Development and Evaluation of a Simple Latex Agglutination Test for Diagnosis of Tuberculosis

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A simple latex agglutination test (SLAT) based on modifications of existing serodiagnostic techniques, in which commercially available reagents are used, was developed for detection of antibodies against *Mycobacterium tuberculosis*. Tests performed on 553 serum samples from 316 individuals, including 117 bacteriologically confirmed active tuberculosis patients, showed 80% positive titers. Sera from 12 patients with arrested tuberculosis showed 91% positive titers. Nonspecific reactions were noted in 5% of 160 sera from selected normal individuals and patients with diseases other than tuberculosis. The antibodies detected by the SLAT method were found to be relatively stable when exposed to low temperatures, whereas high temperatures reduced the antibody titer considerably. Disodium ethylenediaminetetraacetic acid inactivation of serum complement was found to be satisfactory. No variation of tuberculosis antibody titer was noted in tests on multiple specimens from patients whose conditions were stabilized. However, considerable fluctuation was encountered in antibody titers obtained on recently detected individuals. Data obtained in this study indicate that the modified procedures of the SLAT method could replace the tuberculin skin test for simple screening of tuberculosis in adults.

The use of tuberculin skin test procedures has been the basis for the detection of new cases of tuberculosis in mass testing programs in recent years. The Heaf test has been used as the initial screening procedure because of its simplicity. Positive reactors to this test are usually rechecked by the less sensitive Mantoux intradermal tuberculin test. Individuals who have positive reactions with this test are followed up with laboratory studies and X rays. Skin testing programs have been reasonably successful in detecting new cases of tuberculosis in children. Mass skin testing programs in adults have not produced the desired results because many adults have positive reactions to these tests as a result of acquired subclinical infections.

Recognition of the shortcomings of the tuberculin skin test resulted in the development of serodiagnostic methods for the detection of tuberculosis antibodies. Early tests were based on the standard complement-fixation technique. Emphasis later shifted to simpler agglutination procedures such as the hemagglutination test and the bentonite and kaolin flocculation tests. Various fractions and metabolic products of the tubercle bacillus were used to prepare the antigens for these tests. The most successful serodiagnostic procedures for the detection of tuberculosis antibodies have been the gel double-diffusion test devised by Parlett and Youmans (6) and the latex agglutination test of Duboczy and White (3). Although they produce satisfactory results, these tests are complex and require specially prepared reagents. The purpose of this study was to develop a method for the detection of tuberculosis antibodies which would use existing serodiagnostic procedures and make use of commercially available reagents prepared for other tests. Emphasis was placed on simplicity of technique, stability of reagents, and reproducibility of results. The simple latex agglutination

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1 Presented in part at the 71st Annual Meeting of the American Society for Microbiology, Minneapolis, Minn., May 1971.
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test (SLAT) developed is evaluated herein.

**MATERIALS AND METHODS**

The stock tuberculin reagent was obtained from Difco Laboratories and was prepared from a filter-sterilized, dialyzed flash culture of *Mycobacterium tuberculosis* strain H37Ra which had been grown in a modified Beck-Proskauer broth according to the method of Parlett and Youmans (6). This reagent was identified by the manufacturer as Bacto-H37Ra antigen and was originally intended for use in a capillary gel double-diffusion test for the detection of tuberculosis antibodies.

Polystyrene latex particles were obtained from Difco in a stock 3% aqueous suspension. The particle diameter was standardized by the manufacturer at 0.81 μm. This reagent was originally intended for use in the Bacto-RA test for the rheumatoid factor associated with rheumatoid arthritis (Singer et al. [8]).

To determine the optimal concentration of the antigen needed to sensitize the polystyrene latex particles, it was necessary to perform a series of cross-titration studies with positive serum and a standardized concentration of latex particles sensitized with various dilutions of the stock tuberculin reagent. Serial dilutions of the antigen were prepared with physiological saline as the diluent. The initial dilution was 1:10, with a maximal dilution of 1:320. Final volume of each dilution was 10 ml. A 0.1-ml amount of the stock latex reagent was added to each tube of the diluted antigen. A latex control was also prepared by adding 0.1 ml of the stock latex reagent to 10 ml of saline. The antigen-latex suspensions and the latex control suspension were then incubated in a water bath at 37°C for 2 hr.

The antigen titration procedure was performed in triplicate with new, scratch-free serological test tubes (10 by 75 mm). Seven dilutions of eight tubes each were set up. To assure that the seven dilutions would be identical, the serum dilutions were prepared in 5-ml amounts and distributed to appropriately marked tubes for each titration. An initial dilution of 1:2 was used. The diluent was 0.0015 M disodium ethylenediaminetetraacetic acid (EDTA) in physiological saline. Each tube of the first six dilutions received 0.2 ml of the appropriate antigen-latex suspension. The tubes in the seventh series served as latex controls for the detection of nonspecific agglutination and received 0.2 ml of saline-latex suspension.

The optimal incubation conditions were determined by incubating each of the triplicate sets of dilutions under three different conditions: (i) incubated in a water bath overnight at 37°C (approximately 15 hr); (ii) incubated at room temperature (22 to 25°C) for the same length of time; and (iii) allowed to stand at room temperature for 4 hr, and then refrigerated at 4 to 6°C overnight and allowed to warm to room temperature the following morning. Tubes of all three sets of dilutions were centrifuged for 5 min at 2,000 rev/min in a size 1 model SBV International centrifuge and examined macroscopically for evidence of agglutination. The degree of agglutination was graded according to the following criteria: four plus reactions, indicating maximal agglutination, were characterized by the formation of a large, solid mass of latex particles in the bottom of the tube and a clear supernatant fluid; three plus reactions consisted of large aggregates of latex particles and a clear supernatant fluid; two plus reactions were characterized by fine granulations that were easily distinguishable from the control; and plus-minus reactions displayed questionable reactivities difficult to differentiate from the negative control. The titer was determined to be the reciprocal of the highest dilution showing a minimum of a one plus reaction.

Several diluents were evaluated with variable results. Phosphate and borate buffers at pH 8.2, as recommended by Singer and Plotz (7) for use in the RA test, appeared to inhibit agglutination. A glycine buffer (4, 5) was also tested and gave negative results. Physiological saline, as suggested by Carlisle and Saslaw (1) for a diluent in the latex agglutination test for histoplasmosis, was found to work well at pH 6.2. The serum diluent was prepared in 0.0015 M disodium EDTA in physiological saline at pH 6.2.

The individuals furnishing the sera for this study were grouped according to their clinical diagnosis: group 1 included patients with minimal tuberculosis; group 2, patients with moderately advanced pulmonary tuberculosis; group 3, patients with far advanced pulmonary tuberculosis; group 4, patients with mycobacterioses due to organisms other than *M. tuberculosis*; group 5, patients with extrapulmonary tuberculosis; group 6, patients with pulmonary tuberculosis, classification and activity undetermined; group 7, patients with inactive or arrested tuberculosis; group 8, patients admitted to the hospital for the purpose of ruling out tuberculosis; group 9, patients with nontuberculous diseases which might result in abnormal globulin fractions and individuals with high antibody titers resulting from nontuberculous diseases or immunization; and group 10, selected normal controls.

Blood specimens from individuals in test groups 1 through 8 were collected in the laboratory at the Harlingen State Tuberculosis Hospital, Harlingen, Tex. These specimens included patients confined to the hospital as well as those receiving treatment or evaluation in the out-patient and follow-up clinics. Bacteriological data were obtained from the hospital laboratory records. Information pertaining to complement-fixation and latex agglutination tests for deep systemic mycoses was obtained from the same source. Specimens from patients in group 9 were collected at the laboratory of a local hospital in San Benito, Tex., and at the venereal disease clinics held by the Cameron County Health Department in San Benito. Specimens from individuals in group 10 were also collected at the Cameron County Health Department. The collected serum was stored at 4 to 6°C prior to testing, with the exception of those specimens which were to be used to determine the effects of freezing on the antibodies detected by the SLAT method. All specimens were centrifuged at 3,000
rev/min before analysis to remove artifacts which might interfere with the test. The control serum was prepared by pooling sera from patients known to have active tuberculosis as confirmed by positive culture. Sera from patients with positive syphilis, or fungus serology, and those with cultures positive for acid-fast organisms other than \textit{M. tuberculosis} were excluded from the pool.

The test procedure was based on findings obtained in the preliminary titrations for the determination of optimal conditions for the SLAT method. The amount of latex antigen needed to test each bath of specimens was determined and prepared by adding the required amount of Bacto-H37Ra antigen to a known quantity of physiological saline. To simplify this procedure, a tuberculin syringe calibrated in 0.01-ml units was used to transfer the tuberculin reagent from the stock vial to the saline. For each 10 ml of the diluted antigen, 0.1 ml of the stock latex reagent was added. A latex control was prepared by adding 0.1 ml of the stock latex reagent to 10 ml of saline. The latex antigen and the latex control were then incubated in a water bath at 37 C for 2 hr.

Six serological tests tubes were used for each specimen tested. A 0.6-ml amount of the saline-disodium EDTA diluent was pipetted into the first tube of each series. Tubes 2 through 5 received 0.2 ml of the diluent, and tube 6 received none. A 0.2-ml amount of the serum to be tested was pipetted into tube 1 and mixed thoroughly; 0.2-ml samples were transferred to tubes 2 and 6. An additional 0.2 ml was discarded to reduce the volume of diluted serum in tube 1 to 0.2 ml. The contents of tube 2 were mixed, and 0.2 ml was transferred to tube 3. This procedure was repeated through tube 5. To reduce the volume in tube 5 to 0.2 ml, 0.2 ml was discarded. The latex antigen was added to tubes 1 through 5 in 0.2-ml amounts. Tube 6 served as the latex control for the detection of nonspecific agglutination and received 0.2 ml of the latex control suspension. All tubes were thoroughly mixed, allowed to stand at room temperature for 4 hr, and then refrigerated overnight. The tubes were then removed from the refrigerator, allowed to warm to room temperature, centrifuged for 5 min at 2,000 rev/min, and examined macroscopically for agglutination. Titrations which showed evidence of agglutination in the control tube were repeated with the saline-latex suspension in place of the latex antigen to determine the titer of the nonspecific agglutinins responsible for the agglutination in the control tube.

To determine the stability of the prepared latex antigen, a 100-ml quantity was prepared and stored at 4 to 6 C for 138 days. The antigen was checked at various intervals by titrating it against the positive control serum and comparing the results with those obtained with freshly prepared antigen. To determine the stability of antibodies detected by this procedure, samples of the control serum were stored at 4 to 6 C and at –20 C. These sera were tested periodically, and the results were compared with those obtained before storage. An additional group of 18 high-titer sera from tuberculosis patients were tested after storage at –20 C for various periods. The results obtained in these tests after storage were compared with those obtained prior to storage to determine whether there were any significant changes.

### RESULTS

The optimal dilution of Bacto-H37Ra antigen lot 526761 needed to sensitize the polystyrene latex particles was determined to be 1:160, as shown in Table 1. This dilution was found to provide the greatest degree of reactivity regardless of the incubation temperature. It was noted that a prozonelike phenomenon occurred in the 1:4 dilutions of the titrations incubated both at room temperature and at 37 C. This phenomenon was not encountered in the titrations incubated for 4 hr at room temperature and then overnight at 4 to 6 C. It was encountered in the first set of titrations carried out on test sera, and for this reason the 1:4 dilution was discontinued.

The results of titrations carried out on control serum with latex antigen which had been stored up to 138 days indicated that the prepared antigen was relatively stable (Table 2). No signs of deterioration were noted.

The data in Table 3 show that the latex antigen could be prepared repeatedly over an extended period with little variation between lots. Each new quantity of antigen was tested against the positive control serum to compare its reactivity with previously prepared lots. Slight variations noted in the higher dilutions could be attributed to variations in pipetting technique.

Repeated freezing and thawing of the control serum did not appear to affect the results of tests performed over a period of weeks. Variations in the results given in Table 4 should be considered within acceptable limits.

The tests performed on frozen specimens indicated that the antibodies detected by the SLAT method were not destroyed by freezing and thawing during the period tested. Tests performed on sera prior to freezing and those performed after freezing showed only slight changes in titer (Table 5).

Results obtained on sera from patients with infections due to acid-fast organisms other than the tubercle bacillus indicated that the SLAT method, like earlier serological tests, cannot differentiate between tuberculosis and other mycobacterial diseases. The significance of the results obtained on several patients (Table 6) is open to question. The patient with a positive culture for \textit{M. fortuitum} had negative cultures for tuberculosis and other acid-fast bacilli for several years. It is highly prob-
TABLE 1. Optimal dilution of Bacto-H37Ra antigen and optimal incubation temperature for latex antigen titration

| Incubation                  | Dilution of H37Ra antigen | Titer reactions (+, -) |
|-----------------------------|----------------------------|------------------------|
|                             | 1:4 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 | 1:256 | 1:512 |
| Overnight at 37°C           |     | 1:10| 1:20 | 1:40 | 1:80 | 1:160 | 1:320 |     |
| Control                     |     |     |     |     |     |       |       |     |
| Room temperature overnight  |     | 1:10| 1:20 | 1:40 | 1:80 | 1:160 | 1:320 |     |
| Control                     |     |     |     |     |     |       |       |     |
| Room temperature for 4 hr   |     | 1:10| 1:20 | 1:40 | 1:80 | 1:160 | 1:320 |     |
| overnight at 4 to 6°C       |     |     |     |     |     |       |       |     |
| Control                     |     |     |     |     |     |       |       |     |

TABLE 2. Stability of the latex antigen stored at 4 to 6°C for 138 days

| Antigen age (days) | Titer reactions (+, -) |
|-------------------|------------------------|
|                   | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 | 1:256 | Control |
| 0                 |     |     |     |     |     |       |         |
| 3                 |     |     |     |     |     |       |         |
| 15                |     |     |     |     |     |       |         |
| 34                |     |     |     |     |     |       |         |
| 38                |     |     |     |     |     |       |         |
| 58                |     |     |     |     |     |       |         |
| 138               |     |     |     |     |     |       |         |

TABLE 3. Determination of reactivity of latex antigen prepared weekly for 10 weeks

| Test no. | Titer reactions (+, -) |
|----------|------------------------|
|          | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 | 1:256 | Control |
| 1        |     |     |     |     |     |       |         |
| 2        |     |     |     |     |     |       |         |
| 3        |     |     |     |     |     |       |         |
| 4        |     |     |     |     |     |       |         |
| 5        |     |     |     |     |     |       |         |

TABLE 4. Latex agglutination tests on frozen and unfrozen control serum tested weekly for 5 weeks

| Test no. | Titer reactions (+, -) |
|----------|------------------------|
|          | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 | 1:256 | Control |
| Frozen   |     |     |     |     |     |       |         |
| Unfrozen |     |     |     |     |     |       |         |

* Stored at -20°C.
* Stored at 4 to 6°C.

able that M. fortuitum was a contaminant, since the culture showed only one colony. No emphasis can be placed on the results obtained on patients with cultures which were positive for M. aquae and M. vaccae, since these patients also had cultures which were positive for M. tuberculosis. Most physicians treating tuberculous place little importance on cultures yielding saprophytic acid-fast bacilli.
latex agglutination tests obtained on individuals with positive serological tests for the systemic fungi were due to heat-labile antibodies, rather than heat-stable antibodies such as those detected by the complement-fixation procedures. Although four of the sera tested by the SLAT method for tuberculosis antibodies did not become totally negative after being subjected to heat inactivation, titers were considerably reduced. Three of the four sera that

**Table 5. Effect of storage at -20 C on the titer of selected high-titer serum specimens**

| Patient no. | Initial titer | Titer after freezing | Length of storage (days)* |
|-------------|---------------|----------------------|--------------------------|
| 4505        | 1:32          | 1:32                 | 19                       |
| 80120P      | 1:32          | 1:16                 | 48                       |
| 32448       | 1:64          | 1:64                 | 46                       |
| 32645R      | 1:32          | 1:32                 | 48                       |
| 43456R      | 1:64          | 1:32                 | 17                       |
| 42380R      | 1:32          | 1:32                 | 21                       |
| 56893R      | 1:64          | 1:32                 | 10                       |
| 62104R      | 1:64          | 1:32                 | 45                       |
| 62772R      | 1:32          | 1:32                 | 18                       |
| 70911R      | 1:32          | 1:32                 | 48                       |
| 71174       | 1:64          | 1:64                 | 7                        |
| 71231FU     | 1:32          | 1:16                 | 34                       |
| 71478       | 1:64          | 1:64                 | 7                        |
| 71637       | 1:64          | 1:128                | 17                       |
| 72125       | 1:16          | 1:32                 | 30                       |
| 72475       | 1:64          | 1:128                | 18                       |
| 72498       | 1:64          | 1:64                 | 39                       |
| 72536       | 1:128         | 1:128                | 7                        |

* Determined prior to freezing.

* Stored at -20 C.

**Table 6. Latex agglutination tests on patients with mycobacterioses other than tuberculosis**

| Patient no. | Organism isolated | Disease classification and activity | Latex agglutination titer |
|-------------|-------------------|------------------------------------|--------------------------|
|             | M. kansasii       | Moderately advanced active          | 1:32                     |
|             | M. kansasii       | Far advanced active                 | 1:64                     |
|             | M. kansasii       | Far advanced quiescent active       | 1:32                     |
|             | M. kansasii       | Moderately advanced active          | 1:16                     |
|             | M. kansasii       | Moderately advanced quiescent       | +                        |
|             | M. phlei          | Far advanced active                 | 1:32                     |
|             | M. phlei          | Far advanced quiescent active       | ±                        |
|             | M. avium*         | Far advanced active                 | 1:32                     |
|             | M. avium*         | Far advanced active                 | +                        |
|             | M. vaccae*        | Far advanced active                 | 1:64                     |
|             | M. aqae*          | Far advanced active                 | 1:64                     |
|             | M. aqae*          | Activity undetermined               | 1:32                     |
|             | M. aqae*          | Activity undetermined               | 1:32                     |

* Runyon group III or Battey-Avium complex.

* Culture also positive for M. tuberculosis.

**Table 7. Latex agglutination tests on tuberculosis patients with positive fungus serology**

| Patient no. | Fungus culture | Fungus serology* | Latex agglutination test* |
|-------------|----------------|------------------|--------------------------|
|             | HM | HY | BL | CO | HL | CM | Unheated | Heated at 56 C |
| 70658       | +' | 1:8 | -  | -  | 1:64 | -  | +        | 1:16 | -  |
| 69107FU     | -  | -  | 1:8 | -  | -  | -  | -        | 1:64 | 1:8 |
| 69838R      | -  | -  | -  | 1:8 | -  | -  | -        | 1:32 | -  |
| 7163T       | -  | 1:8 | 1:32 | 1:8 | -  | -  | -        | 1:128 | 1:32 |
| 72475       | -  | -  | -  | 1:8 | -  | -  | -        | 1:32 | -  |
| 72788       | -  | 1:8 | -  | -  | -  | -  | -        | 1:32 | -  |
| 67938R      | -  | 1:8 | -  | -  | -  | -  | -        | 1:8  | -  |
| 51612R      | -  | -  | -  | -  | 1:16 | -  | -        | 1:32 | -  |
| 32645R      | -  | -  | -  | -  | -  | -  | -        | 1:16 | -  |
| 69948       | -  | 1:8 | -  | -  | -  | -  | -        | 1:32 | -  |
| 72416       | -  | -  | -  | -  | 1:16 | -  | -        | 1:128 | 1:8 |
| 72948       | -  | 1:8 | -  | 1:8 | -  | -  | -        | -    | ×* |
| 28155       | -  | 1:8 | -  | -  | -  | -  | -        | -    | ×  |
| 72960       | -  | 1:8 | -  | -  | 1:8 | -  | -        | -    | ×  |
| 70078FU     | -  | -  | -  | -  | -  | +  | -        | -    | ×  |
| 72933       | -  | -  | -  | -  | -  | -  | +        | -    | ×  |

* HM = complement-fixation test for Histoplasma capsulatum, mycelial phase; HY = complement-fixation test for H. capsulatum, yeast phase; BL = complement-fixation test for Blastomyces dermatitidis; CO = complement-fixation test for Coccidioides immitis; HL = Histol-latex test; CM = Cocci-latex test.

* Test carried out on both heat-inactivated and chemically inactivated serum.

* Fungus culture positive for C. immitis.

* Test not performed.
had titers after heating originally still showed titers of 1:128. It is possible that additional inactivation at 56 C would have reduced the titer to less than 1:8. A further reason for presenting information on serological tests for the systemic fungi was to show that cross-reactions are often encountered in these tests. Skin testing procedures can have a serious influence on the results of complement-fixation tests for the systemic fungi if the blood specimens are taken after the skin test is performed.

The results in Table 8 demonstrate the need for chemical inactivation of serum complement in the SLAT method. Tests on sera which had not been inactivated in any manner produced no results. The same specimens showed some degree of reactivity when heat-inactivated. The use of chemical inactivation resulted in still higher titers.

The information presented in Table 9 shows

| Patient no. | Non-inactivated* | Heated-inactivated* | Disodium EDTA-inactivated* |
|-------------|------------------|---------------------|-----------------------------|
| 32448       | -                | 1:8                 | 1:32                        |
| 49256R      | -                | 1:16                | 1:32                        |
| 56557       | -                | 1:16                | 1:32                        |
| 62104R      | -                | 1:32                | 1:64                        |
| 64848       | -                | -                   | 1:64                        |
| 71137       | -                | -                   | 1:64                        |
| 71417       | -                | 1:8                 | 1:64                        |
| 71637       | -                | 1:8                 | 1:64                        |
| 72416       | 1:8              | 1:16                | 1:32                        |
| R.M. OP     | -                | 1:8                 | 1:32                        |
| Positive control | -        | -                   | 1:32                        |

* Serum dilutions prepared in physiological saline.
* Serum heat-inactivated at 56 C for 30 min.
* Serum dilutions prepared in 0.0015 M disodium EDTA saline.

The overall results obtained on tests performed on 316 individuals comprising the 10 test groups. The percentages of positive reactions obtained in groups 1 through 7 are based on results obtained on individuals with a clinical diagnosis of tuberculosis but whose cultures may or may not have been positive for M. tuberculosis.

The information presented in Table 10 is based on the results obtained on patients confirmed as having tuberculosis as a result of positive bacteriological studies. When calculated in this manner, the percentage of positive titers obtained shows a slight increase.

Table 11 provides information regarding the individuals in groups 9 and 10. An effort was made to include in these groups only those individuals whose conditions might contribute useful information to the study. Since other investigators obtained false-positive results on patients with liver diseases, syphilis, cancer of the lung, and certain fungus diseases, an effort was made to include individuals with these conditions in this study. No problems were encountered with false-positive reactions.

**DISCUSSION**

Data obtained from preliminary titrations indicated that the antigen prepared for use in the capillary gel double-diffusion test could be adapted to a latex agglutination procedure. The only problem encountered was finding a suitable diluent for the latex test. Efforts to adapt various buffer solutions recommended for use in other latex agglutination procedures failed to yield satisfactory results. Singer and Plotz (7) recommended that a buffer of pH 8.2 be used when working with latex particles to avoid spontaneous agglutination. Their work was with gamma globulin-coated latex particles. Since gamma globulin has an isoelectric point of 6.6, buffers near this pH range should

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**Table 8. Heat and chemical inactivation of serum complement in selected high-titer sera**

**Table 9. Summary of latex agglutination tests on all individuals tested**
Table 10. Summary of latex agglutination tests performed on individuals with bacteriologically confirmed tuberculosis

| Group no. | No. of sera | Distribution of titers | Percentage of tests |
|-----------|-------------|------------------------|---------------------|
|           |             | <1:8 | 1:8 | 1:16 | 1:32 | 1:64 | 1:128 | +   | -   |
| 1         | 2           | 1    | 1   | 0    | 0    | 0    | 0    | 50  | 50  |
| 2         | 20          | 5    | 1   | 3    | 8    | 3    | 0    | 75  | 25  |
| 3         | 43          | 4    | 1   | 3    | 18   | 14   | 3    | 89  | 11  |
| 5         | 2           | 2    | 0   | 0    | 0    | 0    | 0    | 0   | 100 |
| 6         | 50          | 12   | 1   | 6    | 20   | 11   | 1    | 76  | 24  |
| 7         | 12          | 1    | 3   | 2    | 5    | 1    | 0    | 91  | 9   |

Table 11. Simple latex agglutination tests on sera from nontuberculous individuals

| No. tested | Clinical diagnosis                  | Simple latex 1 agglutination test |
|------------|------------------------------------|----------------------------------|
|            |                                    | +   | -   |
| 18         | Syphilis (VDRL, weakly reactive to 1:128; PTA-ABS, reactive)² | 1*  | 17  |
| 1          | Pernicious anemia                   | 0   | 1   |
| 2          | Typhoid fever ("O" titers of 1: 320 and 1:640) | 0   | 2   |
| 2          | Tularemia (titers of 1:640 and 1: 1,280) | 0   | 2   |
| 2          | Brucellosis (titers of 1:1,280 and 1:2,560) | 0   | 2   |
| 1          | Lymphatic leukemia                  | 0   | 1   |
| 1          | Myelocytic leukemia                 | 0   | 1   |
| 3          | Infectious hepatitis                | 1   | 2   |
| 1          | Lymphosarcoma                       | 0   | 1   |
| 1          | Q fever (complement fixation titer, 1:16) | 0   | 1   |
| 1          | Reversed A-G ratio (cause undetermined) | 0   | 1   |
| 5          | Lung cancer                         | 0   | 5   |
| 1          | Pulmonary abscess                   | 0   | 1   |
| 1          | Pleurisy                            | 0   | 1   |
| 7          | Pulmonary disease (tuberculosis ruled out) | 0   | 7   |
| 1          | Rabies antibodies (fluorescent rabies antibody titer of 1:160)² | 0   | 1   |
| 96         | Selected normal controls            | 3   | 93  |

*VDRL = Venereal Disease Research Laboratory test; PTA-ABS = fluorescent treponemal antibody absorption test.

² Probably due to bacterial contamination.
³ Individual undergoing anti-rabies treatment.

be avoided to prevent spontaneous agglutination of the latex particles. Carlisle and Saslaw (1, 2) used physiological saline as the diluent in their latex agglutination test for histoplasmosis. The results they obtained with the latex controls indicated that no problems were encountered when saline was used in place of a buffered diluent.

In the present study, no apparent problems were encountered with sera stored in the frozen state. The results on sera which had been frozen and thawed were comparable to those obtained in tests on fresh specimens. Wallace et al. (9) encountered antibody deterioration in specimens which were frozen and then tested with the bentonite flocculation test.

Inactivation of serum complement was important in the SLAT method. Results indicated that chemical inactivation of the serum complement by use of disodium EDTA was the best method. Titers obtained when heat inactivation was used were considerably lower than those obtained on sera which were chemically inactivated. The use of sera in which the complement had not been inactivated produced no results.

The concentration of latex particles in the preparation of the latex antigen was based on the recommendations by the manufacturer for use in the Bacto-RA test. Information in the literature indicated that a difference of opinion exists between some investigators as to the importance of the concentration. Carlisle and Saslaw (1) found that they could vary the concentration as much as 50% and still obtain good results. Other investigators placed heavy emphasis on using carefully adjusted concentrations of latex, but their methods of preparation were questionable. They used spectrophotometric procedures based on scale readings of less than 20% transmission. The accuracy of readings made in this portion of the scale is generally considered to make them of little value.

The prozone-like phenomenon which was encountered in the preliminary titrations in the present study could possibly be attributed to an excess of protein in the lower dilutions. Dubocz and White (3) noted that an excess of albumin had an inhibitory effect on latex agglutination. Apparently the amount of protein in the 1:8 and higher dilutions was not sufficient to affect the results of the test.
Tests with latex antigen stored at 4 to 6°C for an extended period showed that antigen activity remained constant. No important variations in results were noted in tests performed with the control serum. The results on each new quantity of latex antigen indicated that it could be readily prepared in various amounts with no significant changes in the degree of reactivity.

The results of SLAT tests performed on sera from 316 individuals comprising the 10 test groups are given in Table 9. The values presented on the patients with tuberculosis are based on clinical diagnosis. The results of these tests indicated that patients with far advanced tuberculosis (group 3) had a higher rate of positive tests (89%) than either those patients with moderately advanced pulmonary tuberculosis (group 2, 73% positive) or those with minimal tuberculosis (group 1, 67% positive).

Data on patients with cultures positive for acid-fast bacilli other than M. tuberculosis (group 4) showed that half had positive titers. Four of the six patients with pulmonary infections due to M. kansasii had positive titers. Titers on individuals with cultures which were positive for the saprophytic mycobacteria were questionable. The data obtained from this group were similar to those obtained by Parlett and Youmans (6) in their study of a comparable group.

The two patients tested who had extrapulmonary tuberculosis (group 5) had negative titers. Although the number of patients in this group did not provide sufficient data for definite conclusions, it should be noted that Parlett and Youmans (6) and Duboczy and White (3) found that the percentage of positive tests which were obtained in cases of extrapulmonary tuberculosis was quite low, being 43.9 and 50%, respectively.

The significance of 70% positive titers obtained on patients in group 6 is questionable. This group consisted of patients with a diagnosis of tuberculosis but with no information as to the extent of the disease or the degree of activity. The fact that six of the patients in this group had no record of positive cultures could also cast some doubt on the significance of the rate of positive titers in such patients.

Data on patients in group 7 indicate that, of the 19 individuals tested who had inactive or arrested tuberculosis, 74% had positive titers. In most cases, these patients had been classified as arrested or inactive for not more than 1 year. Parlett and Youmans (6) found positive titers in only 50% of the patients they tested, but some of these individuals had been inactive for over 20 years. Duboczy and White (3) demonstrated antibodies in 38.5% of the inactive patients tested.

Positive titers were found in 3 of 16 patients who were admitted to the hospital for the purpose of ruling out tuberculosis (group 8). No positive cultures were obtained on any of these patients during their confinement to the hospital. Of this group, two died while hospitalized as a result of lung cancer. Three others were found to have lung cancer, another had a pulmonary abscess due to an organism other than one of the mycobacteria, and one had pleurisy. One patient in this group was admitted to the hospital on the basis of a strongly positive reaction with the Mantoux test. He was dismissed from the hospital with a diagnosis of "no evidence of active tuberculosis." The remainder of the patients in this group were also released with the same diagnosis.

The individuals in group 9 were selected for this study because either they had diseases which could result in the formation of abnormal globulin fractions or they had high antibody titers due to nontuberculous diseases. Individuals with high antibody titers resulting from immunization for diseases other than tuberculosis were also included. Only two patients (4%) had positive titers. In one case, the titer could probably have been attributed to bacterial contamination of the serum. Insufficient serum was available for additional studies. The other serum with a positive titer was found to have nonspecific agglutinins. The SLAT method showed a titer of 1:32, and the test for nonspecific agglutinins showed a titer of 1:16. This individual suffered from chronic hepatitis of undetermined etiology.

Group 10 consisted of 96 selected normal controls. Three individuals had weakly positive titers of 1:8 and 1:16. These positive findings are not remarkable, considering that these individuals were from an area where approximately 85% of the adult population has a positive reaction with the Mantoux test.

The data presented in Table 10 are based only on the tests run on patients whose diagnosis of tuberculosis was confirmed by positive bacteriological studies. By using more rigid guidelines in appraising the results obtained by the SLAT method, more significance can be placed on the value of positive findings. An insufficient number of tests was performed on individuals with minimal tuberculosis (group
to provide adequate data on this group. Data from patients with moderately advanced tuberculosis showed a 2% increase in positive results over the values obtained based on clinical diagnosis. The results obtained on patients with far advanced tuberculosis showed no significant change. Of 50 patients with unclassified tuberculosis (group 6), only 38 (76%) had positive titers. This represents a 6% increase over the percentage obtained based on clinical diagnosis. An increase of 17% was noted in the results obtained on patients with arrested or inactive tuberculosis (group 7). The overall percentage of positive titers obtained on patients with bacteriologically confirmed tuberculosis was 80.6% as compared with 75.4% obtained on patients with a clinical diagnosis of tuberculosis.

Twenty-five patients with positive cultures for M. tuberculosis had negative latex agglutination tests. All but three of these patients had positive cultures within the past year. Three patients had only one positive culture, and, of these, two had cultures yielding only one colony.

Multiple specimens were tested from 88 patients. The interval between collection of specimens ranged from 10 to 60 days, with an average of 4 weeks. The data indicated that titers on new patients had a tendency to fluctuate. These changes ranged from only a change in the degree of agglutination in a certain dilution to a considerable change in titer. Little change was noted in the titers obtained on long treated cases with stabilized conditions.

Fluctuation of antibody titers on relatively new patients may have resulted from sporadic proliferation of the tubercle bacilli as the treatment process was initiated. There are several theories regarding the effect of the proliferation of the tubercle bacilli on antibody titers. Wallace et al. (9) reported that an increase in the number of tubercle bacilli in the tissues would increase antibody titers, whereas successful treatment would result in a decrease in the concentration of antigen and a corresponding decrease in antibody titer. Young and Leonard (10) indicated that an increase in the number of tubercle bacilli in the tissue would cause a decrease in the circulating antibodies as a result of antibody neutralization. Successful treatment would reduce the number of tubercle bacilli and allow the circulating antibodies to increase. Since Wallace et al. (9) were using the bentonite flocculation test and Young and Leonard (10) were working with a modification of the Middlebrook-Dubos hemagglutination test, both theories may be correct. Studies have shown that each of these tests appears to detect a different type of antibody. The causes for any fluctuations in antibody titers observed with the SLAT method could be determined only with a long-term controlled study of tuberculosis patients from the time they are diagnosed until their conditions are stabilized.

Table 11 provides data pertaining to the conditions of the individuals included in groups 9 and 10. Both Parlett and Youmans (6) and Duboczy and White (3) found that patients with certain conditions were subject to false-positive reactions. No problems were encountered with false-positive reactions in the tests run on normal, healthy controls by the SLAT method. Three of the individuals had low titers, but, considering that they were from an area where there is a high incidence of tuberculosis, these findings were not highly significant. Only one individual in the group of patients with nontubercular diseases had a titer which was significant. This individual also had nonspecific agglutinins which may have accounted for the high titer in the SLAT method.

No attempts were made during the course of this study to correlate results of the SLAT method with results of the tuberculin skin tests. Parlett and Youmans (6) had previously found that there is no apparent relationship between the results of the skin tests and the concentration of circulating tuberculosis antibodies.

The data obtained in this study indicate that the SLAT method might be satisfactory as a screening procedure for detection of tuberculosis in adults. The results showed it to be comparable to some of the more sophisticated procedures which have been developed. The test is simple enough to be performed in most clinical laboratories since only a minimal amount of equipment is needed. The reagents are readily available from biological supply houses and have been found to be stable both in stock and working solutions for extended periods of time.

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