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Antituberculosis IgG Antibodies as a Marker of Active *Mycobacterium tuberculosis* Disease

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Anti-*Mycobacterium tuberculosis* IgG antibodies may aid in the diagnosis of active *M. tuberculosis* disease. We studied whether anti-*M. tuberculosis* IgG antibodies are elevated in active *M. tuberculosis* disease and assessed factors contributing to false-positive and -negative results. A retrospective study of 2,150 individuals tested by the QuantiFERON-TB Gold In-Tube (QFT-GIT) assay was conducted at the University of Utah, ARUP Laboratories, November 2008 to December 2010. All samples were tested with the InBios Active Tb*Detect* antituberculosis (anti-TB) IgG antibody assay. Of 1,044 patients with a positive QFT-GIT, 59 (5.7%) were positive for *M. tuberculosis* antibodies. Fourteen of 1,106 (1.3%) with a negative or indeterminate QFT-GIT were positive for *M. tuberculosis* antibodies. *M. tuberculosis* antibody tests were positive in 61.5% with confirmed active *M. tuberculosis* disease and other mycobacterial infections. Over half of the false-negative *M. tuberculosis* antibody tests occurred in patients ≥90 years of age. False positives were seen in 12.9% of autoimmune patients. The odds ratio of being positive by the QFT-GIT and the InBios TB IgG assay increased with confirmed *M. tuberculosis* disease or highly suspected *M. tuberculosis* disease and was 86.7 (95% confidence interval [CI], 34.4 to 218.5) in these two groups compared to patients negative by both tests. Although anti-*M. tuberculosis* antibodies can be detected in patients with active *M. tuberculosis* disease, caution should be used with patients where immunoglobulin levels may be decreased or patients with autoantibodies.

Tuberculosis (TB) remains the leading single microbial illness globally, with one-third of the world's population infected with *Mycobacterium tuberculosis* complex. In 2009, there were over 9.4 million new cases and 1.3 million deaths from *M. tuberculosis* (25). While the host's immune system typically prevents the organism from spreading beyond the primary site of infection, 5 to 10% of these latent *M. tuberculosis* infections progress to active disease. Once the disease becomes active, it is contagious and lethal with a mortality rate of greater than 50% in untreated individuals (6). This is in sharp contrast to the <5% mortality rate in regions implementing the guidelines of the World Health Organization (WHO) for the diagnosis and treatment of *M. tuberculosis* (directly observed treatment, short course [DOTS]) (25). Therefore, early diagnosis of active *M. tuberculosis* is a crucial step in the success of treatment through rapid isolation of infected individuals and the early initiation of prophylaxis.

Anti-*M. tuberculosis* IgG antibodies have been shown to increase in patients with active disease (3, 11, 13, 16). While the function of anti-*M. tuberculosis* antibodies in providing protective immunity is still under investigation, it has been proposed that they may be utilized as a diagnostic marker of active disease (1, 2, 7). In response to this research, InBios International (Seattle, WA) has developed the Active Tb*Detect* IgG enzyme-linked immunosorbent assay (ELISA) to identify IgG antibodies against several immunodominant *M. tuberculosis* epitopes (2). In our prior study, we evaluated the Anda-TB IgG and InBios TB IgG assays and the IBL *M. tuberculosis* IgG ELISA in a pilot study of 18 patients positive for *M. tuberculosis* by culture and/or amplified direct detection (ADD) and 88 healthy U.S.-born individuals who tested negative by QuantiFERON-Gold test (which was of the generation of tests that preceded the QuantiFERON-TB Gold In-Tube [QFT-GIT] assay) and had no risk factors for *M. tuberculosis* infection (2). We found that Anda-TB IgG had a sensitivity of 83.3% and a specificity of 72.0%. The InBios TB IgG assay had a sensitivity of 83.3% and a specificity of 98.9%. In that study, we identified an important limitation of the *M. tuberculosis* IgG assays in the fact that both the InBios TB IgG assay and the Anda-TB IgG assay were positive in only 3 of 6 HIV patients with positive *M. tuberculosis* culture and/or ADD for a sensitivity of only 50%. The InBios TB IgG assay, however, showed promise as being a more specific assay than the Anda-TB IgG assay, with a specificity of 98.9%. Therefore, we chose to examine the InBios assay performance characteristics further in our current study.

MATERIALS AND METHODS

Study participants. Sample collection took place from November 2008 to December 2010 on samples originally sent to ARUP Laboratories (Salt Lake City, UT) for *M. tuberculosis* testing with the QFT-GIT assay. Samples (2,150 consecutive samples) were collected. Samples were stored at −70 to −20°C until testing was performed, at which point they were stored at 2 to 4°C until testing was complete. The protocol used was approved by the institutional review board of the University of Utah (IRB #40573).

Following sample collection, histories were obtained through phone interviews with ordering physicians. Relevant clinical information was obtained during the interview process, and doctors were fully informed of what information could be released according to the Health Insurance Portability and Accountability Act (HIPAA) of 1996. Patient classifications are listed in Table 1.

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TABLE 1 Patient classification schema based on physician interviews

Risk of active *M. tuberculosis* (TB) disease (total no. of patients)	Description
Low (790)	Low-active-risk patients being screened for TB including immigrants, students, and health care workers
Medium (95)	Patients with physician-suspected TB with not more than one secondary symptom
High (16)	Patients with physician-suspected TB with two or more secondary symptoms, including night sweats, wt loss, fever, vomiting, severe cough, and unresponsiveness to antibiotics
Confirmed (13)	Patients with physician-diagnosed TB; positive AFB[a] smear, culture, or amplified direct detection method
Autoimmune (33)	Patients being screened for TB before biological therapy for autoimmune disease

[a] AFB, acid-fast bacillus.

QuantiFERON-TB Gold In-Tube assay. The QFT-GIT assay was run according to the manufacturer's protocol. Patients had whole blood collected in three separate tubes: a TB antigen tube containing three *M. tuberculosis*-specific antigens, ESAT-6, CFP-10, and TB7.7; a mitogen tube containing phytohemagglutinin; and a nil tube with no stimulants. Following an incubation of 16 to 24 h, the plasma is separated by centrifugation and run on a gamma interferon (IFN-γ) ELISA. The investigators that performed the QFT-GIT assay were blind to the clinical history of the patients. Patients were considered negative if the antigen value minus the nil value was less than 0.35 IU/ml. Patients were considered positive if the antigen value minus the nil value was greater than 0.35 IU/ml. Patients were considered indeterminate if the mitogen value minus the nil value was less than 0.5 IU/ml or if the nil value was greater than 8.0 IU/ml.

***M. tuberculosis* IgG testing.** *M. tuberculosis* IgG testing was performed with the InBios Active Tb*Detect* IgG ELISA (InBios International, Seattle, WA). The test was performed according to the manufacturer's protocol. Briefly, serum samples were incubated in wells containing several *M. tuberculosis*-specific antigens (Mtb81, Mtb8, Mtb48, DPEP, the 38-kDa protein, and two additional proprietary antigens). Following a conjugate incubation step, the substrate was added and color was allowed to develop. Our previous study concluded that the cutoff of 0.500 optical density (OD) at 450 nm (OD_{450}) maximized sensitivity and specificity (2). The equivocal reference range was defined as 0.425 to 0.499 OD_{450}. First, a cutoff was determined, following the manufacturer's recommendation, to be the average OD of normal serum ($n = 83$) + 3 standard deviations (SD), i.e., 0.450 OD. The equivocal range was then defined as the cutoff OD of 0.450 ± 5.5% (2). At the time the InBios TB IgG assay was performed, the clinical histories of the patients were unknown.

Statistical analysis. Comparison of the InBios TB IgG assay positivity between the QFT-GIT-positive and the QFT-GIT-negative group results was analyzed using Yates' corrected chi-square test. Odds ratios were calculated by comparing the InBios TB IgG assay positivity rate in QFT-GIT-negative samples to results for each category of QFT-GIT-positive patients. Statistical analysis was done using MedCalc version 10.6.1.0 (MedCalc Software, Mariakerke, Belgium). Spreadsheets were constructed and additional calculations were performed using Excel (Microsoft Corp., Redmond, Washington).

RESULTS

Medical histories were obtained for 876 of the 1,044 (83.9%) patients with a positive QFT-GIT result and for a small subset (70 of 1,006; 7.0%) of patients with a negative QFT-GIT result. No histories were obtained for any of the 100 patients with QFT-GIT-indeterminate results. Age and sex information was available for all patients included in the study. The QFT-GIT-positive patients consisted of 46.3% females with a mean age of 44 years (range, <1 year to 97 years). The QFT-GIT-negative patients consisted of 58.0% females with a mean age of 45 years (range, 1 year to 102 years), and the QFT-GIT-indeterminate patients consisted of 50.0% females with a mean age of 47 years (range, < 1 year to 84 years). Of the 876 patients with a positive QFT-GIT result and known history, the WHO region of origin was known for 728 (Table 2).

TABLE 2 WHO region of origin distribution for a subset of patients with a known clinical history and country of origin

WHO region	No. of persons	% of total	% InBiosTB IgG positive (*n*)
U.S.A./Canada	344	46.6	5.3 (18)
Southern Asia/Southeastern Asia	161	22.1	6.3 (10)
Mexico/Central America	74	10.2	9.6 (7)
Africa	62	8.5	9.7 (6)
Central Asia/Eastern Asia/Russia	36	5.0	5.6 (2)
Western Asia	32	4.4	3.1 (1)
Europe	14	1.9	7.1 (1)
Caribbean	5	0.7	40.0 (2)
Oceania	3	0.4	0 (0)
South America	2	0.3	0 (0)
Total	728	100.0	6.5 (47)

Overall, 5.6% of patients positive by the QFT-GIT assay were positive by the InBios TB IgG assay, while only 1.2% of patients negative by the QFT-GIT assay were positive by the InBios TB IgG assay (Table 3). When separated by region, individuals from Africa and Mexico/Central America had the highest positivity rates on the InBios TB IgG assay at 9.7% and 9.6%, respectively. The U.S./Canadian region had the most individuals enrolled (339), where the country of origin was known, and had a positivity rate of 5.3% (Table 2).

Patients were classified in terms of disease state and their reactivity on the QFT-GIT assay. Each individual's active *M. tuberculosis* infection risk status and QFT-GIT result were then compared with their qualitative InBios TB IgG antibody result (Table 4). Patients who were positive by the QFT-GIT assay with low risk, medium risk, and high risk for active *M. tuberculosis* infection had anti-*M. tuberculosis* IgG antibody positivity rates of 3.2%, 10.9%, and 43.8%, respectively. Patients who were positive by the QFT-GIT assay with confirmed active mycobacterial disease had an anti-*M. tuberculosis* IgG antibody positivity rate of 61.5%. Patients who were positive by the QFT-GIT assay and who had been screened prior to biological treatment for preexisting autoimmune disease had an anti-*M. tuberculosis* IgG positivity rate of 12.9%.

TABLE 3 Comparison between the QFT-GIT assay and the InBios TB IgG assay

QFT-GIT status	No. with indicated status by InBios TB IgG assay			
	Positive	Negative	Equivocal	Total
Positive	59	972	13	1,044
Negative	12	971	23	1,006
Indeterminate	2	95	3	100
Total	73	2,038	39	2,150

TABLE 4 InBios TB IgG positivity rate and odds ratios for each category separated by patient histories[a]

Category	TB IgG result[b]		Positivity rate (%)	Odds ratio (95% CI)	*P* value
	Pos	Neg			
QFT-GIT negative	12	971	1.2	1.0	
Screen	23	691	3.2	2.69 (1.33–5.45)	0.006
Medium	10	82	10.9	9.87 (4.14–23.53)	<0.001
High	7	9	43.8	62.94 (20.13–196.80)	<0.001
Confirmed	8	5	61.5	129.47 (36.94–453.71)	<0.001
Autoimmune screen	4	27	12.9	11.99 (3.63–39.58)	<0.001

[a] Equivocal InBios TB IgG and indeterminate QFT-GIT results were excluded.
[b] Pos, positive; neg, negative.

Eight out of 13 (61.5%) patients who were positive by the QFT-GIT assay with known active mycobacterial disease were positive by the InBios TB IgG assay, all with pulmonary disease (Table 5). Two had infections with nontuberculous mycobacteria, *Mycobacterium fortuitum* and *Mycobacterium gordonae*. Infections with nontuberculous mycobacteria have been known to follow *M. tuberculosis* infections. However, there was no information regarding previous *M. tuberculosis* infection in these two patients. Five patients with confirmed active *M. tuberculosis* infections were QFT-GIT positive but negative by the InBios TB IgG antibody assay. Three of the five patients were 90 years of age or greater. The 4th patient negative by the InBios TB IgG antibody assay was immunosuppressed. The 5th patient had no history to suggest an explanation for a negative InBios TB IgG antibody test.

Seven out of 16 patients with physician-suspected active *M. tuberculosis* infection were positive by the InBios TB IgG assay (43.8%) (Table 6). Four of the positive patients had pulmonary disease. One patient was treated for *M. tuberculosis* meningoencephalitis in the past, and another was suspected to have ocular *M. tuberculosis* infection. Nine patients had suspected active *M. tuberculosis* but were negative by the InBios TB IgG antibody assay. Five had pulmonary disease. Two had suspected ocular *M. tuberculosis* infection. One had suspected tuberculous peritonitis, and another had suspected disseminated *M. tuberculosis* infection.

To measure the relationship between disease status and anti-*M. tuberculosis* IgG antibody level, odds ratios and 95% confidence intervals (CI) were calculated using the InBios TB IgG positivity rate among disease-free individuals (QFT-GIT negative) as an OR of 1.00. The crude odds ratio for all QFT-GIT-positive individuals was 4.91 (95% CI, 2.62 to 9.19). Odds ratios varied from 2.69 (95% CI, 1.33 to 5·45) in individuals with a low risk of active disease to 129.47 (95% CI, 36.94 to 453.71) in individuals with confirmed active disease (Table 4).

To assess if *Mycobacterium bovis* BCG vaccination status had an effect on the InBios TB status, patients were further stratified into vaccine status groups, and odds ratios were calculated. The vaccine status was known for 474 of the 1,044 patients who were positive by the QFT-GIT assay. The crude OR for BCG vaccinated QFT-GIT-positive individuals was 2.09 (95% CI, 1.01 to 4.35, $P = 0.05$). However, when subjects were stratified according to active *M. tuberculosis* infection, BCG vaccination was never significantly associated with a positive InBios TB IgG result.

To determine the predictive ability of the quantitative QFT-GIT values to assess active *M. tuberculosis* disease, the means within each category of QFT-GIT-positive patients were compared. Means ranged from a low of 4.24 to a high of 5.28. There was no significant difference in QFT-GIT values between risk groups. As the likelihood of active *M. tuberculosis* infection increased, the mean QFT-GIT result did not increase, and none of the differences were statistically significant when using Student's *t* test to compare the mean result of each category with the low-risk group (Table 7). Additionally, no correlation was seen when anti-*M. tuberculosis* IgG results (OD) were compared with QFT-GIT levels (IU/ml) by scatter plot analysis (linear regression coefficient of determination, $R^2 = 0.0023$).

DISCUSSION

It has been observed that during active *M. tuberculosis* disease, a humoral response occurs in the host, which can be measured using anti-*M. tuberculosis* antibodies. Immunoglobulin G antibodies directed against several *M. tuberculosis* antigens have been proposed as potential markers of tuberculosis, of which Mtb81, Mtb8, Mtb48, DPEP (MPT32), the 38-kDa protein, and two proprietary antigens are contained on the InBios TB IgG assay (2). Both anti-Mtb81 and anti-MPT32 antibodies have been previously shown to

TABLE 5 Clinical histories, PPD, and TB IgG antibody results of QFT-IT positive patients with confirmed mycobacterial infections[a]

Patient no.	Age (yr)	Country of origin	PPD	BCG vaccine	Chest X-ray	TB IgG result	TB IgG interp	History
1	36	U.S.	ND	No	POS	2.141	POS	POS culture TB; pulmonary
2	70	U.S.	NEG	No	POS	1.867	POS	POS ADD; pulmonary
3	22	Mexico	UNK	Yes	POS	1.391	POS	POS smear AFB; pulmonary cavitary lesions on chest X-ray
4	79	U.S.	POS	No	POS	1.282	POS	POS culture *M. fortuitum*; pulmonary
5	67	U.S.	ND	No	UNK	0.968	POS	POS smear AFB TB bronchitis; exposure to active TB
6	55	India	UNK	UNK	UNK	0.940	POS	POS culture *M. gordonae*; pulmonary
7	32	UNK	UNK	UNK	UNK	0.886	POS	POS ADD; pulmonary
8	47	Mexico	POS	Yes	UNK	0.565	POS	POS culture TB; POS smear AFB pulmonary
9	43	Mexico	POS	UNK	POS	0.106	NEG	Immune suppressed; S/P renal transplant; abdominal lymph node POS AFB, POS PCR
10	90	U.S.	ND	No	POS	0.086	NEG	POS smear AFB; pulmonary
11	52	U.S.	POS	No	POS	0.085	NEG	POS culture TB POS ADD; pulmonary
12	90	Vietnam	ND	UNK	UNK	0.064	NEG	Ankle aspirate POS smear AFB
13	92	U.S.	NEG	No	POS	0.061	NEG	POS smear AFB POS ADD; pulmonary

[a] TB, tuberculosis; ADD, amplified direct detection; POS, positive; NEG, negative; ND, not done; UNK, unknown; AFB, acid-fast bacilli; PPD, purified protein derivative; interp, interpretation; S/P, status post.

TABLE 6 Clinical histories, PPD, and TB IgG antibody results of Quantiferon-positive patients with physician-suspected TB[a]

Patient no.	Age (yr)	Country of origin	PPD	BCG vaccine	Chest X-ray	TB IgG result	TB IgG interp	History
1	70	China	POS	Yes	POS	2.111	POS	Chronic cough; chest X-ray suspicious for TB
2	16	Somalia	POS	No	POS	1.065	POS	Pulmonary nodules on chest X-ray; no sputum production
3	55	U.S.	ND	Yes	POS	1.000	POS	Bronchiectasis on chest X-ray; possible exposure to active TB
4	78	U.S.	ND	No	UNK	0·.80	POS	Rapid 15-lb wt loss; exposure to active TB years ago
5	51	India	POS	No	UNK	0.692	POS	Serpiginous chorioretinitis suspicious for ocular TB
6	45	U.S.	ND	No	POS	0.940	POS	Empyema and necrotizing pneumonia on chest X-ray
7	40	Mexico	POS	UNK	POS	0.554	POS	Treated for TB meningoencephalitis in past; new onset hematuria
8	70	Pakistan	UNK	UNK	POS	0.390	NEG	Chronic cough; chest X-ray suspicious for TB
9	64	Mexico	POS	UNK	NEG	0.273	NEG	Suspicious for ocular TB
10	82	U.S.	ND	No	NEG	0.141	NEG	Suspected tuberculous peritonitis; granulomas on biopsy; 25-lb wt loss;
11	50	U.S.	ND	No	UNK	0.138	NEG	Question of disseminated TB; numerous cutaneous lesions; necrotizing granulomas; AFB negative
12	61	U.S.	ND	No	UNK	0.103	NEG	Treated for TB as a child; suspicious for ocular TB
13	92	U.S.	NEG	No	POS	0.099	NEG	Rapid 10-lb wt loss with fatigue; chest CT shows lesions consistent with TB
14	86	U.S.	POS	No	POS	0.085	NEG	Thoracentesis showed lymphocytic exudate consistent with active TB; end-stage renal disease
15	24	Mexico	ND	Yes	POS	0.078	NEG	Pulmonary symptoms, cavitary lesions on chest X-ray; negative smear for AFB
16	22	Ethiopia	POS	UNK	POS	0.063	NEG	9-Week history of nonproductive cough; no sputum production

[a] TB, tuberculosis; ADD, amplified direct detection; POS, positive; NEG, negative; ND, not done; UNK, unknown; AFB, acid-fast bacilli; PPD, purified protein derivative; interp, interpretation; CT, computed tomography.

be highly specific markers of active *M. tuberculosis* disease; however, individually they lack sufficient sensitivity (12, 19). The 38-kDa protein has been well characterized as an immunodominant protein present in *M. tuberculosis* culture filtrates, and although anti-38-kDa protein antibodies offer good specificity, they suffer from low sensitivity when utilized alone (4, 9, 19–21). Individually, these antibodies may be highly specific; however, used alone they lack sensitivity due to the heterogeneous antibody response to *M. tuberculosis* (4, 15). Therefore, InBios developed their assay with a combination of antigens in an attempt to maximize sensitivity and specificity.

Recently, the WHO published a policy statement regarding commercial serodiagnostic tests for diagnosis of tuberculosis. Based on a bivariate meta-analysis of commercially available tests, including 67 studies, the authors of the WHO statement concluded that *M. tuberculosis* antibody tests should not be used for the diagnosis of pulmonary and extrapulmonary *M. tuberculosis* infections (24). In their summary statement, they stated specifically that the Anda-TB IgG (the most commonly evaluated test in their study) had a pooled sensitivity of 76% in smear-positive patients and 59% in smear-negative patients. Only a brief analysis of our previously published smaller pilot study on *M. tuberculosis* IgG antibody testing of three commercial *M. tuberculosis* antibody ELISAs was included in the WHO analysis (2).

The present study has identified some additional limitations of the InBios TB-IgG assay in terms of sensitivity and specificity. Out of 13 individuals with confirmed active disease, 5 were negative by the InBios TB-IgG assay. Three of these five false negatives were in patients 90 years or older. The lack of *M. tuberculosis* antibodies in these individuals may be due to decreased levels of immunoglobins that can be observed in immunosenescence. Several changes in the humoral immune response have been documented in aging individuals, including a decreasing responsiveness to vaccinations and a loss of previously established protective immunity (10, 17, 22, 23). This issue with sensitivity of the assay could be considered a general limitation of all immunoassays that measure antibodies to antigens and not necessarily unique to the InBios TB IgG assay.

TABLE 7 Mean QuantiFERON-Gold In-Tube results

Category	Mean result (IU/ml)	*P* value
Screen	4.27	
Medium	4.24	0.61
High	4.59	0.75
Confirmed	5.28	0.37
Autoimmune screen	4.30	0.96

One of the other two false-negative patients was undergoing immunosuppression therapy for a renal transplant, which could potentially cause a false-negative result on an antibody detection-based assay due to a decrease in IgG levels (5, 8, 18). The final patient had no history that would explain a negative antibody result. Unfortunately, in our present study, no patients with suspected or active *M. tuberculosis* infection were known to be coinfected with HIV. However, as demonstrated in our previous study, HIV patients could potentially be negative by the InBios TB IgG assay due to their immunodeficiency. We conclude that if patients are immunosuppressed, immunodeficient, or at risk for immunosenescence due to advanced age, *M. tuberculosis* antibody tests should not be depended upon for screening for active *M. tuberculosis* disease.

Only 1.2% of QFT-GIT-negative patients and 3.2% of known QFT-GIT-positive low-risk patients were positive with the InBios TB IgG assay, indicating a specificity of greater than 96.8%. However, it should be noted that 12.9% of patients in our study with autoimmune disease were positive with the InBios TB IgG assay. Autoantibodies associated with autoimmune and chronic diseases, especially anti-DNA antibodies and rheumatoid factors, often exhibit polyspecific properties which can cause false-positive results in many ELISAs (14). These autoantibodies are a likely cause of the false positives in these autoimmune patients.

Additional specificity issues with the InBios TB IgG assay were identified concerning cross-reactivity with other mycobacteria. In

the present study, two patients that were positive with both the InBios TB IgG and the QFT-GIT assay, were culture positive with *Mycobacterium fortuitum* and *Mycobacterium gordonae*. The QFT-GIT assay is known to cross-react only with three nontuberculous mycobacteria including *Mycobacterium kansasii*, *Mycobacterium szulgae*, and *Mycobacterium marinum*. Cross-reactions with *M. fortuitum* and *M. gordonae* have not been previously reported with the QFT-GIT assay. Cross-reactions with nontuberculous mycobacteria in the InBios TB IgG assay have not been previously investigated, except with the *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) (2). Since infections with nontuberculous mycobacteria can follow *M. tuberculosis* infections, it is possible that the InBios TB IgG assay was detecting antibodies to a previous or concurrent *M. tuberculosis* infection. However, there was no information regarding previous *M. tuberculosis* infection in these two patients. Further studies of the InBios TB IgG assay will need to be conducted to examine the potential for cross-reactivity.

The InBios TB IgG assay does not appear to cross-react with *Mycobacterium bovis* bacillus Calmette-Guérin (BCG). This is in contrast to the Anda-TB IgG assay, which we found to be highly cross-reactive with BCG in our previous study (2). In the present study, BCG vaccination was never significantly associated with a positive InBios TB IgG result. In our previous study, we found that only 1 of 25 (4%) serum samples from BCG-vaccinated individuals were positive in the InBios TB IgG assay, indicating that the assay did not significantly cross-react with BCG (2). In that same study, the Anda-TB IgG assay detected antibodies in 14 out of the 25 (56.0%) serum samples, indicating a high degree of cross-reaction in BCG-vaccinated individuals.

Some of the overall limitations of the study include the relatively small number of *M. tuberculosis* culture confirmed/ADD cases despite the inclusion of over 2,000 patients in the study. However, the inclusion of a high number of patients at low risk for active *M. tuberculosis* disease makes the analysis of the specificity of the assay very reliable. Our study also had the potential limitation of the possible introduction of bias in the method of medical history collection via phone interviews. Finally, an additional limitation was that there were no children under the age of 16 that had active *M. tuberculosis* disease in the study, which limits any conclusions that can be made about the pediatric population with regard to this assay.

In conclusion, the InBios TB IgG antibody assay could be added to the current established methods for diagnosing *M. tuberculosis* infection with the caveat that false negatives can occur in immunosuppressed patients or elderly patients. Additionally, patients with autoimmune disorders are at risk of having a false-positive result from interference of the assay by autoantibodies.

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