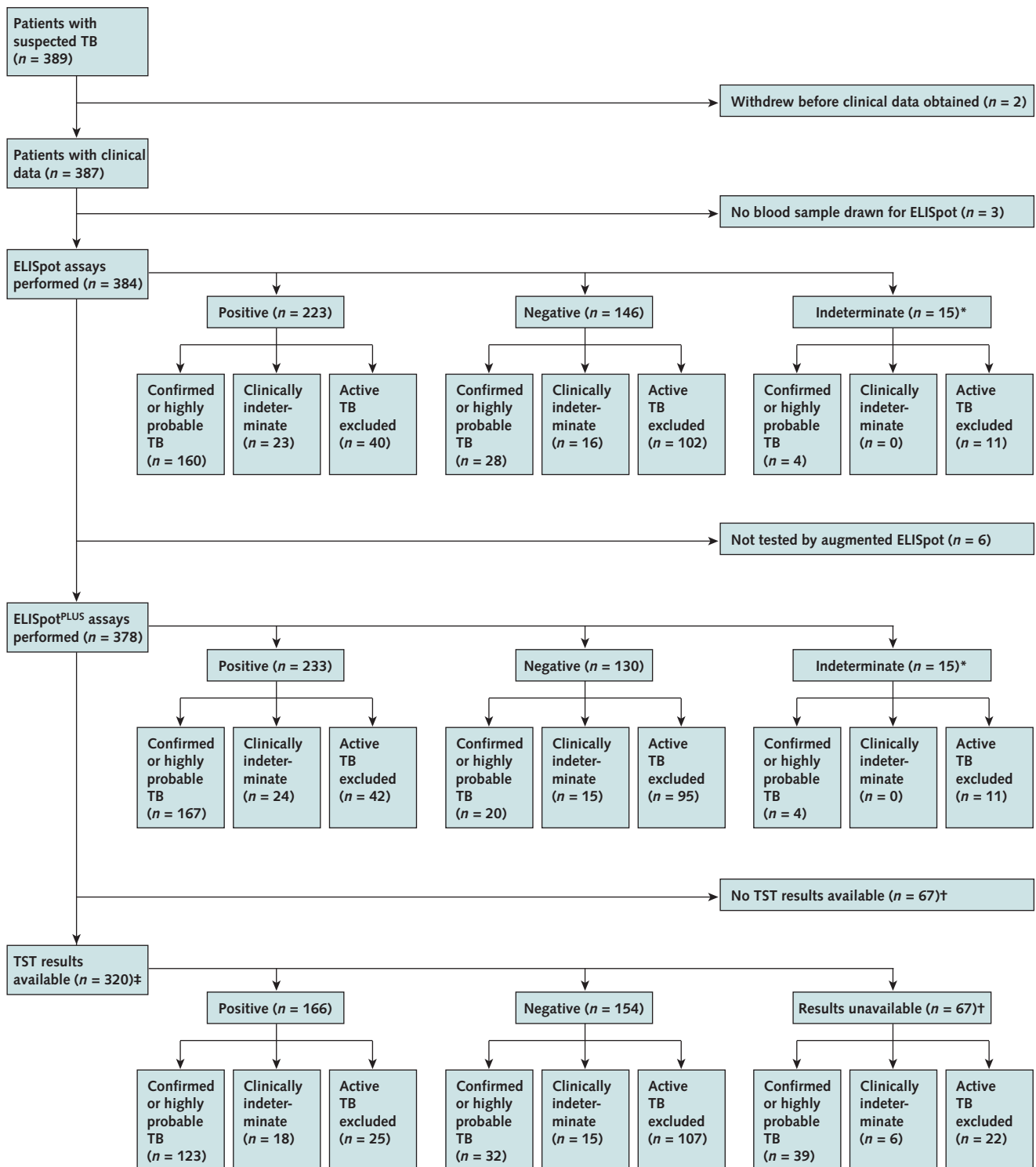
\* We recruited 200 patients from London and 189 from Birmingham; 325 were from inpatient beds and 64 were from clinics. The 2 patients who withdrew before we collected clinical data are not assigned to a diagnostic category but appear in the total column. Approximately 50 additional patients declined consent to the study.

† 105 patients were Pakistani, 89 were Indian, 10 were Sri Lankan, 9 were Bangladeshi, 7 were Afghani, 1 was Nepalese, and 1 was Burmese.

‡ 81 patients were African and 5 were Caribbean.

§ One case each of acute infective hepatitis, α-thalassemia, atrial fibrillation, bronchiectasis, candidiasis, the carpal tunnel syndrome, chronic pancreatitis, chronic obstructive pulmonary disease, hiatal hernia, idiopathic thrombocytopenic purpura, renal transplantation, leprosy, multiple sclerosis, *Mycobacterium avium-intracellulare* infection, osteomyelitis, pancreatectomy, pernicious anemia, the polycystic ovary syndrome, previous breast surgery, previous esophagectomy, previous rheumatic fever, prostatism, salmonella, schizophrenia, sickle cell trait, splenectomy, thyrotoxicosis, ulcerative colitis, urinary tract infection, and Wegener granulomatosis.

Figure 1. Study flow diagram.



ELISpot = enzyme-linked immunospot assay incorporating early secretory antigenic target-6 and culture filtrate protein-10; ELISpot<sup>PLUS</sup> = enzyme-linked immunospot assay incorporating early secretory antigenic target-6, culture filtrate protein-10, and Rv3879c; TB = tuberculosis; TST = tuberculin skin testing. \*Results were indeterminate because of no achievement of positive control (11 patients), high background (1 patient), peptide contamination (1 patient), inconclusive assay (1 patient), or defective ELISpot plate (1 patient). †Results were not available because of history of TB (clinically contraindicated) (45 patients), patient did not return for reading (8 patients), result not recorded (8 patients), reason unknown (3 patients), death (1 patient), test performed elsewhere (1 patient), or patient declined consent (1 patient). ‡Tuberculin skin test results were based on a 15-mm cutoff point and considered positive if induration was  $\geq 15$  mm on the Mantoux test or grade 3 to 4 on the Heaf test regardless of bacille Calmette–Guérin vaccination status.

**Table 3. Final Diagnoses of Study Participants**

Category	Patients, n (%)
<b>Confirmed or highly probable tuberculosis (n = 194)</b>	
Site of disease	
Pulmonary	140 (72.2)
Lymphatic	18 (9.3)
Disseminated	10 (5.2)
Pleural	9 (4.6)
Pulmonary and pleural	3 (1.5)
Bone	2 (1.0)
Genitourinary	2 (1.0)
Abdominal	1 (0.5)
Breast and pericardial	1 (0.5)
Joint	1 (0.5)
Meningeal	1 (0.5)
Pericardial	1 (0.5)
Peritoneal and pleural	1 (0.5)
Pulmonary and lymphatic	1 (0.5)
Retropharyngeal	1 (0.5)
Thyroid	1 (0.5)
Psoas	1 (0.5)
<b>Active tuberculosis excluded (n = 154)</b>	
Pneumonia	45 (29.2)
Cancer*	8 (5.2)
Other viral infection	8 (5.2)
Bronchiectasis	7 (4.5)
Sarcoidosis	6 (3.9)
Lung abscess	5 (3.2)
Lymphoma	4 (2.6)
Inactive tuberculosis only	4 (2.6)
Typhoid	4 (2.6)
Urinary tract infection	4 (2.6)
Chronic obstructive pulmonary disease	3 (1.9)
Healthy	3 (1.9)
Hepatic abscess†	3 (1.9)
Bacterial meningitis	2 (1.3)
Bronchitis	2 (1.3)
Hepatitis	2 (1.3)
<i>Mycobacterium avium-intracellulare</i>	2 (1.3)
Osteomyelitis	2 (1.3)
Pancreatitis	2 (1.3)
<i>Pneumocystis carinii</i> pneumonia	2 (1.3)
Other‡	36 (23.4)

\* Three cases of pulmonary cancer (1 squamous cell, 1 small cell, and 1 unspecified); 1 case each of esophageal, ovarian, and colon cancer; and 2 cases in which the primary site was unknown.

† One pyogenic, 1 cryptococcal, and 1 unspecified.

‡ One case each of adult Still disease, alcohol-related symptoms, aspergilloma, bacterial endocarditis, benign spinal tumor, bronchial adenoma, cellulitis, cytomegalovirus, Crohn disease, cystic hygroma, dengue fever, deep venous thrombosis, viral encephalitis, epilepsy, hypervolemia, foot ulcer, the Goodpasture syndrome, hemangioma, hematoma, Henoch-Schönlein purpura, hydrocele, hydradenitis suppurativa, hypertension-related headache, hyperthyroidism, ileopsoas inflammation, laryngitis, paratyphoid, pericarditis, pyelonephritis, salmonella sepsis, septicemia, sinusitis, sterile pyuria, streptococcal-associated reactive arthritis, subacute pericarditis, and viral labyrinthitis.

**RESULTS**

We enrolled 389 consenting adult patients, whose demographic characteristics are shown in **Table 2**. Blood was unavailable from 3 patients, tuberculin skin test results were unavailable in 67 patients, and 15 ELISpot assays (4%) were indeterminate (**Figure 1**). The 67 patients without tuberculin skin test results were similar to those with skin test results in terms of age, sex, ethnicity, presence of tuberculosis, site of disease, and presence of comorbid con-

ditions (data provided on request). We classified 154 patients (40%) as having culture-confirmed tuberculosis, 40 (10%) as having highly probable tuberculosis, and 39 (10%) as clinically indeterminate; we excluded active tuberculosis in 154 patients (40%) (**Table 1** and **Appendix Figure 1**, available at [www.annals.org](http://www.annals.org)). Twenty-eight percent of tuberculosis cases were extrapulmonary; **Table 3** shows the clinical subtypes of tuberculosis and alternative diagnoses for patients without tuberculosis.

For culture-confirmed and highly probable cases, diagnostic sensitivities were 89% (95% CI, 84% to 93%) with ELISpot<sup>PLUS</sup>, 85% (CI, 79% to 90%) with standard ELISpot, 79% (CI, 72% to 85%) with 15-mm threshold tuberculin skin testing, and 83% (CI, 77% to 89%) with stratified 10-mm threshold tuberculin skin testing (**Table 4**). The ELISpot<sup>PLUS</sup> assay was more sensitive than tuberculin skin testing with the 15-mm threshold ( $P = 0.01$ ) but not with the stratified 10-mm threshold ( $P = 0.10$ ). The ELISpot<sup>PLUS</sup> assay was more sensitive than tuberculin skin testing with a 15-mm threshold in the 297 patients with results from both tests (89% [CI, 83% to 94%] versus 81% [CI, 73% to 87%];  $P = 0.04$ ), but not with stratified-10 mm threshold (85% [CI, 78% to 90%];  $P = 0.2$ ). The sensitivity of ELISpot<sup>PLUS</sup> was 4.2% higher than that of standard ELISpot ( $P = 0.02$ ). Seven patients with active tuberculosis had ELISpot responses to novel peptides but not to early secretory antigenic target-6 or culture filtrate protein-10, of whom 4 were positive to Rv3873, 3 to Rv3878, and all 7 to Rv3879c.

In a sensitivity analysis to investigate whether inclusion of highly probable cases (category 2, which was biased in favor of the tuberculin skin test because a diagnosis of highly probable tuberculosis was based in part on skin test results) affected performance estimates, we reestimated sensitivity by using only culture-confirmed cases (category 1). Sensitivities were 91% (CI, 85% to 95%) with ELISpot<sup>PLUS</sup>, 87% (CI, 80% to 92%) with standard ELISpot, 79% (CI, 71% to 86%) with tuberculin skin testing using the 15-mm threshold, and 82% (CI, 74% to 89%) with the stratified 10-mm threshold. The ELISpot<sup>PLUS</sup> assay was more sensitive than tuberculin skin testing using either the 15-mm ( $P = 0.005$ ) or the stratified 10-mm threshold ( $P = 0.03$ ) (**Table 4**).

Tuberculin skin testing and ELISpot had higher sensitivity when used in combination. For culture-confirmed or highly probable diagnoses of tuberculosis, sensitivity of ELISpot<sup>PLUS</sup> with tuberculin skin testing was 99% (CI, 95% to 100%) and standard ELISpot with tuberculin skin testing was 97% (CI, 93% to 99%) (**Figure 2**). A negative result on both tests was associated with likelihood ratios of 0.02 (CI, 0 to 0.09) for ELISpot<sup>PLUS</sup> with tuberculin skin testing and 0.04 (CI, 0.02 to 0.12) for standard ELISpot with tuberculin skin testing. Positive results from both tests provided likelihood ratios of 5.5 (CI, 3.2 to 9.5) for ELISpot<sup>PLUS</sup> with tuberculin skin testing and 5.7 (CI, 3.2 to 9.9) for standard ELISpot with tuberculin skin testing,

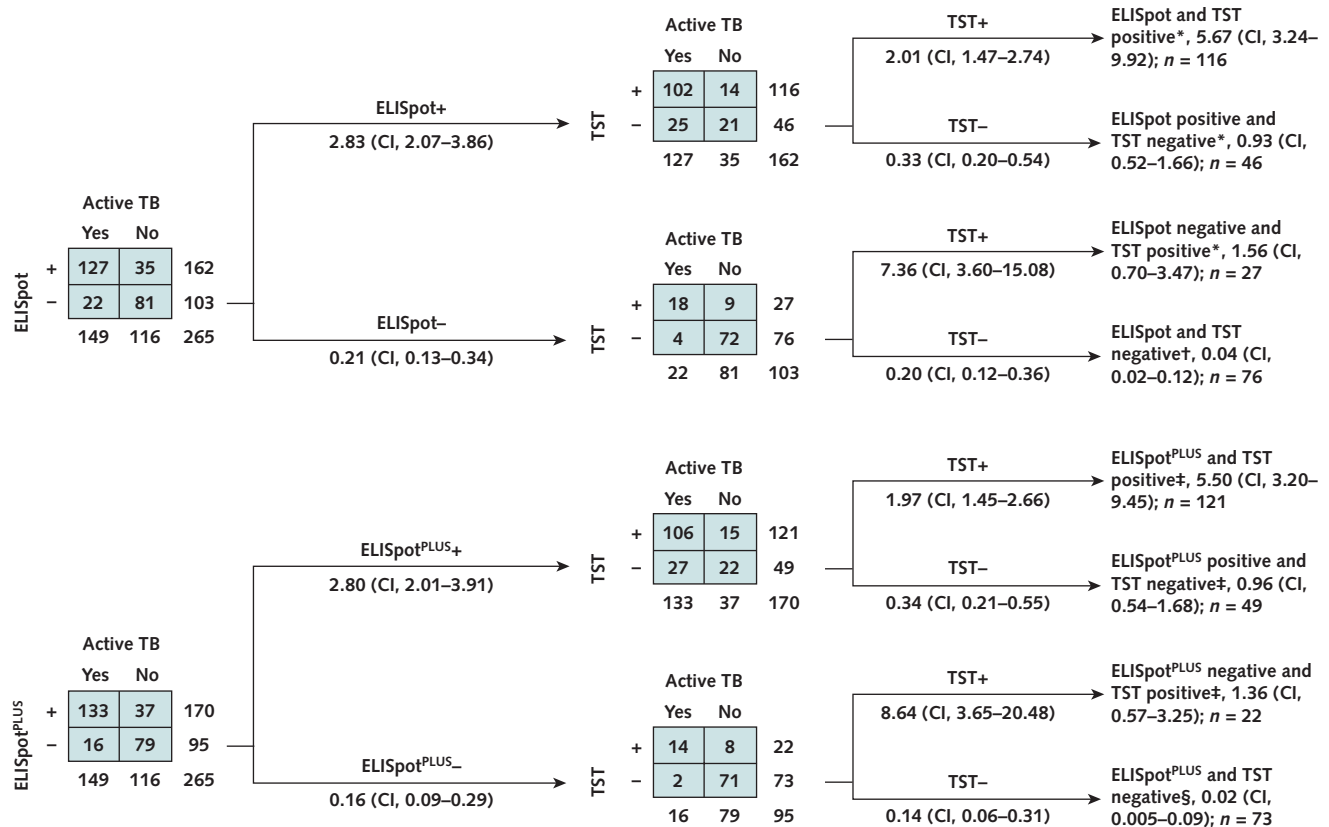
whereas discordant results (likelihood ratios, 0.93 to 1.56) had no diagnostic value. **Figure 2** shows sequential likelihood ratios when ELISpot assays are followed by tuberculin skin testing, and **Appendix Figure 2** (available at [www.annals.org](http://www.annals.org)) shows corresponding results when tuberculin skin testing is used first.

The high sensitivity of the combined tests reflects the fact that false-negative tuberculin skin test results and false-negative ELISpot results occurred in different patient populations. In univariate analysis among culture-confirmed and highly probable cases, the presence of factors associated with cutaneous anergy (5) or risk for progression from latent infection to active tuberculosis (35) was associated with false-negative tuberculin skin test results ( $P = 0.001$ ) but not with false-negative ELISpot results ( $P = 0.55$ ). Specifically, 28% (CI, 11% to 44%) of patients with false-negative tuberculin skin test results had factors associated with cutaneous anergy (3 had diabetes and 3 were HIV-

positive), compared with 5.0% (CI, 1.1% to 8.8%) of those with true-positive results. Eleven patients with tuberculosis were deemed immunocompromised on the basis of being HIV-positive (8 patients) or receiving long-term corticosteroid treatment (3 patients); 10 had positive ELISpot results and all 11 had positive ELISpot<sup>PLUS</sup> results. Neither age nor presence of any comorbid condition was associated with false-negative tuberculin skin test or ELISpot results ( $P > 0.11$  in all cases).

The sensitivity of culture was 79% (CI, 73% to 85%) for confirmed and highly probable tuberculosis, which was lower than that of both ELISpot assays; this difference was significant for ELISpot<sup>PLUS</sup> ( $P = 0.005$ ). Microscopy had a sensitivity of 39% (CI, 32% to 46%). Microbiological techniques had lower sensitivity in extrapulmonary disease than in pulmonary disease (culture, 53% [CI, 39% to 67%] vs. 89% [CI, 83% to 94%], respectively [ $P < 0.001$ ]; microscopy, 14% (CI, 5.5% to 26%) vs. 49% (CI,

**Figure 2.** Likelihood ratios, sensitivities, and specificities of tests used in combination, using ELISpot or ELISpot<sup>PLUS</sup> first.



Data are for patients in whom results on both tests were available ( $n = 265$ ). Except where stated, values are likelihood ratios with 95% CIs. Tuberculin skin test thresholds for positivity were induration  $\geq 15$  mm on the Mantoux test or grade 3 to 4 on the Heaf test. ELISpot = enzyme-linked immunospot incorporating early secretory antigenic target-6 and culture filtrate protein-10; ELISpot<sup>PLUS</sup> = enzyme-linked immunospot incorporating early secretory antigenic target-6, culture filtrate protein-10, and Rv3879c; TB = tuberculosis; TST = tuberculin skin testing. **Top.** ELISpot followed by TST. \*Combined sensitivity of 1 or more positive results from tests used in combination, 97% (CI, 93% to 99%). †Combined specificity for a double-negative result from tests used in combination, 62% (CI, 53% to 71%). **Bottom.** ELISpot<sup>PLUS</sup> followed by TST. ‡Combined sensitivity of 1 or more positive results from tests used in combination, 99% (CI, 95% to 100%). §Combined specificity for a double-negative result from tests used in combination, 61% (CI, 52% to 70%).

**Table 4. Accuracy for the Diagnosis of Active Tuberculosis\***

Statistic	TST [95% CI], n (%)	
	15-mm Threshold†	10-mm Threshold‡
<b>Sensitivity for diagnosis of active tuberculosis</b>		
Confirmed tuberculosis	94/119 (79.0 [70.6–85.9])	98/119 (82.4 [74.3–88.7])
Confirmed and highly probable tuberculosis	123/155 (79.4 [72.1–85.4])	129/155 (83.4 [77.1–89.3])
<b>Specificity for diagnosis of active tuberculosis</b>		
Category 4 (active tuberculosis excluded)§	107/132 (81.1 [73.3–87.4])	97/132 (73.5 [65.1–80.8])
Category 4D (active tuberculosis excluded, TST negative, no risk factors for latent tuberculosis infection)	25/25 (100)¶	25/25 (100)¶
<b>Predictive value</b>		
Positive predictive value	123/148 (83.1 [76.1–88.8])	130/165 (78.8 [71.8–84.8])
Negative predictive value	107/139 (77.0 [69.1–83.4])	97/122 (79.5 [71.3–86.3])
<b>Likelihood ratios</b>		
Positive likelihood ratio	287 (4.19 [2.92–6.02])	287 (3.16 [2.36–4.27])
Negative likelihood ratio	287 (0.25 [0.19–0.35])	287 (0.22 [0.15–0.32])

\* Data are for patients in whom results are available for the test in question, and in whom a definite diagnosis was available ( $n \leq 348$ ). Values are for confirmed and highly probable tuberculosis unless stated otherwise. ELISpot = enzyme-linked immunospot incorporating early secretory antigenic target-6 and culture filtrate protein-10; ELISpot<sup>PLUS</sup> = enzyme-linked immunospot incorporating early secretory antigenic target-6, culture filtrate protein-10, and Rv3879c; TST = tuberculin skin test.

† Threshold for positivity was induration  $\geq 15$  mm on the Mantoux test or grade 3–4 on the Heaf test.

‡ Stratified 10-mm cutoff threshold, in which induration  $\geq 15$  mm on the Mantoux test or grade 3–4 on the Heaf test was considered positive in patients who had had bacille Calmette–Guérin vaccination and values  $\geq 10$  mm or grade 2–4 were considered positive in unvaccinated patients.

§ Proportion of patients with negative results among those in whom active tuberculosis was excluded.

|| Proportion of patients with negative results among those in whom active tuberculosis was excluded, tuberculin skin testing was negative, and no risk factors for latent tuberculosis infection were identified.

¶ Patients in category 4D must have negative skin test results if a skin test is performed.

40% to 58%), respectively [ $P < 0.001$ ]), whereas ELISpot<sup>PLUS</sup> had nonsignificantly higher sensitivity in extrapulmonary disease (94% [CI, 84% to 99%]) than in pulmonary disease (88% [CI, 81% to 93%]) ( $P = 0.19$ ). Among patients with suspected pulmonary tuberculosis, the sensitivity of sputum microscopy with ELISpot<sup>PLUS</sup> was 94% (CI, 89% to 98%) for culture-confirmed and highly probable cases, generating a negative likelihood ratio of 0.10 and a negative predictive value of 90%, compared with a sensitivity of 78% (CI, 69% to 85%) for microscopy combined with tuberculin skin testing ( $P < 0.001$ ).

Specificity of ELISpot<sup>PLUS</sup> was 69% (CI, 61% to 77%) for active disease in all category 4 patients, which was lower than the specificity of tuberculin skin testing (81% [CI, 73% to 87%];  $P = 0.03$ ). Although ELISpot<sup>PLUS</sup> gave more diagnostic negative likelihood ratios and negative predictive values than tuberculin skin testing, positive likelihood ratios and positive predictive values were somewhat lower, most likely because of detection of latent infection in category 4 patients; this finding is consistent with the 84% (CI, 60% to 97%) specificity of ELISpot<sup>PLUS</sup> calculated by using category 4D patients (who were considered least likely to have latent infection). The specificity of standard ELISpot was similar to that of ELISpot<sup>PLUS</sup> (Table 4). Three of 21 category 4D patients had false-positive results by standard ELISpot, and 3 of 19 had false-positive results by ELISpot<sup>PLUS</sup>. All 3 individuals who had false-

positive ELISpot<sup>PLUS</sup> results were United Kingdom–born white persons with 0 mm of induration on the Mantoux test, and 2 were 75 years of age or older. Only 2 category 4D patients who were negative by standard ELISpot were positive to any of the new antigens; both responded to Rv3873 alone. Thus, inclusion of Rv3879c alone in ELISpot<sup>PLUS</sup> enhanced diagnostic sensitivity without compromising specificity.

Our comparison of the Heaf test and the Mantoux test with a 15-mm induration threshold for patients with culture-confirmed and highly probable cases of tuberculosis revealed a nonsignificant increase in sensitivity for the Heaf test (86% [CI, 74% to 82%] for the Heaf test vs. 76% [CI, 66% to 84%] for the Mantoux test;  $P = 0.14$ ) and a higher specificity for the Mantoux test (66% [CI, 46% to 82%]) for the Heaf test vs. 85% [CI, 77% to 92%] for the Mantoux test;  $P = 0.02$ ) (subgroup data provided on request). Receiver-operator characteristic analysis indicated that the area under the curve for both skin test formats was identical at 0.84. Subgroup analysis of combined use of standard ELISpot or ELISpot<sup>PLUS</sup> followed by Mantoux testing (for the 184 patients who received Mantoux testing) gave similar final likelihood ratios to those observed in the whole study population tested with Mantoux or Heaf methods (265 patients; subgroup data provided on request).

Table 4—Continued

Patients [95% CI], n (%)	ELISpot		Patients [95% CI], n (%)	ELISpot <sup>PLUS</sup>	
	Chi-Square P Value			Chi-Square P Value	
	Versus TST 15-mm Threshold	Versus TST 10-mm Threshold		Versus TST 15-mm Threshold	Versus TST 10-mm Threshold
128/148 (86.5 [79.9–91.6])	0.10	0.35	134/147 (91.2 [85.4–95.2])	0.005	0.03
160/188 (85.1 [79.2–89.9])	0.16	0.63	167/187 (89.3 [84.0–93.3])	0.01	0.10
102/142 (71.8 [63.8–79.1])	0.07	0.76	95/137 (69.3 [60.9–76.9])	0.03	0.45
18/21 (85.7 [63.7–97.0])	–	–	16/19 (84.2 [60.4–96.6])	–	–
160/200 (80.0 [73.8–85.3])	0.46	0.78	167/209 (79.9 [73.8–85.1])	0.45	0.79
102/130 (78.5 [70.4–85.2])	0.77	0.84	95/115 (82.6 [74.4–89.0])	0.27	0.54
330 (3.02 [2.31–3.96])	0.15	0.97	324 (2.91 [2.25–3.77])	0.11	0.68
330 (0.21 [0.15–0.30])	0.46	0.86	324 (0.15 [0.10–0.24])	0.06	0.19

Overall, 4.7% of positive results on standard ELISpot or ELISpot<sup>PLUS</sup> were dependent on a single peptide pool response of 5 to 10 spot-forming cells per well. These results might be considered borderline positive.

## DISCUSSION

This prospective study has defined a role for T-cell–based interferon- $\gamma$  release assay testing in the diagnostic evaluation of patients with suspected tuberculosis. The 89% sensitivity of ELISpot<sup>PLUS</sup> for a diagnosis of culture-confirmed or highly probable tuberculosis was higher than that of the standard ELISpot, tuberculin skin testing, and culture. The combined sensitivity of ELISpot<sup>PLUS</sup> and tuberculin skin testing in confirmed and highly probable cases was 99%.

The negative likelihood ratio of 0.15 with ELISpot<sup>PLUS</sup> means that a negative result would reduce the odds of tuberculosis by 6.5-fold, which allows it to be used as a rule-out test in patients with a moderate or low pretest probability of tuberculosis. Given the high pretest probability of tuberculosis (0.50) in our patient population (patients attending urban infectious disease units), the negative predictive value of ELISpot<sup>PLUS</sup> alone was modest at 83%. However, the 99% combined sensitivity of tuberculin skin testing and ELISpot<sup>PLUS</sup> and the corresponding negative likelihood ratio of 0.02 allow the combined use of these assays as a rule-out test for tuberculosis, even in populations with high pretest probabilities, such as our study sample. Pretest probabilities in other settings, such as general medical or primary care clinics in low-prevalence countries, will be much lower, and the negative predictive value of ELISpot<sup>PLUS</sup>, alone or in combination with skin testing, will be correspondingly higher. The higher sensitivity of ELISpot<sup>PLUS</sup> compared with standard ELISpot

was entirely attributable to the addition of 1 antigen, Rv3879c (to which Rv3873 and Rv3878 provided no further incremental sensitivity), which supports inclusion of Rv3879c in T-cell–based interferon- $\gamma$  release assays.

Analysis of tuberculin skin testing using the lower 10-mm induration threshold in patients not vaccinated with bacille Calmette–Guérin showed a nonsignificant increase in sensitivity and decrease in specificity. Whereas ELISpot<sup>PLUS</sup> was not significantly more sensitive than skin testing with the stratified 10-mm threshold in patients with confirmed and highly probable tuberculosis, it was significantly more sensitive in the patients with culture-confirmed disease. This is probably because the diagnosis of highly probable tuberculosis was based in part on skin test results, which biases results in favor of the skin test.

Our results are probably generalizable to clinical units evaluating patients with suspected tuberculosis in other low-prevalence countries because we performed our study in routine clinical practice and included a wide spectrum of clinical cases and alternative diagnoses (36, 37). This may also account for the lower sensitivity of standard ELISpot in our study compared with earlier, smaller studies of more selected patient groups (16, 18, 38). Because our population comprised relatively few immunocompromised patients, our results cannot be generalized to populations with a higher prevalence of HIV co-infection, although previous studies of larger numbers of patients with tuberculosis who were co-infected with HIV suggest that ELISpot has high sensitivity in this group (25, 28). Our study population was predominantly South Asian and black; further studies are required in other ethnic groups. Results will be less generalizable outside low-prevalence countries, because the utility of ELISpot in endemic regions will be limited by higher background rates of latent

tuberculosis (29, 39). This reduces the utility of a positive result; however, negative results (although proportionally fewer) would still be useful to rule out a diagnosis of tuberculosis (8, 25, 40).

Any T-cell–based test with a high sensitivity for *M. tuberculosis* infection will detect latent infection in patients who are suspected of having tuberculosis but in whom active tuberculosis has been excluded. Because our study population predominantly comprised patients with suspected tuberculosis from ethnic groups with a high prevalence of tuberculosis and latent tuberculosis, patients without active tuberculosis had a much higher risk for latent infection than would healthy control patients selected for very low risk for infection. This explains the 66% to 86% specificities of ELISpot and tuberculin skin testing when used as markers of active tuberculosis. Hence, when used to support a diagnosis of active tuberculosis, immune-based tests of infection should be interpreted in the context of the overall clinical picture and diagnostic work-up (40), as is recognized for tuberculin skin testing.

Given that culture remains the diagnostic gold standard and is required for identifying drug resistance, we believe our findings will have the following effects on clinical decision making. First, a negative ELISpot<sup>PLUS</sup> result can assist in excluding tuberculosis when the pretest probability is low-to-moderate. When pretest probabilities are higher, combined use of ELISpot<sup>PLUS</sup> and tuberculin skin testing excludes tuberculosis with a greater degree of certainty (Figure 2). The likelihood ratio of 0.02 to 0.04 refines a pretest probability of 50% to a posttest probability of approximately 2% (41) in patients with double-negative results, who constituted more than one quarter of those tested in our study. Conversely, a positive ELISpot<sup>PLUS</sup> (or standard ELISpot) result with a corresponding likelihood ratio of 2.8 is of limited value. However, in patients with positive results on both ELISpot and the tuberculin skin test, who constituted nearly one half of those tested, the corresponding likelihood ratio of 5.5 increased the probability of active tuberculosis by approximately 30% (41). Thus, double-positive results may help guide decisions about early initiation of presumptive treatment in severe disease while awaiting culture results and in extrapulmonary disease, in which culture is frequently negative. Combined testing also generates some discordant results (Figure 2). In these cases, which amounted to one quarter of persons tested, the near-unity likelihood ratios do not alter pretest probabilities and do not contribute to the diagnostic work-up.

The rate of indeterminate ELISpot results in our study was similar to the 3% to 4% rates observed in other studies (42, 43) and less than the 11% to 21% rates observed with whole-blood ELISA in routine practice (42, 44). Indeterminate ELISA results are associated with immunosuppressive therapies or conditions (42, 44), but no such association was seen for ELISpot in this or other studies (25, 42, 43, 45, 46). Moreover, the proportion of patients with

tuberculosis was no higher among those with indeterminate results. As expected, false-negative skin test results were associated with factors known to cause anergy or progression to active disease. These factors did not confound the ELISpot results, consistent with previous findings (10, 15, 42).

The 99% combined sensitivity of tuberculin skin testing and ELISpot reflects the fact that patients who had a false-negative result on one test were distinct from those who had a false-negative result on the other. This implies that distinct immunologic processes underlie failure of these different, yet complementary, immune-based tests.

The main weakness of our study arises from the problem of differing standards for tuberculin skin testing. Standards vary worldwide, differing by type of tuberculin, production standards, method of administration, dose, and skin test cutoff points (13, 23, 24, 31, 35, 47). Where possible, we used a 10-tuberculin unit PPD–Siebert Mantoux test (in accordance with the United Kingdom standard at the time) and corresponding cutoff points for induration. In one quarter of patients, a Heaf test was used for logistical reasons. When standard thresholds for Mantoux test equivalence were used (23, 24), the Heaf test showed a nonsignificant increase in diagnostic sensitivity and a significant decrease in specificity relative to the Mantoux test. Subgroup analysis of patients who had Mantoux testing showed that conclusions for combined testing with ELISpot or ELISpot<sup>PLUS</sup> followed by Mantoux testing were similar to those for the study population as a whole. Parallel testing with the alternative whole-blood ELISA T-cell–based interferon- $\gamma$  release assay would have been of interest, but neither this test nor the T-SPOT.TB (which is based on the standard ELISpot used here) was available when our study began. The modest sample size, limited number of immunocompromised patients, and lack of a definitive gold standard are also limitations of our study. Because many patients with tuberculosis are culture-negative (48), a microbiological gold standard used alone is too insensitive to evaluate new diagnostic tests. We therefore included highly probable cases in our composite reference standard, which better reflects the clinically relevant population, and our sensitivity analysis confirmed that estimates based on the composite reference standard were similar to those based on the microbiological reference standard. Finally, because this is only the second study to evaluate Rv3879c (21) and the first prospective study, further validation of its incremental sensitivity is required.

In summary, we found that ELISpot<sup>PLUS</sup> was a sensitive and clinically useful diagnostic test for evaluation of patients with suspected tuberculosis. Incorporation of a novel antigen, Rv3879c, conferred improved sensitivity over standard ELISpot. Combined use of tuberculin skin testing with either ELISpot<sup>PLUS</sup> or ELISpot enabled rapid exclusion of tuberculosis in patients with moderate to high probability of infection.

From Tuberculosis Immunology Group, Imperial College London, London; University of Oxford, Oxford; Birmingham Heartlands Hospital and University of Birmingham, Birmingham; and Northwick Park Hospital, Harrow, United Kingdom.

**Note:** Drs. Dosanjh and Hinks contributed equally to this work.

**Acknowledgment:** The authors thank the study participants; Sarah Gooding, for collection and processing of some samples; and Muhunthan Thillai, for critical appraisal and revision of the final manuscript.

**Grant Support:** By the Wellcome Trust (Dr. Lalvani is a Wellcome Senior Research Fellow in Clinical Science), the Sir Halley Stewart Trust (Dr. Dosanjh's studentship), a Wellcome Trust PhD Prize Studentship (Dr. Millington), and a United Kingdom Department of Health Senior Fellowship in Evidence Synthesis (Dr. Deeks).

**Potential Financial Conflicts of Interest:** *Consultancies:* A. Lalvani (Oxford Immunotec Ltd. [nonexecutive director from 2003 to 2007]). *Honoraria:* G. Pasvol (Transactions of the Royal Society of Tropical Medicine). *Stock ownership or options (other than mutual funds):* D.P.S. Dosanjh (Oxford Immunotec Ltd.), A Lalvani (Oxford Immunotec Ltd.), University of Oxford (Oxford Immunotech Ltd.). *Patents received:* A. Lalvani (T-cell–based diagnosis of tuberculous infection), University of Oxford (T-cell–based diagnosis of tuberculous infection). *Patents pending:* D.P.S. Dosanjh (T-cell–based diagnosis of tuberculous infection), A. Lalvani (T-cell–based diagnosis of tuberculous infection), University of Oxford (T-cell–based diagnosis of tuberculous infection).

**Reproducible Research Statement:** The analytic data set of all participants' diagnoses, tuberculin skin test results, ELISpot results, and ELISpot<sup>PLUS</sup> results can be made available to academic investigators on request by written agreement.

**Requests for Single Reprints:** Ajit Lalvani, DM, Tuberculosis Immunology Group, Department of Respiratory Medicine, Faculty of Medicine, Imperial College London, St. Mary's Campus, Norfolk Place, London W2 1PG, United Kingdom; e-mail, a.lalvani@imperial.ac.uk.

Current author addresses and author contributions are available at [www.annals.org](http://www.annals.org).

## References

1. The STOP TB Partnership. The global plan to stop TB, 2006–2015. Geneva: World Health Organization; 2006. Accessed at [www.who.int/tb/publications/global\\_plan\\_to\\_stop\\_tb/en/](http://www.who.int/tb/publications/global_plan_to_stop_tb/en/) on 2 January 2008.
2. Schluger NW, Perez D, Liu YM. Reconstitution of immune responses to tuberculosis in patients with HIV infection who receive antiretroviral therapy. *Chest*. 2002;122:597-602. [PMID: 12171838]
3. Steingart KR, Henry M, Ng V, Hopewell PC, Ramsay A, Cunningham J, et al. Fluorescence versus conventional sputum smear microscopy for tuberculosis: a systematic review. *Lancet Infect Dis*. 2006;6:570-81. [PMID: 16931408]
4. Dinnes J, Deeks J, Kunst H, Gibson A, Cummins E, Waugh N, et al. A systematic review of rapid diagnostic tests for the detection of tuberculosis infection. *Health Technol Assess*. 2007;11:1-196. [PMID: 17266837]
5. Huebner RE, Schein MF, Bass JB Jr. The tuberculin skin test. *Clin Infect Dis*. 1993;17:968-75. [PMID: 8110954]
6. Menzies D. Interpretation of repeated tuberculin tests. Boosting, conversion, and reversion. *Am J Respir Crit Care Med*. 1999;159:15-21. [PMID: 9872812]
7. Menzies D, Pai M, Comstock G. Meta-analysis: new tests for the diagnosis of latent tuberculosis infection: areas of uncertainty and recommendations for research. *Ann Intern Med*. 2007;146:340-54. [PMID: 17339619]
8. Lalvani A. Diagnosing tuberculosis infection in the 21st century: new tools to tackle an old enemy. *Chest*. 2007;131:1898-906. [PMID: 17565023]
9. Pai M, Riley LW, Colford JM Jr. Interferon-gamma assays in the immunodiagnosis of tuberculosis: a systematic review. *Lancet Infect Dis*. 2004;4:761-76. [PMID: 15567126]
10. Richeldi L. An update on the diagnosis of tuberculosis infection. *Am J Respir Crit Care Med*. 2006;174:736-42. [PMID: 16799073]
11. Behr MA, Wilson MA, Gill WP, Salamon H, Schoolnik GK, Rane S, et al. Comparative genomics of BCG vaccines by whole-genome DNA microarray. *Science*. 1999;284:1520-3. [PMID: 10348738]
12. Richeldi L, Ewer K, Losi M, Roversi P, Fabbri LM, Lalvani A. Repeated tuberculin testing does not induce false positive ELISPOT results [Letter]. *Thorax*. 2006;61:180. [PMID: 16443712]
13. Mori T, Sakatani M, Yamagishi F, Takashima T, Kawabe Y, Nagao K, et al. Specific detection of tuberculosis infection: an interferon-gamma-based assay using new antigens. *Am J Respir Crit Care Med*. 2004;170:59-64. [PMID: 15059788]
14. Arend SM, Thijsen SF, Leyten EM, Bouwman JJ, Franken WP, Koster BF, et al. Comparison of two interferon-gamma assays and tuberculin skin test for tracing tuberculosis contacts. *Am J Respir Crit Care Med*. 2007;175:618-27. [PMID: 17170386]
15. Lalvani A, Pathan AA, Durkan H, Wilkinson KA, Whelan A, Deeks JJ, et al. Enhanced contact tracing and spatial tracking of *Mycobacterium tuberculosis* infection by enumeration of antigen-specific T cells. *Lancet*. 2001;357:2017-21. [PMID: 11438135]
16. Lalvani A, Pathan AA, McShane H, Wilkinson RJ, Latif M, Conlon CP, et al. Rapid detection of *Mycobacterium tuberculosis* infection by enumeration of antigen-specific T cells. *Am J Respir Crit Care Med*. 2001;163:824-8. [PMID: 11282752]
17. Ewer K, Deeks J, Alvarez L, Bryant G, Waller S, Andersen P, et al. Comparison of T-cell-based assay with tuberculin skin test for diagnosis of *Mycobacterium tuberculosis* infection in a school tuberculosis outbreak. *Lancet*. 2003;361:1168-73. [PMID: 12686038]
18. Meier T, Eulenbruch HP, Wright-Smith P, Enders G, Regnath T. Sensitivity of a new commercial enzyme-linked immunospot assay (T SPOT-TB) for diagnosis of tuberculosis in clinical practice. *Eur J Clin Microbiol Infect Dis*. 2005;24:529-36. [PMID: 16133410]
19. Mazurek GH, Jereb J, Lobue P, Iademarco MF, Metchock B, Vernon A. Division of Tuberculosis Elimination, National Center for HIV, STD, and TB Prevention, Centers for Disease Control and Prevention (CDC). Guidelines for using the QuantiFERON-TB Gold test for detecting *Mycobacterium tuberculosis* infection, United States. *MMWR Recomm Rep*. 2005;54:49-55. [PMID: 16357824]
20. National Collaborating Centre for Chronic Conditions. Tuberculosis: clinical diagnosis and management of tuberculosis, and measures for its prevention and control. London: Royal Coll of Physicians; 2006.
21. Liu XQ, Dosanjh D, Varia H, Ewer K, Cockle P, Pasvol G, et al. Evaluation of T-cell responses to novel RD1- and RD2-encoded *Mycobacterium tuberculosis* gene products for specific detection of human tuberculosis infection. *Infect Immun*. 2004;72:2574-81. [PMID: 15102765]
22. Rose AM, Watson JM, Graham C, Nunn AJ, Drobniewski F, Ormerod LP, et al. Public Health Laboratory Service/British Thoracic Society/Department of Health Collaborative Group. Tuberculosis at the end of the 20th century in England and Wales: results of a national survey in 1998. *Thorax*. 2001;56:173-9. [PMID: 11182007]
23. Control and prevention of tuberculosis in the United Kingdom: code of practice 2000. Joint Tuberculosis Committee of the British Thoracic Society. *Thorax*. 2000;55:887-901. [PMID: 11050256]
24. Department of Health. Immunisation against infectious disease. London: HMSO; 1996.
25. Liebeschuetz S, Bamber S, Ewer K, Deeks J, Pathan AA, Lalvani A. Diagnosis of tuberculosis in South African children with a T-cell-based assay: a prospective cohort study. *Lancet*. 2004;364:2196-203. [PMID: 15610806]
26. Pathan AA, Wilkinson KA, Klenerman P, McShane H, Davidson RN, Pasvol G, et al. Direct ex vivo analysis of antigen-specific IFN-gamma-secreting CD4 T cells in *Mycobacterium tuberculosis*-infected individuals: associations with clinical disease state and effect of treatment. *J Immunol*. 2001;167:5217-25. [PMID: 11673535]
27. Soysal A, Millington KA, Bakir M, Dosanjh D, Aslan Y, Deeks JJ, et al. Effect of BCG vaccination on risk of *Mycobacterium tuberculosis* infection in children with household tuberculosis contact: a prospective community-based study. *Lancet*. 2005;366:1443-51. [PMID: 16243089]

28. Chapman AL, Munkanta M, Wilkinson KA, Pathan AA, Ewer K, Ayles H, et al. Rapid detection of active and latent tuberculosis infection in HIV-positive individuals by enumeration of *Mycobacterium tuberculosis*-specific T cells. *AIDS*. 2002;16:2285-93. [PMID: 12441800]
29. Lalvani A, Nagvenkar P, Udawadia Z, Pathan AA, Wilkinson KA, Shastri JS, et al. Enumeration of T cells specific for RD1-encoded antigens suggests a high prevalence of latent *Mycobacterium tuberculosis* infection in healthy urban Indians. *J Infect Dis*. 2001;183:469-77. [PMID: 11133379]
30. Richeldi L, Ewer K, Losi M, Bergamini BM, Roversi P, Deeks J, et al. T cell-based tracking of multidrug resistant tuberculosis infection after brief exposure. *Am J Respir Crit Care Med*. 2004;170:288-95. [PMID: 15130907]
31. Diagnostic Standards and Classification of Tuberculosis in Adults and Children. This official statement of the American Thoracic Society and the Centers for Disease Control and Prevention was adopted by the ATS Board of Directors, July 1999. This statement was endorsed by the Council of the Infectious Disease Society of America, September 1999. *Am J Respir Crit Care Med*. 2000;161:1376-95. [PMID: 10764337]
32. Chan SF, Deeks JJ, Macaskill P, Irwig L. Three methods to construct predictive models using logistic regression and likelihood ratios to facilitate adjustment for pretest probability give similar results. *J Clin Epidemiol*. 2008;61:52-63. [PMID: 18083462]
33. Knottnerus JA. Application of logistic regression to the analysis of diagnostic data: exact modeling of a probability tree of multiple binary variables. *Med Decis Making*. 1992;12:93-108. [PMID: 1573985]
34. Albert A. On the use and computation of likelihood ratios in clinical chemistry. *Clin Chem*. 1982;28:1113-9. [PMID: 7074890]
35. Targeted tuberculin testing and treatment of latent tuberculosis infection. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. This is a Joint Statement of the American Thoracic Society (ATS) and the Centers for Disease Control and Prevention (CDC). This statement was endorsed by the Council of the Infectious Diseases Society of America. (IDSA), September 1999, and the sections of this statement. *Am J Respir Crit Care Med*. 2000;161:S221-47. [PMID: 10764341]
36. Jaeschke R, Guyatt GH, Sackett DL. Users' guides to the medical literature. III. How to use an article about a diagnostic test. B. What are the results and will they help me in caring for my patients? The Evidence-Based Medicine Working Group. *JAMA*. 1994;271:703-7. [PMID: 8309035]
37. Small PM, Perkins MD. More rigour needed in trials of new diagnostic agents for tuberculosis. *Lancet*. 2000;356:1048-9. [PMID: 11009137]
38. Lee JY, Choi HJ, Park IN, Hong SB, Oh YM, Lim CM, et al. Comparison of two commercial interferon-gamma assays for diagnosing *Mycobacterium tuberculosis* infection. *Eur Respir J*. 2006;28:24-30. [PMID: 16611658]
39. Kang YA, Lee HW, Hwang SS, Um SW, Han SK, Shim YS, et al. Usefulness of whole-blood interferon-gamma assay and interferon-gamma enzyme-linked immunospot assay in the diagnosis of active pulmonary tuberculosis. *Chest*. 2007;132:959-65. [PMID: 17505029]
40. Barnes PF. Diagnosing latent tuberculosis infection: the 100-year upgrade [Editorial]. *Am J Respir Crit Care Med*. 2001;163:807-8. [PMID: 11282742]
41. Grimes DA, Schulz KF. Refining clinical diagnosis with likelihood ratios. *Lancet*. 2005;365:1500-5. [PMID: 15850636]
42. Ferrara G, Losi M, D'Amico R, Roversi P, Piro R, Meacci M, et al. Use in routine clinical practice of two commercial blood tests for diagnosis of infection with *Mycobacterium tuberculosis*: a prospective study. *Lancet*. 2006;367:1328-34. [PMID: 16631911]
43. Piana F, Codecasa LR, Cavallerio P, Ferrarese M, Migliori GB, Barbarano L, et al. Use of a T-cell-based test for detection of tuberculosis infection among immunocompromised patients. *Eur Respir J*. 2006;28:31-4. [PMID: 16540502]
44. Ferrara G, Losi M, Meacci M, Meccugni B, Piro R, Roversi P, et al. Routine hospital use of a new commercial whole blood interferon-gamma assay for the diagnosis of tuberculosis infection. *Am J Respir Crit Care Med*. 2005;172:631-5. [PMID: 15961696]
45. Dheda K, Lalvani A, Miller RF, Scott G, Booth H, Johnson MA, et al. Performance of a T-cell-based diagnostic test for tuberculosis infection in HIV-infected individuals is independent of CD4 cell count. *AIDS*. 2005;19:2038-41. [PMID: 16260914]
46. Piana F, Codecasa LR, Besozzi G, Migliori GB, Cirillo DM. Use of commercial interferon-gamma assays in immunocompromised patients for tuberculosis diagnosis [Letter]. *Am J Respir Crit Care Med*. 2006;173:130; author reply 130-1. [PMID: 16368794]
47. Lee E, Holzman RS. Evolution and current use of the tuberculin test. *Clin Infect Dis*. 2002;34:365-70. [PMID: 11774084]
48. Thwaites GE, Chau TT, Stepniewska K, Phu NH, Chuong LV, Sinh DX, et al. Diagnosis of adult tuberculous meningitis by use of clinical and laboratory features. *Lancet*. 2002;360:1287-92. [PMID: 12414204]

#### SERVICES FOR PDA USERS

See the "PDA Services" heading at the top of the *Annals* home page at [www.annals.org](http://www.annals.org) to learn more about the following services available to PDA users.

##### *Annals* Reader for Palm PDA

Editors' Notes, abstracts, and full texts. Images and references are not available.

Reminders from PDA to the desktop computer about articles. The reminders include a link to the full text of the article at [www.annals.org](http://www.annals.org).

Storage of multiple issues of *Annals*. You can control how many issues to store on your PDA or have the reader automatically delete back issues.

Issues may be stored on external memory. Back issues are available starting with July 2003.

Storage of favorite articles (separate from storage of back issues).

Storage of collections of previously published articles from *Annals*.

Wireless access to the current issue for an Internet-enabled PDA

**Current Author Addresses:** Dr. Dosanjh: University of Oxford Medical School, William Osler House, John Radcliffe Hospital, Oxford OX3 9DU, United Kingdom.

Dr. Hinks: Allergy, Inflammation, and Repair Group, Level D, Southampton General Hospital, Southampton SO16 6YD, United Kingdom.

Dr. Innes and Ms. Hackforth: Department of Infection & Tropical Medicine, Birmingham Heartlands Hospital, Bordsley Green East, Birmingham B9 5SS, United Kingdom.

Dr. Deeks: Department of Public Health and Epidemiology, University of Birmingham, Edgbaston, Birmingham B15 2TT, United Kingdom.

Dr. Pasvol and Ms. Varia: Department of Infection and Tropical Medicine, Northwick Park Hospital, Imperial College London, Watford Road, Harrow HA1 3UJ, United Kingdom.

Drs. Millington and Lalvani: Department of Respiratory Medicine, Imperial College London, St. Mary's Campus, Norfolk Place, London W2 1PG, United Kingdom.

Dr. Gunatheesan: University of Melbourne Medical School, Melbourne, Australia.

Dr. Guyot-Revol: Genopoietic, 1390 rue Centrale-Beynost, 01708 Miribel Cedex, France.

**Author Contributions:** Conception and design: J.A. Innes, A. Lalvani. Analysis and interpretation of the data: D.P.S. Dosanjh, T.S.C. Hinks, J.J. Deeks, K.A. Millington, A. Lalvani.

Drafting of the article: D.P.S. Dosanjh, T.S.C. Hinks, G. Pasvol, A. Lalvani.

Critical revision of the article for important intellectual content: D.P.S. Dosanjh, T.S.C. Hinks, J.A. Innes, J.J. Deeks, G. Pasvol, K.A. Millington, A. Lalvani.

Final approval of the article: D.P.S. Dosanjh, J.J. Deeks, G. Pasvol, V. Guyot-Revol, A. Lalvani.

Provision of study materials or patients: J.A. Innes, G. Pasvol, S. Hackforth, H. Varia, A. Lalvani.

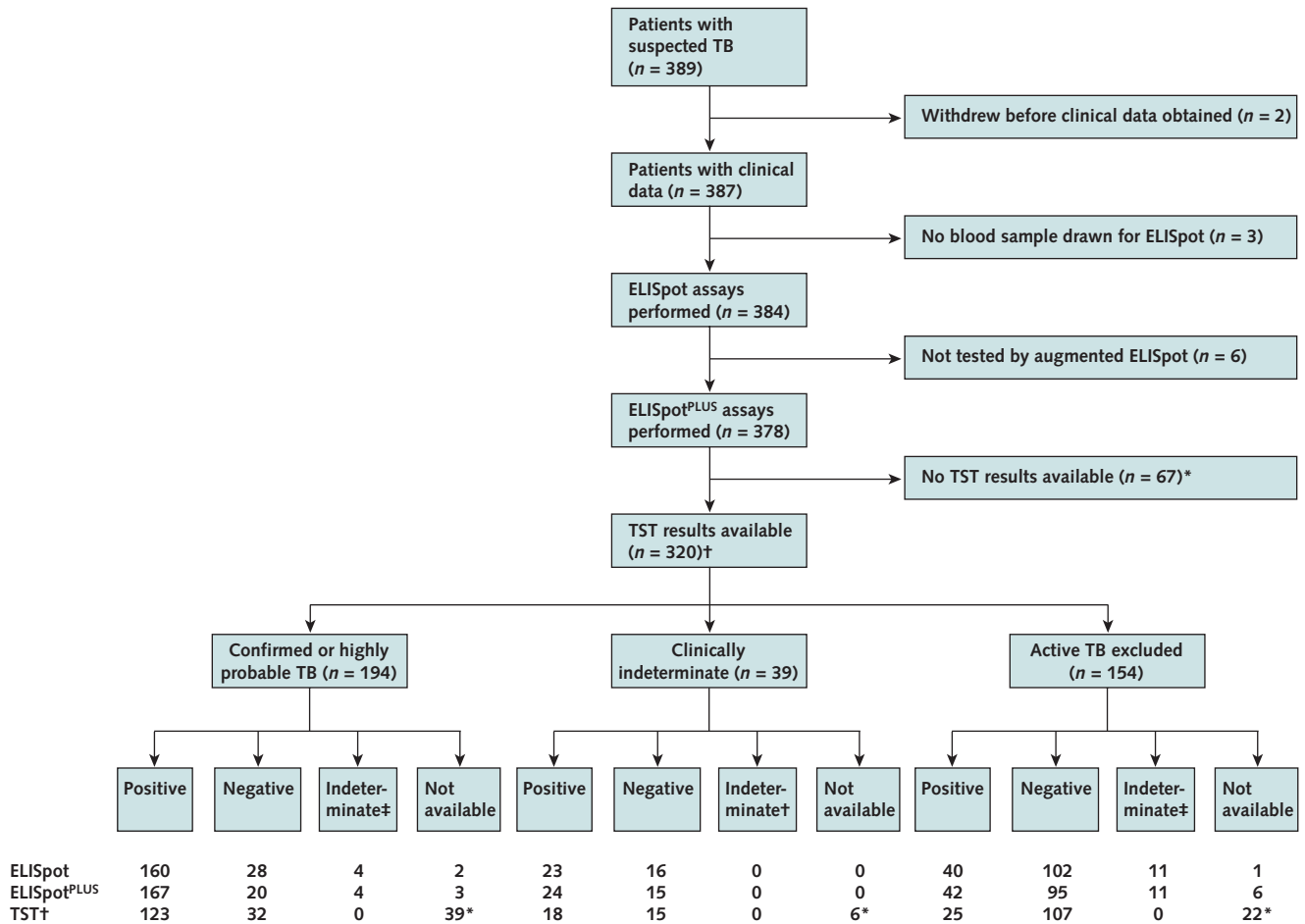
Statistical expertise: D.P.S. Dosanjh, J.J. Deeks.

Obtaining of funding: A Lalvani.

Administrative, technical, or logistic support: D.P.S. Dosanjh, G. Pasvol, S. Hackforth, K.A. Millington, A. Lalvani.

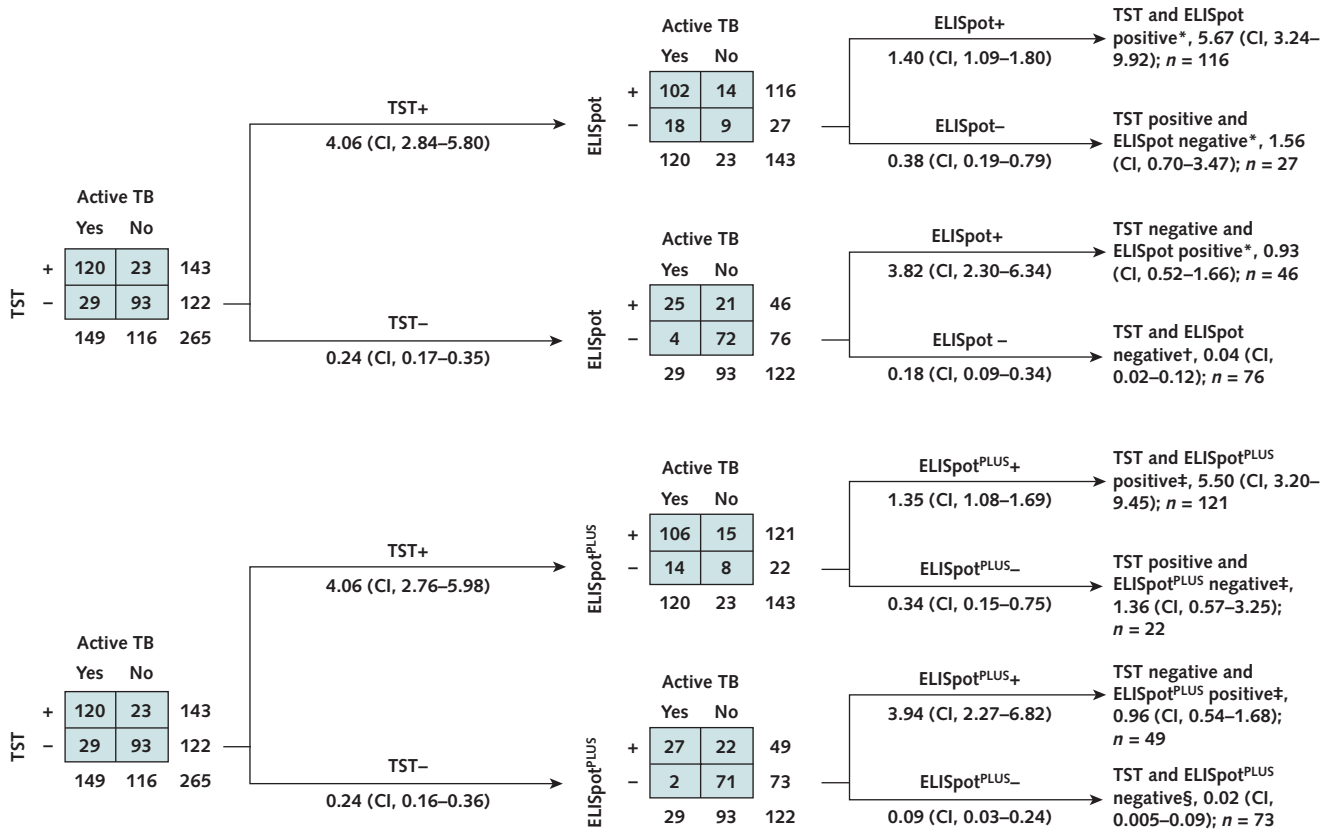
Collection and assembly of data: D.P.S. Dosanjh, T.S.C. Hinks, S. Hackforth, K.A. Millington, V. Guyot-Revol.

Appendix Figure 1. Study flow diagram, stratified by final diagnosis and then by test result.



These are the same data shown in Figure 1 but displayed in a different manner to allow scrutiny of the entire raw data set. ELISpot = enzyme-linked immunospot assay incorporating early secretory antigenic target-6 and culture filtrate protein-10; ELISpot<sup>PLUS</sup> = enzyme-linked immunospot assay incorporating early secretory antigenic target-6, culture filtrate protein-10, and Rv3879c; TB = tuberculosis; TST = tuberculin skin testing. \*Results were not available because of history of TB (clinically contraindicated) (45 patients), patient did not return for reading (8 patients), result not recorded (8 patients), reason unknown (3 patients), death (1 patient), test performed elsewhere (1 patient), or patient declined consent (1 patient). †Tuberculin skin test results were based on a 15-mm cutoff point and considered positive if induration was  $\geq 15$  mm on the Mantoux test or grade 3 to 4 on the Heaf test regardless of bacille Calmette–Guérin vaccination status. ‡Results were indeterminate because of no achievement of positive control (11 patients), high background (1 patient), peptide contamination (1 patient), inconclusive assay (1 patient), or defective ELISpot plate (1 patient).

**Appendix Figure 2. Likelihood ratios, sensitivities, and specificities of tests used in combination for diagnostic evaluation, using tuberculin skin testing (TST) as the first test.**



Data are for patients in whom results on both tests were available ( $n = 265$ ). Except where stated, numbers are likelihood ratios with 95% CIs. Tuberculin skin test thresholds for positivity were induration  $\geq 15$  mm on the Mantoux test or grade 3 to 4 on the Heaf test. ELISpot = enzyme-linked immunospot incorporating early secretory antigenic target-6 and culture filtrate protein-10; ELISpot<sup>PLUS</sup> = enzyme-linked immunospot incorporating early secretory antigenic target-6, culture filtrate protein-10, and Rv3879c; TB = tuberculosis. **Top.** TST followed by ELISpot. \*Combined sensitivity of 1 or more positive results from tests used in combination, 97% (CI, 93% to 99%). †Combined specificity for a double-negative result from tests used in combination, 62% (CI, 53% to 71%). **Bottom.** TST followed by ELISpot<sup>PLUS</sup>. ‡Combined sensitivity of 1 or more positive results from tests used in combination, 99% (CI, 95% to 100%). §Combined specificity for a double-negative result from tests used in combination, 61% (CI, 52% to 70%).