

# Enzyme-linked Immunospot and Tuberculin Skin Testing to Detect Latent Tuberculosis Infection

Homayoun Shams\*, Stephen E. Weis\*, Peter Klucar, Ajit Lalvani, Patrick K. Moonan, Janice M. Pogoda, Katie Ewer, and Peter F. Barnes

Center for Pulmonary and Infectious Disease Control, and Departments of Microbiology and Immunology and Medicine, University of Texas Health Center at Tyler, Tyler; Department of Internal Medicine, University of North Texas Health Science Center, Fort Worth, Texas; Nuffield Department of Clinical Medicine, University of Oxford, and John Radcliffe Hospital, Oxford, United Kingdom; and Statology, Ventura, California

**Rationale:** Diagnosis of latent tuberculosis infection (LTBI) is currently based on the tuberculin skin test. The enzyme-linked immunospot assay (ELISPOT) is a new blood test to diagnose LTBI.

**Objective:** To compare the ELISPOT and the tuberculin skin test for detecting LTBI in contacts of patients with tuberculosis.

**Methods:** Prospective study of 413 contacts of patients with tuberculosis.

**Measurements and Main Results:** Because there is no gold standard for LTBI, the sensitivity and specificity of the ELISPOT and tuberculin skin test cannot be directly measured. For each contact, we therefore estimated the likelihood of having LTBI by calculating a contact score that quantified exposure to and infectiousness of the index case. We analyzed the relationship of contact score to ELISPOT and tuberculin skin test results. The likelihood of a positive ELISPOT ( $p = 0.0005$ ) and a tuberculin skin test ( $p = 0.01$ ) increased significantly with rising contact scores. The contact score was more strongly related to the ELISPOT than to the tuberculin skin test results, although this difference was not statistically significant. Among U.S.-born persons and those who were not vaccinated with bacille Calmette-Guérin, approximately 30% had positive ELISPOT or tuberculin skin test results. Foreign-born, bacille Calmette-Guérin-vaccinated persons were significantly more likely to have a positive tuberculin skin test than a positive ELISPOT result ( $p < 0.0001$ ).

**Conclusions:** Compared with the tuberculin skin test, the ELISPOT appears to be at least as sensitive for diagnosis of LTBI in contacts of patients with tuberculosis.

**Keywords:** blood test; contact investigation; diagnosis

Tuberculosis case rates continue to decline in most industrialized nations, but persons with latent tuberculosis infection (LTBI) constitute a vast pool of individuals who may develop tuberculosis, particularly when the immune response is suppressed. Diagnosis and treatment of LTBI are therefore increasingly important goals of tuberculosis control (1). Unfortunately, the standard method to diagnose LTBI is the tuberculin skin test, which has

many shortcomings. Two visits are required, and skilled personnel are essential for proper placement and interpretation of the test. In addition, because purified protein derivative of tuberculin contains many antigens that are shared with other mycobacteria, the skin test does not reliably distinguish LTBI from prior immunization with *Mycobacterium bovis* bacille Calmette-Guérin (BCG) or infection with environmental mycobacteria (2). This is a major problem in most developed countries because a growing proportion of those with LTBI are foreign-born persons from high-incidence countries, most of whom received BCG vaccination during childhood (3, 4). A more accurate and convenient test to diagnose LTBI would greatly enhance tuberculosis control efforts (5).

In persons with LTBI, memory T cells produce IFN- $\gamma$  in response to *Mycobacterium tuberculosis* antigens. A major scientific advance was the identification of the 6-kD *M. tuberculosis* early-secreted antigenic target protein (ESAT-6) and the 10-kD culture filtrate protein (CFP10), which are absent from BCG and most environmental mycobacteria (6, 7).

Tests can now detect T cells that produce IFN- $\gamma$  in response to ESAT-6 and CFP10 using the ELISA to measure IFN- $\gamma$  concentrations in supernatants (QuantiFeron-TB Gold; Cellestis Ltd., St. Kilda, Australia) or the enzyme-linked immunospot assay (ELISPOT) to detect individual IFN- $\gamma$ -producing T cells (T SPOT-TB; Oxford Immunotec, Oxford, UK) (8–11). The Food and Drug Administration has approved the QuantiFeron-TB Gold test and is evaluating the T Spot-TB test, which is approved for use in Europe. These tests are positive in most persons with a high likelihood of LTBI and are negative in BCG-vaccinated individuals with a low likelihood of LTBI. In a school tuberculosis outbreak, ELISPOT results correlated significantly better than tuberculin skin test results with the degree of exposure to the index case (12), suggesting that the ELISPOT is a better marker of LTBI than the tuberculin skin test. However, the sensitivity of the tuberculin skin test may have been underestimated in this study, because tuberculin reactivity was assessed by the Heaf test, which may be inferior to the Mantoux method (2).

We wished to compare the ability of the ELISPOT and the tuberculin skin test to detect LTBI in a routine clinical setting where contacts of patients with tuberculosis are evaluated. Although there is no gold standard for identifying LTBI, the percentage of infected contacts is higher if the patient with tuberculosis has a positive sputum smear for acid-fast bacilli (13, 14). The frequency of LTBI also rises in those with increasing duration and proximity of contact with the patient (15, 16). We developed a formula to calculate a “contact score” for each contact on the basis of information obtained by a standardized interview. This score quantified the extent of exposure to the index case, as well as the degree of infectiousness of the index case. Using the contact score as a surrogate measure for likelihood of LTBI, we compared the ELISPOT and tuberculin skin test results with this contact score to determine if either test was superior in detecting LTBI.

(Received in original form May 12, 2005; accepted in final form August 4, 2005)

Supported by a cooperative agreement from the Centers for Disease Control (U90/CCU620916), the National Institutes of Health (AI44935), the Cain Foundation for Infectious Disease Research, the Wellcome Trust, the Center for Pulmonary and Infectious Disease Control, and the American Lung Association. P.F.B. holds the Margaret E. Byers Cain Chair for Tuberculosis Research. A.L. is a Wellcome Senior Research Fellow in Clinical Science.

\* These authors contributed equally to this article.

Correspondence and requests for reprints should be addressed to Peter F. Barnes, M.D., CPIDC, University of Texas Health Center, 11937 U.S. Highway 271, Tyler, TX 75708–3154. E-mail: peter.barnes@uthct.edu

This article has an online supplement, which is accessible from this issue's table of contents at [www.atsjournals.org](http://www.atsjournals.org)

Am J Respir Crit Care Med Vol 172, pp 1161–1168, 2005

Originally Published in Press as DOI: 10.1164/rccm.200505-748OC on August 4, 2005

Internet address: [www.atsjournals.org](http://www.atsjournals.org)

## METHODS

### Study Subjects

This study was approved by the institutional review boards of the University of North Texas Health Science Center at Fort Worth and the University of Texas Health Center at Tyler, and all subjects provided written, informed consent. Contact investigations were performed as described in the Tuberculosis Curriculum training modules of the Centers for Disease Control and Prevention. All contact investigators completed these modules at the time of their employment. We enrolled 416 recent contacts, aged 12 yr or older, of 72 cases of culture-proven pulmonary tuberculosis evaluated at the Tarrant County Public Health Department in Fort Worth from January 30, 2002, through September 3, 2003. We excluded persons with a history of tuberculosis, a positive tuberculin skin test, or prior exposure to a patient with tuberculosis. Fifty-five (12%) eligible subjects refused to participate in the study.

### Interview and Calculation of Contact Score

On the basis of our clinical experience and our literature review regarding factors that affect transmissibility of tuberculosis (13–17), we derived a method to calculate a contact score before analysis of study data. All contacts were interviewed using a standardized instrument to determine the extent of contact with the index case during a typical week. Contact scores were then calculated using three variables that are critical risk factors for infection: the relationship of the contact to the patient with tuberculosis, the infectivity of the patient, and the extent of exposure to the patient (Table 1). We reasoned that spouses and other household contacts were likely to be closer to the patient than nonhousehold contacts. We therefore assigned increased weights to persons with a close relationship to the patient with tuberculosis. The magnitude of these weights was based on clinical intuition, because published literature does not provide information on this issue (Table 1). On the basis of published data, contacts of patients with positive sputum acid-fast smears are approximately four times more likely to be infected with *M. tuberculosis* than contacts of patients with negative sputum acid-fast smears (13, 14), so this was incorporated into the contact score (Table 1). Time spent with the patient with tuberculosis in different settings was weighted on the basis of clinical intuition (Table 1). Because the risk of acquiring LTBI is proportional to the closeness of the relationship to the patient, the infectivity of the patient, and the extent of exposure, we used the following formula: contact score = relationship × infectivity × extent of exposure. Extent of exposure was calculated as  $(4 \times 3 \text{ ft}) + \text{sum}(\text{room type} \times \text{room weight}) + \text{building} + (0.25 \times \text{outdoors})$ , 3 ft = h/wk spent indoors, within 3 ft of the patient; room type = h/wk spent in a specific type of room or in a car with the patient; room weights are shown in Table 1; building = h/wk spent in a different room in the same building as the patient; and outdoors = h/wk spent outdoors with the patient.

**TABLE 1. WEIGHTS AND VARIABLES USED TO CALCULATE THE CONTACT SCORE**

Variable	Weight
Relationship to patient with tuberculosis	
Household sexual partner	3
Other household member	2
Nonhousehold contact	1
Infectivity of patient with tuberculosis	
Sputum acid-fast smear positive	4
Sputum acid-fast smear negative	1
Type of exposure to patient with tuberculosis	
Within 3 ft	4
Car	3.5
Hospital room, crack house, jail cell, restroom	3
Room in private residence	2.5
Bar, restaurant, cafeteria, school, office	2
Shelter, factory, indoor construction site, church, movie theater, store, garage	1.5
Different room in same building	1
Outdoors	0.25

For example, consider a nonhousehold contact of a patient with positive sputum acid-fast smears who rode in a car with the patient 5 h/wk, worked in a different room in the same building as the patient 40 h/wk, and spent 4 h/wk in the yard with the patient. For this contact, the relationship weight is 1, the infectivity is 4, and the extent of exposure is  $0 + (5 \times 3.5) + (40 \times 1) + (4 \times 0.25)$ , or 58.5. The contact score is  $1 \times 4 \times 58.5 = 234$ .

### Tuberculin Skin Testing

A tuberculin skin test was administered to all contacts by the Mantoux method using 0.1 ml of Tubersol (5 tuberculin units), and interpreted 48 to 72 h later. The tests were performed by two trained health care workers, each of whom performed more than 1,000 skin tests annually. A diameter of 5 mm of induration or more was considered positive (18). Of the 205 persons with initially negative tuberculin skin tests, a second skin test was placed in 136 contacts,  $87 \pm 50$  (mean  $\pm$  SD) d later. A clinical evaluation for tuberculosis and a chest radiograph were performed in all persons with positive tuberculin skin tests.

### ELISPOT

Blood samples were obtained for ELISPOT after placement of the tuberculin skin test in 411 of 416 subjects, within 2 wk after skin test placement in 268 subjects and after 2 wk in 143 subjects. In a previous study, 44 adults had negative ELISPOT and tuberculin skin tests 3 mo after exposure to a patient with tuberculosis. Tuberculin skin testing and ELISPOT were repeated 9, 15, and 24 mo after exposure. At 24 mo, all 44 individuals remained ELISPOT-negative (A.L., unpublished data). Therefore, tuberculin skin testing does not affect subsequent ELISPOT results. Blood samples were transported to the University of Texas Health Center at Tyler, where ELISPOT was performed in a research laboratory 2 to 8 h later in 411 cases, and 9 to 10 h later in five cases. A second blood sample was obtained for 200 cases  $104 \pm 62$  d after obtaining the first sample.

The assay was performed as previously described (*see METHODS* in the online supplement) (11, 12, 19). The mean number of spots in duplicate negative control wells was six or less in 406 of 413 cases. In these 406 cases, ELISPOT results were considered to be positive if the ESAT-6 or CFP10 wells contained a mean of at least seven more spots than the mean of the negative control wells. A result of seven spots was 3.1 SD above the mean value for control wells containing no antigen. In addition, this cut-off showed an excellent correlation with reactivity to peptides of ESAT-6 and CFP10 in pilot experiments (data not shown). In prior studies, a well was considered positive if it contained at least five spots more than negative control wells, and at least twice the number of spots in negative control wells (10, 20). For the seven persons with 7 to 32 spots in control wells, a result was considered positive if the ESAT-6 or CFP10 wells contained more than twice the number of spots in duplicate negative control wells. All ELISPOT assays were scored without reference to personal identifiers, and all cut-offs were defined before unblinding the clinical and demographic data.

### Statistical Analysis

Logistic regression was used to calculate maximum likelihood estimates of odds ratios and 95% confidence intervals, relating quartiles of contact score to ELISPOT or tuberculin skin test results. Contact score as an ordinal variable was used for trend tests. To determine whether the effect of contact score on test result (positive or negative) differed by test type (tuberculin skin test or ELISPOT), generalized estimating equations were used to perform a repeated-measures logistic regression with test result as the dependent variable, "subject" as a random effect, and independent terms for contact score, test type, and the interaction between contact score and test type. Generalized estimating equations were also used to evaluate the effect of test (ELISPOT vs. tuberculin skin test) on test result (positive or negative). Fisher's exact tests or  $\chi^2$  tests of association were used for contingency table analyses. Pairwise differences in means were tested using the Tukey-Kramer approach. All analyses were done with SAS statistical software, version 9.0 (SAS Institute, Inc., Cary, NC). A significance level of 0.05 was used for all tests.

## RESULTS

### Demographics and Risk Factors for Development of Tuberculosis

Blood samples were obtained from 416 subjects. Insufficient blood was obtained in two cases and mononuclear cells could not be isolated in one additional case. Data are reported for the remaining 413 subjects, all of whom had evaluable results. A total of 226 subjects (55%) were male, and their age was  $35.3 \pm 15$  (mean  $\pm$  SD) yr. Forty-five (11%) contacts were younger than 18 yr, and the youngest person was 12 yr. On the basis of self-report, 219 contacts (53%) were Hispanic, 88 (21%) were African American, 57 (14%) were white non-Hispanic, and 49 (12%) were Asian. One hundred and eighty-five persons (45%) were born in the United States, and 228 were foreign-born. Of the latter, 163 (72%) were from Mexico, eight (4%) from other Latin American countries, 45 (20%) from Asia, 11 (5%) from Africa, and one (0.5%) from Europe. Therefore, almost all foreign-born subjects were from countries where the incidence of tuberculosis is high.

Two hundred and four persons had a history of BCG vaccination, and 172 (85%) had a BCG scar. One hundred and eighty-six persons were vaccinated once, 13 were vaccinated twice, and two were vaccinated three times. Fifty-five persons received the first vaccination at age 2 yr or younger, 92 at ages 3 to 6, 50 at ages 7 to 12, and one at age 25 yr. The time of BCG vaccination was unknown in six persons.

Of 149 individuals who reported being tested for HIV infection, three reported a positive result. Eleven (3%) and nine (2%) subjects gave histories of liver and kidney disease, respectively. In the preceding month, nine (2%) persons received prednisone and none received chemotherapy for cancer. Six (1%) contacts developed tuberculosis during the study period. There were 72 index cases of tuberculosis, 46 of whom had positive sputum smears for acid-fast bacilli.

The demographics of the 55 persons who refused to take part in the study were similar to those of the 413 study subjects (*see* RESULTS in the online supplement).

### Initial ELISPOT and Tuberculin Skin Test Results

Of the 413 subjects, the tuberculin skin test and ELISPOT were positive in 208 (50%) and 163 (39%) persons, respectively. Of those with positive ELISPOT results, only the response to ESAT-6 was positive in 21 cases, and only that to CFP10 was positive in 49 cases. For the 250 persons with negative ELISPOT results, the tuberculin skin test size was  $5 \pm 8$  mm (mean  $\pm$  SD; median, 0 mm; range, 0–32 mm). For the 163 persons with a positive ELISPOT result, the mean skin test size was  $18 \pm 13$  mm (median, 20 mm; range, 0–72 mm;  $p < 0.0001$ , Wilcoxon rank sum test) compared with those with a negative ELISPOT. The distribution of tuberculin skin test sizes showed no evidence of terminal digit preference.

The contacts were divided into quartiles on the basis of the contact score. Those in the lowest quartile, who should have the lowest risk for acquiring LTBI from recent exposure, were considered to be the reference group. The odds ratio for having a positive tuberculin skin test rose with each increment in contact score quartile and was 1.9 in the highest quartile (Table 2;  $p$  trend = 0.03). Although the ELISPOT results appeared more strongly associated with contact score than did the tuberculin skin test results, with an odds ratio of 2.3 in the highest contact score quartile ( $p$  trend = 0.001), these differences were not significant ( $p = 0.36$ ). In the entire study population, 208 (50%) contacts had positive tuberculin skin tests and 163 (39%) had positive ELISPOT results ( $p < 0.0001$ , repeated measures logistic regression). Because prior studies using the tuberculin skin test have

estimated that approximately one-third of household contacts of patients with tuberculosis are infected with *M. tuberculosis* (13), this suggests that some positive ELISPOT and tuberculin skin test results may have resulted from LTBI acquired before the current episode of exposure.

Previous studies have shown that the likelihood of LTBI in a contact is higher if the patient with tuberculosis has a positive sputum smear for acid-fast bacilli (13, 14). LTBI is also more common among household contacts than casual contacts (15, 16). In our population, there were 75 contacts of 26 smear-negative patients (2.9 contacts/patient) and 338 contacts of 46 smear-positive patients (7.3 contacts/patient). Thirty-nine (52%) contacts of smear-negative cases were household contacts, compared with only 91 (27%) contacts of smear-positive cases. These differences reflect the more extensive efforts of public health authorities to identify nonhousehold contacts of smear-positive cases, because of their greater infectivity. Because this differential screening introduces bias, we did not analyze ELISPOT or tuberculin skin test results according to household versus nonhousehold contact, or according to the sputum smear status of the patient.

### Subgroup Analysis by Country of Birth and BCG Vaccination Status

Persons born in the United States are less likely to have been previously infected with *M. tuberculosis* than those born in high-incidence countries. Therefore, we further analyzed the ELISPOT and tuberculin skin test results for the 185 persons born in the United States, only three (2%) of whom were BCG-vaccinated (Table 3). Fifty-five (30%) persons had positive tuberculin skin tests and 57 (31%) had positive ELISPOT results. The odds ratio in the highest contact score quartile increased to 2.7 for a positive ELISPOT ( $p$  trend = 0.02) and to 1.9 for a positive tuberculin skin test ( $p$  trend = 0.15). However, these trends were not significantly different ( $p = 0.29$ ).

Among the 228 foreign-born persons, of whom 201 (88%) were BCG-vaccinated, the contact score had a borderline significant association with the ELISPOT results and no significant association with tuberculin skin test results (Table 4). One hundred and fifty-three (67%) persons had positive tuberculin skin tests and 106 (46%) had positive ELISPOT tests ( $p < 0.0001$ , repeated measures logistic regression). The high percentage of positive tests and lack of strong association with extent of recent exposure to tuberculosis suggest that some of these individuals had preexisting LTBI.

Because BCG vaccination may cause positive tuberculin skin tests but should not give positive ELISPOT results (10, 11), we further analyzed the results for 209 persons without prior BCG vaccination, 182 (87%) of whom were born in the United States (Table 5). Sixty-six (32%) subjects had positive tuberculin skin tests and 64 (31%) had positive ELISPOT results. Among contacts in the highest contact score quartile, the odds ratio for a positive ELISPOT result increased to 3.0 ( $p$  trend = 0.01), and that for a positive tuberculin skin test increased to 2.2 ( $p$  trend = 0.04). These trends were not significantly different ( $p = 0.37$ ).

Among the 204 BCG-vaccinated persons, 142 (70%) had positive tuberculin skin tests and 99 (49%) had positive ELISPOT results ( $p < 0.0001$ , repeated measures logistic regression). Neither the tuberculin skin test nor the ELISPOT related to the contact scores (Table 6). All but three BCG-vaccinated persons were foreign-born.

For the six contacts who developed tuberculosis during the study, five had positive ELISPOT and four had positive tuberculin skin test results at enrollment. For the three HIV-infected subjects, none of whom developed tuberculosis during the study,

**TABLE 2. ODDS RATIOS AND 95% CONFIDENCE INTERVALS FOR A POSITIVE TUBERCULIN SKIN TEST OR ELISPOT IN ALL 413 SUBJECTS**

Test	Contact Score, Quartile	Positive, No. (%)	Negative, No. (%)	OR (95% CI)	p Value*
Tuberculin skin test	1st (0–65)	42 (20)	61 (30)	1.0	0.03
	2nd (66–246)	53 (25)	50 (24)	1.5 (0.9, 2.7)	
	3rd (247–590)	54 (26)	49 (24)	1.6 (0.9, 2.8)	
	4th (> 590)	59 (28)	45 (22)	1.9 (1.1, 3.3)	
ELISPOT	1st (0–65)	31 (19)	72 (29)	1.0	0.001
	2nd (66–246)	35 (21)	68 (27)	1.2 (0.7, 2.2)	
	3rd (247–590)	45 (28)	58 (23)	1.8 (1.0, 3.2)	
	4th (> 590)	52 (32)	52 (21)	2.3 (1.3, 4.1)	

Definition of abbreviations: CI = confidence interval; ELISPOT = enzyme-linked immunospot assay; OR = odds ratio.  $\kappa$  for agreement between the tuberculin skin test and the ELISPOT = 0.49.

\* Test for trend, using contact score as an ordinal variable.

all had negative ELISPOT and two had negative tuberculin skin test results.

### Sensitivity Analyses

The relationship between contact score and both the ELISPOT and tuberculin skin tests was strongest in persons born in the United States and in persons without BCG vaccination (Tables 3 and 5). To evaluate the robustness of these associations, we performed sensitivity analyses, varying the weights of different factors used in computing the contact score. The following changes were made individually: (1) time spent within 3 ft of the patient was multiplied by 2 and by 1, instead of 4; (2) time spent outdoors was multiplied by 0.5 instead of 0.25; (3) the weight for contact in a car was 3 instead of 3.5, that for contact in a room in a private residence was 2 instead of 2.5, and that for contact in a shelter, factory, indoor construction site, church, movie theater, store, or garage was 1 instead of 1.5; (4) the weight for a spouse or household sexual partner was reduced from 3 to 2, and that of other household members from 2 to 1.5; (5) the weight was eliminated for all household contacts; and (6) the infectivity weight for a patient with positive sputum acid-fast smears was changed from 4 to 8 or to 2 in separate analyses. None of these changes substantially altered the relationship between ELISPOT or tuberculin skin test results and contact score quartile in persons born in the United States (data not shown). In all analyses, the odds ratio for a positive ELISPOT test was 2.6 to 3.3 in the highest contact score quartile (p trend = 0.01–0.02), and the odds ratio for a positive tuberculin skin test was 1.7 to 2.3 (p trend = 0.08–0.20).

Nine persons had tuberculin skin test results that showed 5 to 9 mm of induration, which may be more likely due to prior BCG vaccination or to exposure to environmental mycobacteria.

Therefore, we repeated the analyses performed in Tables 2 through 6, considering 10 mm instead of 5 mm as a cutoff for a positive tuberculin skin test, but found no differences in the results (data not shown).

We considered the possibility that young persons were more likely to have positive tuberculin skin tests from BCG vaccination because less time had elapsed since vaccination. However, when we excluded persons aged 18 or younger, and repeated the analyses performed in Tables 2 through 6, the results were unchanged (data not shown).

### Discrepant ELISPOT and Tuberculin Skin Test Results

For the 413 contacts, both ELISPOT and tuberculin skin test results were positive in 133 and both were negative in 175 contacts. Only the tuberculin skin test was positive in 75 cases (18%) and only the ELISPOT test was positive in 30 cases (7%). Approximately 80% of persons with only positive tuberculin skin tests were Hispanic or Asian, compared with only 40% of those with only positive ELISPOT results (Table 7). Foreign birth and BCG vaccination were also significantly more common in persons with only positive tuberculin skin test results. The contact scores of those with only positive ELISPOT results were similar to those of persons with only positive tuberculin skin tests.

### Repeat ELISPOT and Tuberculin Skin Test Results

Of the 413 contacts, 200 persons consented to repeat ELISPOT testing 3 to 6 mo after the initial test. For these 200 persons, the results were the same as the initial test in 84% of contacts (168 cases: positive in 57 cases and negative in 111 cases). Sixteen cases had initial positive but repeat negative ELISPOT results, and 16 cases had initial negative but repeat positive ELISPOT

**TABLE 3. ODDS RATIOS AND 95% CONFIDENCE INTERVALS FOR A POSITIVE TUBERCULIN SKIN TEST OR ELISPOT IN 185 PERSONS BORN IN THE UNITED STATES**

Test	Contact Score, Quartile	Positive, No. (%)	Negative, No. (%)	OR (95% CI)	p Value*
Tuberculin skin test	1st (0–50)	10 (18)	36 (28)	1.0	0.15
	2nd (51–212)	13 (24)	33 (25)	1.4 (0.6, 3.7)	
	3rd (213–475)	16 (29)	30 (23)	1.9 (0.8, 4.9)	
	4th (> 475)	16 (29)	31 (24)	1.9 (0.7, 4.7)	
ELISPOT	1st (0–50)	10 (18)	36 (28)	1.0	0.02
	2nd (51–212)	12 (21)	34 (27)	1.3 (0.5, 3.3)	
	3rd (213–475)	15 (26)	31 (24)	1.7 (0.7, 4.4)	
	4th (> 475)	20 (35)	27 (21)	2.7 (1.1, 6.6)	

For definition of abbreviations, see Table 2.

$\kappa$  for agreement between the tuberculin skin test and the ELISPOT = 0.51.

\* Test for trend, using contact score as an ordinal variable.

**TABLE 4. ODDS RATIOS AND 95% CONFIDENCE INTERVALS FOR A POSITIVE TUBERCULIN SKIN TEST OR ELISPOT IN 228 FOREIGN-BORN PERSONS**

Test	Contact Score, Quartile	Positive, No. (%)	Negative, No. (%)	OR (95% CI)	p Value*
Tuberculin skin test	1st (0–75)	32 (21)	23 (31)	1.0	0.23
	2nd (76–260)	41 (27)	18 (24)	1.6 (0.8, 3.5)	
	3rd (261–670)	40 (26)	16 (21)	1.8 (0.8, 4.0)	
	4th (> 670)	40 (26)	18 (24)	1.6 (0.7, 3.5)	
ELISPOT	1st (0–75)	21 (20)	34 (28)	1.0	0.06
	2nd (76–260)	23 (22)	36 (30)	1.0 (0.5, 2.2)	
	3rd (261–670)	33 (31)	23 (19)	2.3 (1.1, 5.0)	
	4th (> 670)	29 (27)	29 (23)	1.5 (0.7, 3.1)	

For definition of abbreviations, see Table 2.

$\kappa$  for agreement between the tuberculin skin test and the ELISPOT = 0.43.

\* Test for trend, using contact score as an ordinal variable.

results. For the 153 persons who had repeat tuberculin skin testing, the results were the same as the initial test in 86% of cases (positive in 12 and negative in 120 cases). Five persons had initial positive and repeat negative tuberculin skin tests, and 16 had initial negative and repeat positive tuberculin skin tests.

We repeated the analysis performed in Table 2, considering the ELISPOT or tuberculin skin test to be positive if either the initial or repeat test was positive. The associations between contact score quartile and either the ELISPOT or tuberculin skin test results were slightly stronger, compared with the analysis using the initial results only (Table E1 in the online supplement and Table 2). When subgroup analyses were performed by country of birth and BCG vaccination status, results were similar to those in Tables 3 through 6 (data not shown).

## DISCUSSION

The ELISPOT has been used in research settings and in institutional outbreaks, and is being evaluated by the Food and Drug Administration for the diagnosis of LTBI. However, it has not previously been used in contact investigations in the United States or under routine program conditions. Our study is the first to address these critical issues. Because of the lack of a gold standard for LTBI, sensitivity and specificity of the ELISPOT and tuberculin skin tests cannot be calculated. Therefore, we derived a contact score that quantified the risk of acquiring recent tuberculosis infection on the basis of data from prospectively obtained, standardized interviews. We investigated the relationship between ELISPOT and tuberculin skin test results and this contact score, which served as a surrogate measure for likelihood of LTBI. ELISPOT results were more strongly associated with the contact score than were tuberculin skin test

results in the entire study group and in all subpopulations evaluated, although the differences between these two tests were not statistically significant.

Persons born in the United States and those who were not vaccinated with BCG were at relatively low risk for preexisting LTBI, and their principal risk for LTBI was from recent exposure to a patient with tuberculosis. In this setting, higher contact scores were significantly associated with positive ELISPOT and tuberculin skin test results (Tables 3 and 5). The contact score appeared to relate more strongly to the ELISPOT than to the tuberculin skin test results, but this difference was not statistically significant. In these subpopulations, approximately 30% had positive skin tests, consistent with prior studies indicating that 30 to 40% of close contacts of patients with tuberculosis become infected with *M. tuberculosis* (13, 15). The frequencies of positive ELISPOT and tuberculosis skin tests were essentially identical in those in the lower quartiles of contact score. However, the ELISPOT was positive in 43% (20/47) of U.S.-born persons and 45% (24/53) of non-BCG-vaccinated persons in the highest contact score quartile. The tuberculin skin test was positive in 34% (16/47) and 38% (20/53) of these groups, respectively. This suggests that the ELISPOT may be more sensitive than the tuberculin skin test for detecting LTBI in contacts of patients with tuberculosis. Alternatively, persons who are transiently infected with *M. tuberculosis* may mount a T-cell response that yields a positive ELISPOT but not a positive tuberculin skin test. Long-term follow-up studies of contacts with positive ELISPOT results but negative tuberculin skin tests are critical to separate these possibilities.

Among foreign-born and BCG-vaccinated persons (99% of whom were foreign-born), in the two lowest contact score

**TABLE 5. ODDS RATIOS AND 95% CONFIDENCE INTERVALS FOR A POSITIVE TUBERCULIN SKIN TEST OR ELISPOT IN 209 PERSONS WITHOUT A HISTORY OF BACILLE CALMETTE-GUÉRIN VACCINATION**

Test	Contact Score, Quartile	Positive, No. (%)	Negative, No. (%)	OR (95% CI)	p Value*
Tuberculin skin test	1st (0–55)	11 (17)	40 (28)	1.0	0.04
	2nd (56–220)	14 (21)	37 (26)	1.4 (0.6, 3.4)	
	3rd (221–485)	21 (32)	33 (23)	2.3 (1.0, 5.5)	
	4th (> 485)	20 (30)	33 (23)	2.2 (0.9, 5.3)	
ELISPOT	1st (0–55)	11 (17)	40 (28)	1.0	0.01
	2nd (56–220)	14 (22)	37 (26)	1.4 (0.6, 3.4)	
	3rd (221–485)	15 (23)	39 (27)	1.4 (0.6, 3.4)	
	4th (> 485)	24 (38)	29 (20)	3.0 (1.3, 7.1)	

For definition of abbreviations, see Table 2.

$\kappa$  for agreement between the tuberculin skin test and the ELISPOT = 0.53.

\* Test for trend, using contact score as an ordinal variable.

**TABLE 6. ODDS RATIOS AND 95% CONFIDENCE INTERVALS FOR A POSITIVE TUBERCULIN SKIN TEST OR ELISPOT IN 204 BACILLE CALMETTE-GUÉRIN-VACCINATED PERSONS**

Test	Contact Score, Quartile	Positive, No. (%)	Negative, No. (%)	OR (95% CI)	p Value*
Tuberculin skin test	1st (0–68)	30 (21)	21 (34)	1.0	0.56
	2nd (69–250)	40 (28)	10 (16)	2.8 (1.2, 6.8)	
	3rd (251–672)	38 (27)	14 (23)	1.9 (0.8, 4.4)	
	4th (> 672)	34 (24)	17 (27)	1.4 (0.6, 3.1)	
ELISPOT	1st (0–68)	19 (19)	32 (30)	1.0	0.17
	2nd (69–250)	23 (23)	27 (26)	1.4 (0.7, 3.2)	
	3rd (251–672)	34 (34)	18 (17)	3.2 (1.4, 7.1)	
	4th (> 672)	23 (23)	28 (27)	1.4 (0.6, 3.1)	

For definition of abbreviations, see Table 2.

κ for agreement between the tuberculin skin test and the ELISPOT = 0.39.

\* Test for trend, using contact score as an ordinal variable.

quartiles, approximately 70% had positive tuberculin skin tests and 40% had positive ELISPOT results. These findings suggest a high prevalence of preexisting LTBI, which may explain the lack of a strong relationship between both ELISPOT and tuberculin skin test results and the contact score in these subpopulations (Tables 4 and 6), because neither test distinguishes recent from longstanding LTBI. Overall, in foreign-born and BCG-vaccinated persons, approximately two-thirds had positive tuberculin skin tests and half had positive ELISPOT results ( $p < 0.0001$ ). Seventy-one percent of those with positive tuberculin skin tests and negative ELISPOT results were BCG-vaccinated, compared with only 33% of those with positive ELISPOT results and negative tuberculin skin tests (Table 7). These findings, prior publications (8–11), and the fact that the CFP10 and ESAT-6 antigens used in the ELISPOT are not present in BCG support the contention that BCG vaccination can result in positive tuberculin skin tests but not positive ELISPOT results. The improved specificity of the ELISPOT suggests that using it to screen foreign-born and BCG-vaccinated contacts of patients with tuberculosis would reduce the number of persons incorrectly diagnosed with LTBI, limiting the cost and toxicity of inappropriately treating these individuals. However, positive ELISPOT results will identify persons with LTBI acquired either recently or in the distant past.

In evaluating contacts of patients with tuberculosis who are initially tuberculin-negative, skin testing is repeated 12 wk later, because the delayed-type hypersensitivity response to mycobacterial antigens may not be detectable until this time. We found that repeat ELISPOTs and tuberculin skin tests showed the same results as initial tests in 84 to 86% of cases. Persons with initial

positive and subsequent negative ELISPOT results may have transient infection that causes temporary T-cell reactivity to mycobacterial antigens. Initial negative and subsequent positive ELISPOT results may reflect the fact that T-cell reactivity to mycobacterial antigens does not occur immediately after infection, as with contacts who develop skin test conversion after an initial negative test. Among persons born in the United States with a low prevalence of prior LTBI, seven of nine ELISPOT conversions and five of seven tuberculin skin test conversions occurred in persons in the two highest contact score quartiles (compare Table 3 and Table E1). This suggests that conversions resulted from recent infection by the index case. The odds ratio for having a positive initial or repeat ELISPOT was 3.9 in the highest contact score quartile, compared with 2.7 when only the initial ELISPOT results were used. The odds ratio for having a positive initial or repeat tuberculin skin test was 2.4 in the highest contact score quartile, compared with 1.9 when only the initial tuberculin skin test was used. Therefore, as with the tuberculin skin test, a repeat ELISPOT probably permits detection of additional cases of recent tuberculosis infection.

Most studies comparing the results of new blood tests for LTBI with those of tuberculin skin tests have evaluated results in groups of persons with differing risk of LTBI, on the basis of broad risk factors such as household contact with patients with tuberculosis, country of birth, and intravenous drug use (20, 21). The current study has taken the evaluation of blood tests an important step further by developing a contact score on the basis of prospectively obtained interview data, which provides an estimate of an individual's likelihood for having LTBI after contact with a patient with tuberculosis. It will be important

**TABLE 7. DEMOGRAPHICS AND CONTACT SCORES OF SUBJECTS WITH DISCREPANT TUBERCULIN SKIN TESTS OR ELISPOT RESULTS**

Characteristic	TST + E- (n = 75)	TST - E+ (n = 30)	p Value*
Male sex, no. (%)	37 (49)	15 (50)	1.00
Age in yr, mean (SD)	32.4 (13.1)	37.0 (14.4)	0.13
Race, no. (%)			
African American	9 (12)	12 (40)	0.001
Hispanic	47 (63)	9 (30)	
White	5 (7)	6 (20)	
Asian	13 (17)	3 (10)	
Other	1 (1)	0 (0)	
Foreign-born, no. (%)	57 (76)	10 (33)	< 0.0001
BCG vaccination, no. (%)	53 (71)	10 (33)	0.0008
Mean log-transformed contact score, mean (SD)	5.2 (1.8)	5.6 (1.7)	0.26

Definition of abbreviations: BCG = bacilli Calmette-Guérin; E = ELISPOT; TST = tuberculin skin test.

\* Wilcoxon rank sum test for age; Fisher's exact test for all others.

to evaluate the validity of the contact score in other populations. A potential shortcoming of this approach is the imprecision inherent in estimating exposure to tuberculosis. Information obtained regarding contact with the source case may not have been completely accurate, and we did not consider the duration of infectiousness of the index case, because this variable could not be precisely ascertained. However, we do not believe that this substantially affected our results because they were robust in sensitivity analyses that incorporated wide variations in the method to compute the contact score. Another possible problem is that our study population included a relatively high percentage of Hispanic subjects, and the results may differ in other ethnic groups.

We observed that the contact score may be more strongly associated with the ELISPOT than with the tuberculin skin test results in the entire population and in all subgroups evaluated, although these differences were not statistically significant. Ewer and colleagues (12) reported similar findings in a tuberculosis outbreak at a school, except that the extent of exposure to tuberculosis correlated significantly better with ELISPOT than with tuberculin skin test results. The clearer superiority of the ELISPOT in Ewer and colleagues' study may have resulted from several factors. First, extent of exposure to tuberculosis in the outbreak was based largely on student classroom schedules and was probably more accurately quantified than in our contacts, who were evaluated under routine program conditions. Second, tuberculin skin testing in the outbreak was performed by the Heaf method, which is probably less accurate than the Mantoux technique that we used (2). Finally, Ewer and colleagues evaluated a larger population of contacts at low risk for preexisting LTBI (459 students born in the United Kingdom), compared with 185 U.S.-born persons in the current report. If our findings are extrapolated to a larger population, 1,300 subjects are needed to have 80% power to show that the ELISPOT is superior to the tuberculin skin test. With 185 subjects, we had only 18% power to detect this difference.

Our findings contrast with those of a study in Gambia comparing the tuberculin skin test and the ELISPOT in 735 household contacts of patients with tuberculosis (20). In this setting, the tuberculin skin test appeared to correlate more closely than the ELISPOT with extent of exposure to the index case, although the difference was not statistically significant. The authors suggested that the sensitivity of the ELISPOT was reduced because it uses only two antigens, ESAT-6 and CFP10. We believe that this is unlikely given our current findings and prior reports demonstrating that the ELISPOT is of equal or greater sensitivity than the tuberculin skin test for detection of LTBI (11, 12, 22). The reasons for the apparently reduced sensitivity of the ELISPOT in the Gambian study remain speculative.

LTBI was recently diagnosed in 32 persons evaluated during a school tuberculosis outbreak by measuring IFN- $\gamma$  concentrations in supernatants of whole blood incubated with ESAT-6 and CFP10 (8), and this test (QuantiFeron-TB Gold) is available in Europe and the United States. Unlike this test, the ELISPOT measures the number of IFN- $\gamma$ -producing cells. Until recently, ELISPOT required manual isolation of mononuclear cells from blood. However, it has now been simplified, using automated cell separation vacutainers, and is marketed as the T Spot-TB test, which has European regulatory approval and is being evaluated by the Food and Drug Administration.

We found that ELISPOT results were interpretable in more than 99% of cases. In contrast, in most clinical settings, a large proportion of tuberculin skin tests are not interpreted because the patient does not return for the second visit. Our results reflect optimal performance of the tuberculin skin test, which was performed by trained, experienced individuals. In many health

care settings, tuberculin skin tests are performed by personnel with variable expertise, and the reliability of skin testing is likely to be substantially lower under these circumstances. As a result, the ELISPOT is logistically superior to the tuberculin skin test because it requires a single visit and is less operator-dependent.

In conclusion, in a large group of contacts of patients with tuberculosis evaluated in a routine clinical setting, the ELISPOT appeared to be at least as sensitive as the tuberculin skin test for detection of LTBI in U.S.-born persons, and was more specific than the skin test in BCG-vaccinated persons. The ELISPOT was technically feasible and robust, and is likely to be a more accurate means to diagnose LTBI than the tuberculin skin test. The improved specificity of the ELISPOT will reduce the number of false-positive diagnoses of LTBI in BCG-vaccinated individuals. As the prevalence of LTBI declines in low-prevalence countries, an increasing proportion of positive tuberculin skin test results will be due to prior BCG vaccination. Therefore, the specificity of the ELISPOT makes it an important tool for control programs aimed at eliminating tuberculosis.

**Conflict of Interest Statement:** H.S. does not have a financial relationship with a commercial entity that has an interest in the subject matter of this manuscript. S.E.W. does not have a financial relationship with a commercial entity that has an interest in the subject matter of this manuscript. P.K. does not have a financial relationship with a commercial entity that has an interest in the subject matter of this manuscript. A.L. is named inventor on several patents related to T-cell diagnosis filed by the University of Oxford. Regulatory approval for ELISPOT has been undertaken by Oxford Immunotec, in which he is a shareholder and to which he acts as a scientific advisor. P.K.M. does not have a financial relationship with a commercial entity that has an interest in the subject matter of this manuscript. J.M.P. does not have a financial relationship with a commercial entity that has an interest in the subject matter of this manuscript. K.E. is named inventor on a pending patent related to T-cell diagnosis filed by the University of Oxford. P.F.B. owns 4,000 shares of Cellestis, Ltd., which produces QuantiFeron-TB and QuantiFeron-TB Gold tests.

**Acknowledgment:** The authors thank Mabtech and Staffan Paulie for providing precoated IFN- $\gamma$  ELISPOT plates.

## References

- Centers for Disease Control and Prevention. CDC's response to ending neglect: the elimination of tuberculosis in the United States. Atlanta, GA: Department of Health and Human Services; 2002.
- American Thoracic Society. Diagnostic standards and classification of tuberculosis. *Am Rev Respir Dis* 1990;142:725-735.
- Talbot EA, Moore M, McCray E, Binkin NJ. Tuberculosis among foreign-born persons in the United States, 1993-1998. *JAMA* 2000;284:2894-2900.
- Broekmans JF, Migliori GB, Rieder HL, Lees J, Ruutu P, Loddenkemper R, Raviglione MC. European framework for tuberculosis control and elimination in countries with a low incidence: recommendations of the World Health Organization (WHO), International Union Against Tuberculosis and Lung Disease (IUATLD) and Royal Netherlands Tuberculosis Association (KNCV) working group. *Eur Respir J* 2002; 19:765-775.
- Institute of Medicine. Ending neglect: the elimination of tuberculosis in the United States. Washington, DC: National Academy Press; 2000.
- Colangeli R, Spencer JS, Bifani P, Williams A, Lyashchenko K, Keen MA, Hill PJ, Belisle J, Gennaro ML. MTSA-10, the product of the Rv3874 gene of *Mycobacterium tuberculosis*, elicits tuberculosis-specific, delayed-type hypersensitivity in guinea pigs. *Infect Immun* 2000;68:990-993.
- Harboe M, Oettinger T, Wiker HG, Rosenkrands I, Andersen P. Evidence for occurrence of the ESAT-6 protein in *Mycobacterium tuberculosis* and virulent *Mycobacterium bovis* and for its absence in *Mycobacterium bovis* BCG. *Infect Immun* 1996;64:16-22.
- Brock I, Weldingh K, Lillebaek T, Follmann F, Andersen P. Comparison of tuberculin skin test and new specific blood test in tuberculosis contacts. *Am J Respir Crit Care Med* 2004;170:65-69.
- Mori T, Sakatani M, Yamagishi F, Takashima T, Kawabe Y, Nagao K, Shigeto E, Harada N, Mitarai S, Okada M, *et al.* Specific detection of tuberculosis infection with an interferon-gamma assay using novel antigens. *Am J Respir Crit Care Med* 2004;170:59-64.
- Lalvani A, Nagvenkar P, Udawadia Z, Pathan AA, Wilkinson KA, Shastri JS, Ewer K, Hill AVS, Mehta A, Rodrigues C. 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;183:469–477.
11. Lalvani A, Pathan AA, McShane H, Wilkinson RJ, Latif M, Conlon CP, Pasvol G, Hill AV. Rapid detection of *M. tuberculosis* infection by enumeration of antigen-specific cells. *Am J Respir Crit Care Med* 2001;163:824–828.
  12. Ewer K, Deeks J, Alvarez L, Bryant G, Waller S, Andersen P, Monk P, Lalvani A. Comparison of T-cell-based assay with tuberculin skin test for diagnosis of *Mycobacterium tuberculosis* infection in a school tuberculosis outbreak. *Lancet* 2003;361:1168–1173.
  13. Grzybowski S, Barnett GD, Styblo K. Contacts of patients with active tuberculosis. *Bull Int Union Tuberc* 1975;60:90–106.
  14. Behr MA, Warren SA, Salamon H, Hopewell PC, Ponce de Leon A, Daley CL, Small PM. Transmission of *Mycobacterium tuberculosis* from patients smear-negative for acid-fast bacilli. *Lancet* 1999;353:444–449.
  15. Lutong L, Bei Z. Association of prevalence of tuberculin reactions with closeness of contact among household contacts of new smear-positive pulmonary tuberculosis patients. *Int J Tuberc Lung Dis* 2000;4:275–277.
  16. Sepkowitz KA. How contagious is tuberculosis? *Clin Infect Dis* 1996;23:954–962.
  17. Hopewell PC. Factors influencing the transmission and infectivity of *Mycobacterium tuberculosis*: implications for clinical and public health management. In: Sande MA, Hudson LD, Root RD, editors. Respiratory infections. New York: Churchill Livingstone; 1986. pp. 191–216.
  18. American Thoracic Society. Targeted tuberculin testing and treatment of latent tuberculosis infection. *Am J Respir Crit Care Med* 2000;161:S221–S247.
  19. Shams H, Klucar P, Weis SE, Lalvani A, Moonan PK, Safi H, Wigel B, Ewer K, Nepom GT, Lewinsohn DM, et al. Characterization of a *Mycobacterium tuberculosis* peptide that is recognized by human CD4+ and CD8+ T-cells in the context of multiple HLA alleles. *J Immunol* 2004;173:1966–1977.
  20. Hill PC, Brookes RH, Fox A, Fielding K, Jeffries DJ, Jackson-Sillah D, Logos MD, Owiafe PK, Donkor SA, Hammond AS, et al. Large-scale evaluation of enzyme-linked immunospot assay and skin test for diagnosis of *Mycobacterium tuberculosis* infection against a gradient of exposure in the Gambia. *Clin Infect Dis* 2004;38:966–973.
  21. Mazurek GH, LoBue PA, Daley CL, Bernardo J, Lardizabal AA, Bishai WR, Iademarco MF, Rothel JS. Comparison of a whole-blood interferon  $\gamma$  assay with tuberculin skin testing for detecting latent *Mycobacterium tuberculosis* infection. *JAMA* 2001;286:1740–1747.
  22. Richeldi L, Ewer K, Losi M, Bergamini BM, Roversi P, Deeks J, Fabbri LM, Lalvani A. T cell-based tracking of multidrug resistant tuberculosis infection after brief exposure. *Am J Respir Crit Care Med* 2004;170:288–295.