Comparing Interferon-γ Release Assay with Tuberculin Skin Test Readings at 48–72 Hours and 144–168 Hours with Use of 2 Commercial Reagents

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Background. Despite widespread use, the tuberculin skin test (TST) has many limitations, including a requirement for a second visit between 48 and 72 hours. The goal of this study was to determine the reliability of a TST reading between 144 and 168 hours.

Methods. Tuberculin antigen was applied into both forearms (Aplisol in one arm and Tubersol in the other, from single lots of each product) by the Mantoux method. Blood samples were obtained for interferon-γ release assay. Subjects were seen at 48–72 hours for the initial (day 2) TST reading and returned at 144–168 hours for a second (day 7) reading.

Results. A total of 116 subjects at increased risk for tuberculosis were studied; 25 (22%) had positive results at day 2 with Tubersol and 27 (23%) had positive results at day 2 with Aplisol. Overall agreement between Tubersol and Aplisol at day 2 was 93% (κ = 0.80) and at day 7 was 94% (κ = 0.76). Overall agreement between day 2 and day 7 was 89% for Tubersol and 86% for Aplisol. Discordant results between day 2 and day 7 occurred mostly in persons with a history of bacille Calmette-Guérin vaccination.

Conclusions. Subjects who fail to present at 48–72 hours for TST reading may still have a reliable TST reading at up to 168 hours. Aplisol and Tubersol reagents produce comparable results when compared with the interferon-γ release assay.

The diagnosis of latent Mycobacterium tuberculosis infection serves as the basis of preventative therapy. Identifying persons with latent M. tuberculosis infection is crucial to the goal of tuberculosis elimination, because the development of active tuberculosis in this population can be prevented with treatment. The tuberculin skin test (TST) has served as a mainstay in the screening of patients at risk for tuberculosis [1–3]. It is an intradermal injection of a PPD from broth culture of M. tuberculosis. Two companies manufacture tuberculin in the United States: Parkdale Pharmaceuticals (Aplisol) and Pasteur Mérieux Connaught (Tubersol).

Despite its widespread use, the TST has many limitations. Difficulty in application and lack of reproducible results can be drawbacks [4–9]. Interobserver variability among health care professionals also poses a significant issue in the accurate interpretation of TST results [1, 10, 11]. Interpretation bias and digit preference can alter the TST result [2, 12]. A source of false-positive TST results is contact with mycobacteria that share common antigens with M. tuberculosis [13, 14]. Given the degree of cross-reactivity of antigen from different mycobacterial species, the bacille Calmette-Guérin (BCG) vaccination may adversely affect the specificity of the TST [1, 10, 11, 15–18]. Finally, the need to assess the tuberculin response at 48–72 h [2, 3, 19] can be troublesome, especially in an indigent population [20]. Because of these shortcomings, numerous assays have been developed to quantify the response to tuberculous antigens [12, 15, 21–29].

QuantiFERON-TB (Cellestis) is a test to aid the detection of persons infected with M. tuberculosis. The test involves overnight incubation of whole, heparin-
ized blood with stimulation antigens (negative and positive controls, *M. tuberculosis* PPD and *Mycobacterium avium* PPD) followed by an interferon-γ release assay (IGRA) in the plasma supernatant by EIA. In numerous studies, IGRA has demonstrated sensitivity and specificity comparable to that of TST in its ability to detect latent *M. tuberculosis* infection [12, 21–24]. One advantage of the IGRA is that it eliminates the need for a return visit for test reading. Another advantage is that the IGRA is less affected by BCG vaccination and has the ability to discriminate responses caused by nontuberculous mycobacteria [12, 15, 24].

Because the TST remains a cost-effective method to identify persons with latent *M. tuberculosis* infection, its total elimination would not be feasible. Currently, 3 situations regarding a delayed TST reading (beyond 72 h) remain problematic. First, there are persons who do not return within the 48- to 72-h time frame but return later and are found to have nonreactive results; these persons are usually required to have a repeat TST. Some data suggest that a nonreactive TST read up to 7 days after placement is likely to represent a true-negative result [30, 31]. Second, there are persons who do not return for a reading until after 72 h and have a reactive TST; these individuals are generally accepted as having true-positive results without repeating the TST [1–3]. Third, a few persons with nonreactive results at 48–72 h return with significant induration between 72 and 168 h. There are few data regarding the significance of such an occurrence, although the recommendation is to consider these persons as having positive reactions [32].

Our aims were, first, to determine the reliability of a TST read after 72 h. Also, we sought to determine the reliability of Aplisol versus Tubersol, compared with IGRA at 2 different time frames. Finally, we wanted to identify any subject-related variables that might contribute to test discordance.

**METHODS**

This study was conducted at the Dayton Veteran’s Affairs Medical Center in affiliation with the Wright State University School of Medicine (Dayton, OH). Approval for the study was obtained from the institutional review board at the Wright State University. We enrolled 117 subjects (50 male and 67 female). We recruited subjects who were considered to be at increased risk for latent *M. tuberculosis* infection (i.e., health care workers, laboratory personnel, patient care technicians, those born in a country with a high prevalence of tuberculosis, and other ancillary hospital personnel with patient contact). Participants were excluded if they were positive for HIV antibody, were pregnant, had a malignancy, had a history of previous severe reaction to a TST (i.e., blistering, scarring, or anaphylaxis), or were receiving immunosuppressive medications.

After providing written informed consent, study subjects completed a detailed questionnaire about tuberculosis risk factors, including recent exposure to tuberculosis, prior TST results, previous BCG vaccination, and exclusion criteria. Subject age, place of birth, and occupation were also recorded.

Before administration of the TST, consenting subjects underwent venipuncture for the IGRA. Only single lots of the Aplisol and Tubersol were used. The injection site for each of the 2 tuberculin reagents—the right or left forearm—was randomized by a coin flip. The investigator interpreting the results was blinded to the identity of the reagent used for each respective forearm. Transverse induration at each TST site was measured 48–72 h (day 2) after injection by use of the risk stratified interpretation of induration, as recommended by the American Thoracic Society/Centers for Disease Control guidelines [1]. Subjects were instructed to return at 144–168 h (day 7) after injection for a second reading with use of the same guidelines. A positive TST reaction was defined as induration of ≥10 mm in diameter. For our population with tuberculosis risk factors, a conditional positive result of IGRA was considered a positive reaction.

IGRA was done and interpreted according to the manufacturer’s instructions [22, 23]. On the basis of prior studies, a positive test for *M. tuberculosis* infection is defined by a percentage human response (human PPD response divided by mitogen response) of >15% and a percentage avian difference (human PPD response minus avian PPD response, divided by human PPD response) of −10% or more [21, 23]. In addition, *M. avium* reactivity was defined as a percentage avian response (avian PPD response divided by mitogen response) of ≥20% and a percentage avian difference of −10% or less. Calculations and interpretations were done with a QuantiFERON-TB calculator (version 1.51; Cellestis).

The strength of agreement between the TST and IGRA results was evaluated by use of κ coefficients. A κ value of .75 represents excellent agreement beyond chance, a κ value between .40 and .75 represents fair to good agreement, and a κ value of <.40 represents poor agreement beyond chance [33]. McNemar’s test was used to determine whether the strength of agreement differed between the 2 time points. χ² test of independence and Fisher’s exact test were used for contingency table analysis.

**RESULTS**

One of the 117 subjects was excluded from the analyses because she did not appear at a follow-up appointment for the TST readings. Another subject had the second reading done on day 4 after injection (rather than day 7) but was included in the study group. The remaining 116 participants consisted of persons ≥18 years of age (median age, 38 years; range, 23–69 years) and included 50 men and 66 women. There were 35 persons born in countries with high prevalence of tuberculosis (≥10 cases per 100,000 population) [2, 34] and 28 subjects who...
Table 1. Comparison of results of Tubersol antigen tuberculin skin test at 48–72 h (day 2) and 144–168 h (day 7) after injection.

<table>
<thead>
<tr>
<th></th>
<th>Day 2</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Positive</td>
<td>(n = 25)</td>
<td>15 (60)</td>
</tr>
<tr>
<td>Negative</td>
<td>(n = 91)</td>
<td>10 (40)</td>
</tr>
</tbody>
</table>

NOTE. Data are no. (%) of subjects. Overall agreement, 89%; k coefficient (95% CI), .63 (.45–.81).

reported receiving the BCG vaccine (26 of whom were born in countries with high prevalence of tuberculosis).

Twenty-five subjects (22%) who underwent TST with Tubersol and 27 (23%) who had testing with Aplisol had a positive result at day 2. Overall agreement between Tubersol and Aplisol at day 2 was 93% (κ = .80) and at day 7 was 94% (κ = .76). Of the 8 subjects with discordant readings between Aplisol and Tubersol TST at day 2, results of IGRA agreed with Aplisol and Tubersol TST 4 times each. Comparison of day 2 and day 7 Tubersol results are shown in table 1. There was 86% agreement between day 2 and day 7 for Aplisol (κ = .56). IGRA results are shown compared with Tubersol results in tables 2 and 3. Overall agreement between IGRA and Aplisol at day 2 was 88% (κ = .67) and at day 7 was 85% (κ = .54). Thirteen subjects (11%) had discordant Tubersol readings between day 2 and day 7; of these, results of IGRA agreed with the day 2 reading for 8 subjects. Ten subjects had a positive reaction at day 2 and a negative reaction at day 7; of these, results of IGRA agreed with the day 2 reading for only 5 subjects.

**DISCUSSION**

The primary goal of this study was to determine the validity of TST results obtained at day 7 after injection. Secondarily, we wanted to determine if there were factors correlating with a different reading on day 7, compared with day 2. For convenience, many health care providers have accepted TST readings obtained >72 h after injection as valid interpretations without full knowledge of the implications. Unfortunately, the lack of a reference standard in the diagnosis of latent *M. tuberculosis* infection makes the determination of the validity of TST results obtained on day 7 more difficult. Overall agreement between IGRA and TST results obtained on day 2 was good. Mazurek et al. [12] found a similar level of agreement in their analysis of persons being screened for latent *M. tuberculosis* infection (84.7%; κ = .55). Streton et al. [21] found a slightly better overall agreement (91%). Because the IGRA has established reliability, compared with TST results obtained at day 2, it was used as a basis of comparison between day 2 and day 7 results. Although the observed κ value at the later time is slightly lower than at the earlier time, there is considerable overlap between the 95% CIs. Therefore, we conclude that a TST that is not read at day 2 may still provide a reliable TST reading at day 7. At the same time, a TST reading at day 7 must be regarded with caution. Previous studies have shown that some persons without significant induration at 48–72 h subsequently develop induration, and some individuals with significant induration at 48–72 h have no induration at 7 days after injection [31, 32]. We have shown a small decrease in induration between day 2 and day 7. There was a corresponding trend toward more subjects’ results changing from positive to negative than from negative to positive. Even though more subjects showed a decrease in induration from day 2 to day 7, we found a small number of subjects with a change from a negative TST result at day 2 to a positive TST result at day 7. Results of IGRA were negative in each of these instances, suggesting that these are false-positive TST results at day 7.

Although there were not enough subjects with discordant readings between day 2 and day 7 to allow multivariate analysis, persons with a history of BCG vaccination (or birth in a country with high prevalence of tuberculosis) had a high discordance between day 2 and day 7 readings (table 4). Previous studies have shown that BCG vaccination [16, 35] or exposure to other mycobacteria [14] may affect TST results. Mazurek et al. [12] suggested that reactivity to nontuberculous mycobacteria may be the cause of a positive TST result in one-fifth of non—BCG-vaccinated subjects. Johnson et al. [15] have shown that BCG vaccination decreases the specificity of TST and IGRA based on PPD (QuantiFERON-TB), but that IGRA based on 2 *M. tuberculosis*-specific antigens, ESAT-6 and MPT-64, was not affected by BCG vaccination. This more specific whole-blood test is now commercially available under the trademark QuantiFERON-TB Gold [24], but it was not available to us when we were planning the current study.

Some previous studies have shown discordant results when comparing Aplisol with Tubersol [36–38]. In particular, Grabau et al. [36] found 40% discordance when those who had positive test results with Aplisol were retested with Tubersol. This dis-

Table 2. Comparison of interferon-γ release assay (IGRA) and Tubersol antigen tuberculin skin test (TST) readings at 48–72 h (day 2) and 144–168 h (day 7) after injection.

<table>
<thead>
<tr>
<th>IGRA result</th>
<th>TST result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 2</td>
</tr>
<tr>
<td>Positive</td>
<td>18</td>
</tr>
<tr>
<td>Negative</td>
<td>7</td>
</tr>
</tbody>
</table>

NOTE. Data are no. of subjects. For day 2, overall agreement is 88%; κ coefficient (95% CI) is .66 (.49–.83). For day 7, overall agreement is 85%; κ coefficient (95% CI) is .54 (.34–.73).
complex.
tuberculin skin test.

Table 3. Comparison of results of Tubersol antigen tuberculin skin test at 48–72 h (day 2) and 144–168 h (day 7) after injection.

<table>
<thead>
<tr>
<th>Variable</th>
<th>IGRA result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive (n = 25)</td>
</tr>
<tr>
<td>Born in country with high prevalence of tuberculosis(^a)</td>
<td>14 (56)</td>
</tr>
<tr>
<td>Bacille Calmette-Guérin vaccination(^b)</td>
<td>10 (40)</td>
</tr>
<tr>
<td>Recent exposure to tuberculous</td>
<td>4 (16)</td>
</tr>
<tr>
<td>Reactivity to Mycobacterium avium complex</td>
<td>0(^c)</td>
</tr>
</tbody>
</table>

Reaction to Tubersol antigen TST at time point, mean mm
- 48–72 h: 14.0 | 1.5
- 144–168 h: 8.3 | 1.0

**NOTE.** Data are no. (%) of subjects, unless otherwise indicated. TST, tuberculin skin test.

\(^a\) \(P < 0.001; \chi^2\) test of independence (Fisher’s exact test for sparse tables).
\(^b\) \(P = 0.024; \chi^2\) test of independence (Fisher’s exact test for sparse tables).
\(^c\) IGRA result is negative by definition for those reacting to *M. avium* complex.

Discrepancies, whether obtained at day 2 or day 7. The IGRA is a newer test and has the potential to resolve this uncertainty.

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