

# Improved serodiagnosis of tuberculosis using two assay test

E KRAMBOVITIS,\* M HARRIS,† DTD HUGHES†

*From the \*Wellcome Research Laboratories, Beckenham, Kent, and †The London Hospital, London*

**SUMMARY** An antigen capture immunoassay was developed for the detection of mycobacterial antigens in sera from patients with tuberculosis. The assay was evaluated together with an antibody measuring enzyme immunoassay in a clinical trial for serodiagnosis of tuberculosis.

Sensitivity of the antibody assay for active pulmonary tuberculosis, including relapsed infections, was 75%, and specificity with other lung diseases was 97%. Sensitivity for extrapulmonary tuberculosis was 84.5% and specificity 84%. Sensitivity of the antigen assay for active tuberculosis was 45% with no false positive reactions. Combination of the results from the two assays increased total sensitivity to 96.5% with a positive predictive value of 0.81 and a negative predictive value of 0.98.

The two assay test was relatively simple to perform and offered improved serological diagnosis of tuberculosis over a single antibody test.

Tuberculosis remains a major disease with many socioeconomic implications. The clinical spectrum in developed countries has changed: more than 30% of patients with pulmonary tuberculosis may present atypical chest x-rays,<sup>1</sup> and extrapulmonary disease represents an increasing proportion of all cases,<sup>2</sup> which makes diagnosis more difficult.

Standard bacteriological techniques are generally unsatisfactory. Microscopic examination of sputum specimens may give a less than 50% positive yield,<sup>3,4</sup> and culture techniques, though more sensitive and providing definitive diagnosis,<sup>4</sup> are expensive and require prolonged incubations.<sup>5</sup>

The development of enzyme linked immunosorbent assays (ELISA) has stimulated renewed interest in serological tests for the diagnosis of tuberculosis.<sup>6-12</sup> Although the reported results are encouraging, specific antibodies at levels of diagnostic importance are not detected in a relatively high proportion of patients with tuberculosis despite general hypergammaglobulinaemia.<sup>13-15</sup> The reported inverse relation between antibody and immune complexes<sup>16</sup> and the association of low antibody titres with heavily infected patients<sup>12</sup> suggests that mycobacterial antigens are released into the blood circulation. Mycobacterial plasma membrane antigens were detected in the cerebrospinal fluid of patients with

tuberculous meningitis with a latex particle agglutination test.<sup>17</sup> We developed an antigen capture immunoassay (ACIA) for detecting plasma membrane antigens in patient sera and evaluated the diagnostic potential of the assay together with that of an antibody immunoassay (TB-ELISA)<sup>12</sup> in a clinical trial.

## Material and methods

### PATIENTS

Sera were collected and tested prospectively and blindly for antibody from 66 patients suspected of having tuberculosis on admission to hospital or during the course of outpatient treatment. The following patient groups were defined:

- 1 Patients with pulmonary tuberculosis who were all culture positive (new and relapsed cases).
- 2 Patients who were presumed tuberculous and subsequently treated but who, in fact, were subsequently found to be negative by direct microscopy and culture. (Such patients were excluded from this study.)
- 3 Patients who were suspected of having pulmonary tuberculosis but were definitely negative, as confirmed by negative direct microscopy and cultures and follow up for at least one year.
- 4 Patients with extrapulmonary tuberculosis that was confirmed either by biopsy or culture, or both.
- 5 Patients with non-tuberculous (atypical) mycobacterial infections, all of whom were positive

by culture.

6 A small number of patients were excluded because inadequate details were available.

This left a total of 55 patients who were included in the study. Subsequently, 37 of these were confirmed as having active disease by bacteriological or histological evidence, or both.

Samples were also obtained from several subjects with confirmed inactive tuberculosis, all of whom were followed up for at least one year. Control sera were also collected from 16 patients with other lung diseases and from 25 patients treated for diseases not associated with the lung.

#### ELISA FOR ANTIBODY ACTIVITY (TB-ELISA)

The assay was performed in round bottomed polystyrene microtitre plates. (Titertek, Flow Laboratories), as described previously.<sup>12</sup> The wells were sensitised with 50 µg/ml plasma membrane antigen. Test samples and the reference serum were diluted 1/40 and tested in duplicate. IgG antibody was measured with peroxidase labelled sheep antihuman IgG.<sup>18</sup> Antibody activity was calculated from the optical density (OD) readings:

$$\frac{\text{OD of test sample}}{\text{OD of reference serum}} \times 40$$

to reduce plate to plate and day to day variations. All samples were tested blind to eliminate operator bias.

#### ANTIGEN CAPTURE IMMUNOASSAY (ACIA)

Microtitre plates were coated with 150 µg/ml purified rabbit antimycobacterial plasma membrane immunoglobulin in 50 mM sodium carbonate-bicarbonate buffer (pH 9.5). The plates were sensitised at 37°C for three hours followed by 18 hours at 4°C. They were blocked with 0.5% bovine serum albumin for one hour. Treated serum samples and dilutions of a standard concentration of plasma membrane extract were placed into appropriate wells and incubated at 37°C for three hours. Antigen was detected with peroxidase

labelled rabbit antimycobacterial plasma membrane immunoglobulin<sup>18</sup> used at 20 µg/ml, incubated at room temperature for one hour. The reaction was visualised with a mixture of hydrogen peroxide and 5-aminosalicylic acid.<sup>12</sup>

#### TREATMENT OF SERA FOR ACIA

Sera were treated according to the method of Smith *et al* (Smith LB, *et al*. Abstract of the annual meeting of ASM, 1981). Test samples were diluted 1/4 with 100 mM edetic acid in 66 mM sodium phosphate buffer (pH 7.4) and heated at 80°C for 10 minutes. The denatured material was centrifuged at 3000 g for 15 minutes and the resultant supernatant was dialysed for two hours in phosphate buffer.

#### Results

Table 1 shows the clinical details and the radiological and bacteriological results of the patients with tuberculosis who were studied. Fig. 1 shows antibody activity measured by TB-ELISA.

Taking 50 as the cut off, 75% of the patients with confirmed active pulmonary tuberculosis gave a positive antibody test. One of the highest results was from a patient whose sputum samples were negative by direct microscopy and culture on two occasions but from whom positive cultures were obtained at necropsy. In the same group the two patients with the lowest antibody activity were terminally ill with lung carcinoma and Crohn's disease, respectively, in addition to tuberculosis. The third lowest antibody titre was from a patient with adenocarcinoma. The remaining three false negative samples and most of the sera with intermediate antibody activity were from microscopy and culture positive cases and with no other complications. All cases of relapsed pulmonary tuberculosis and 90% of extrapulmonary cases gave a positive antibody test.

In the inactive tuberculosis group the three sera with highest antibody activity were from patients who

Table 1 Cases of tuberculosis studied

	Sex		Origin		Mean age (SD) range	Chest x-ray		Microscopy		Culture or biopsy or both	
	M	F	European	Asian		Normal	Abnormal	Positive	Negative	Positive	Negative
Patients with new pulmonary tuberculosis	17	2	18	1	51 (13) 22-76	1	18	16	3	19	0
Patients with relapsed pulmonary tuberculosis	4	1	4	1	49 (15) 37-79	0	5	3	2	5	0
Patients with extrapulmonary tuberculosis	5	5	2	8	38 (14) 21-56	3	7	3	7	10	0
Patients with inactive tuberculosis	6	1	5	2	44 (15) 22-64	0	7	0	7	0	7
Patients with atypical mycobacterial infections	2	1	3	0	67 (10) 53-77	0	3	3	0	3	0

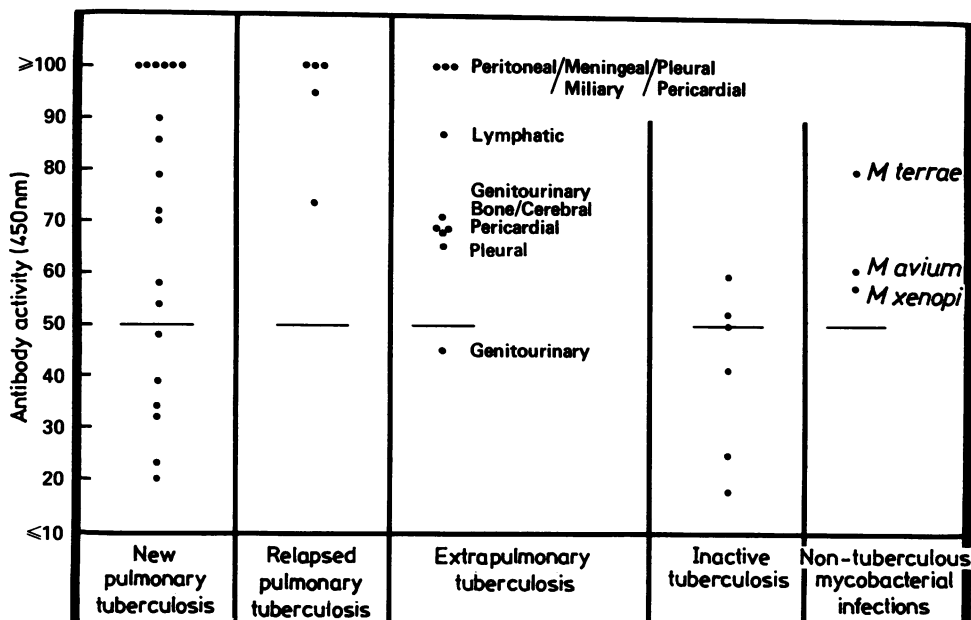


Fig. 1 Antibody activity of sera from patients with tuberculosis as measured by TB-ELISA. Each point represents one patient.

whereas the remaining samples were from patients who had completed treatment five to 10 years earlier.

The sera from the atypical mycobacterial infection group gave intermediate antibody activity. In one patient *Mycobacterium terrae* was isolated in two sputum samples. This organism was suspected to be a contaminant, particularly as this patient was also alcoholic. He discharged himself from the hospital soon after, and no further samples were available.

Table 2 shows the clinical details of the control patients. The patients suspected of tuberculosis because of abnormal chest radiographs but found not to have active disease were included in this Table because they constituted an important control group.

Fig. 2 shows the antibody activity. Specificity for active tuberculosis in the group with lung abnormalities was 97% with one false positive result from a patient with pneumonia. In the other control group (non-lung disease) specificity was 84%. Of the four false positive sera, the highest antibody activity was obtained from a 48 year old Asian patient treated for hypothyroidism. Two further samples taken three and six months later were also strongly positive, but the radiological and bacteriological results were negative. The three other patients were treated for diabetes mellitus, hypertension, and Crohn's disease.

Twenty nine sera from the groups with tuberculosis, including the false negative samples and 40

Table 2 Control cases studied

	Sex		Origin		Mean age (SD) range	Chest x ray		Microscopy		Culture	
	M	F	European	Asian		Normal	Abnormal	Positive	Negative	Positive	Negative
Suspected but found not to be infected with tuberculosis	14	4	13	5	45 (16) 14-75	0	18	0	18	0	18
Other lung diseases:											
Sarcoidosis	1	6	5	2	40 (17) 21-65	0	7	0	7	0	7
Carcinoma	2	1	2	1	55 (2) 52-57	0	3	0	3	0	3
Chronic obstructive airways disease	3	0	3	0	69 (8) 59-80	0	3	0	3	0	3
Pneumonia	1	2	2	1	55 (11) 52-70	2	1				
Diseases not associated with the lung	11	14	15	10	49-(17)	25	0				

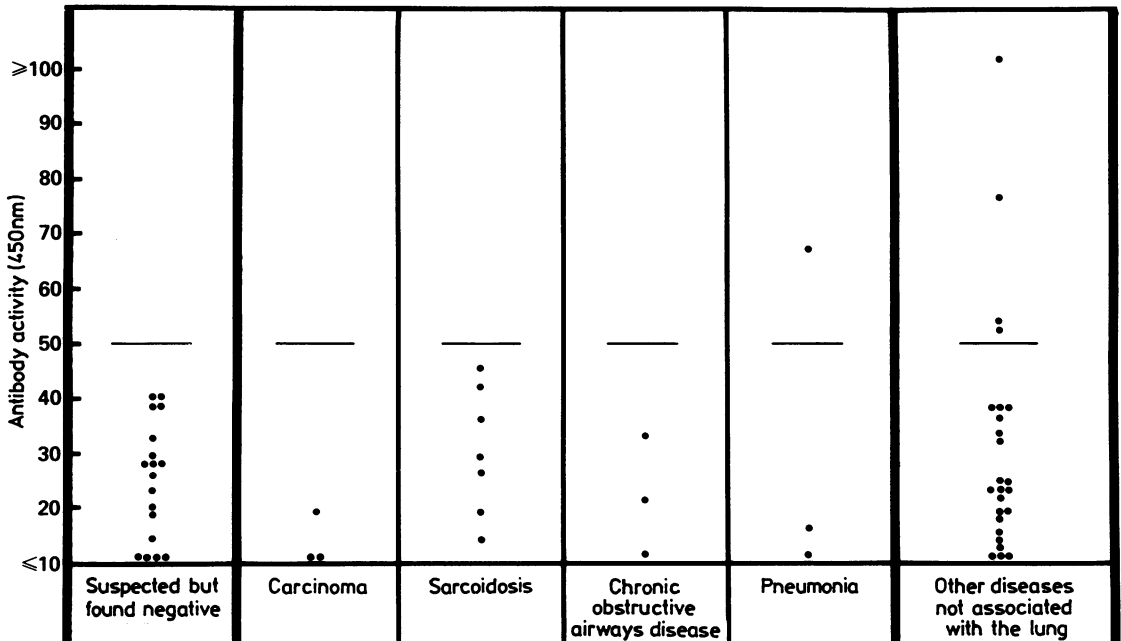


Fig. 2 Antibody activity of sera from control patients as measured by TB-ELISA. Each point represents one patient.

sera from the control group, including the false positive samples, were tested by ACIA. The frequency distribution for the control group had a sample median of 0.14 and a range of 0.08 to 0.20. It was assumed that this group consisted of subjects totally lacking in mycobacterial antigens. Optical density of 0.22 was chosen as the baseline of positivity, which was the value of the mean (0.147) plus three standard deviations.

Thirteen (45%) of the serum samples from the patients with tuberculosis were ACIA positive (Table 3). This low test sensitivity for active disease was considered to be of limited diagnostic value. When the results of the ACIA were combined with those of the

TB-ELISA, however (Fig. 3), 28 samples (96.5%) were positive in either or both assays. Only one serum was negative in both tests and that was from a patient with genitourinary tuberculosis.

The mean antibody activity of the sera from the tuberculosis patients in the group positive for ACIA was 52 (SD 21) and that of the group negative for ACIA 98 (26). This strongly suggests that antigen detected in the serum may be inversely related to antibody activity. The only exception was a sample from a patient with disseminated disease with foci in both the lung and bone that was strongly positive in both assays.

Table 3 Distribution and concentration of mycobacterial antigen detected by antigen capture immunoassay in tuberculosis and control patient sera

	Optical density range (450 nm) of antigen capture immunoassay								
	$\leq 0.2$	$\leq 0.4$	$\leq 0.6$	$\leq 0.8$	$\leq 1.0$	$\leq 1.2$	$\leq 1.4$	$\leq 1.6$	$\leq 1.8$
Antigen concentration (ng/ml)*									
Tuberculosis sera (n = 29)	16	4	3	2	10		2	1	100
Control sera (n = 40)	40								

\*Reactivity was determined after spiking a negative control serum with antigen.

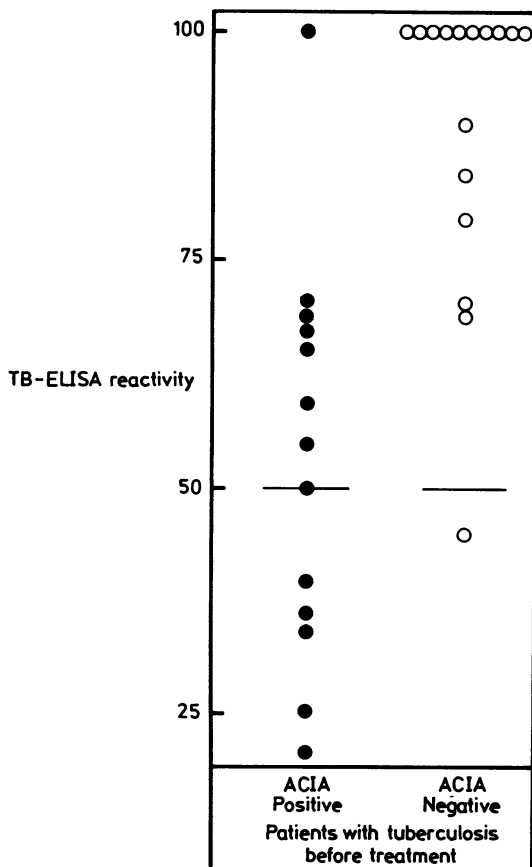


Fig. 3 Comparison of antigen capture immunoassay (ACIA) positive and ACIA negative sera in relation to their respective antibody activity, as measured by TB-ELISA.

### Discussion

Enzyme immunoassay techniques have been developed for tuberculosis, which are almost exclusively used to measure antibody titres. Promising results have been reported in these studies using crude mycobacterial extracts such as filtrates<sup>6</sup> and cell sonicates,<sup>19–21</sup> purified protein derivative,<sup>9–11 22–24</sup> or highly purified antigens including glycolipids<sup>25</sup> and cytoplasmic proteins,<sup>7 8 11</sup> which clearly show the spectrum of humoral responses in tuberculosis. The average sensitivity of most of these assays was about 85%, but because of limited clinical information it is difficult to establish why a substantial proportion of patients failed to produce antibodies at a significantly high titre.

In a recent study Krambovitis<sup>12</sup> reported that low antibody activity in patients with pulmonary tuber-

culosis was associated with heavy infections and with microscopy positive culture positive sputa. It was suggested that release of mycobacterial antigens may lead to formation of immune complexes with the more reactive antibodies, resulting in a considerable reduction of specific antibody activity. Carr *et al*<sup>16</sup> found that 68% of patients with tuberculosis had high titres of circulating immune complexes, as measured by C1q binding. In many of these patients there was an inverse relation between immune complexes and specific antibody titres. Brostoff *et al*<sup>26</sup> did not find such a correlation, but some patients with high values of immune complexes had no detectable antibody, indicating that immune complex formation may be responsible for some of the antibody negative cases. Measurement of C1q binding has limited diagnostic application in tuberculosis because of autoantibody complex formation,<sup>27 28</sup> as well as immune complexes due to antigen antibody interaction. Samuel *et al*<sup>29</sup> recently measured specific antibody and mycobacterial antigens that had been isolated from polyethylene glycol precipitated circulating immune complexes. Using radioimmunoassay techniques, they reported higher antigen titres in sputum positive cases as compared with sputum negative cases, a gradual decrease in antigen concentrations, and an increase in specific antibody during treatment. In the present study free antibody and mycobacterial plasma membrane antigens released by direct inactivation of serum were investigated. Release of antigens was facilitated by the use of edetic acid simplifying the extraction procedure. Enzyme assays were also preferred because of the drawbacks of radioimmunoassays.

The sensitivity of the antibody assay for active pulmonary tuberculosis, including relapsed infections, was 75%, and the specificity with other lung diseases 97%. Using the same cut off the sensitivity for extrapulmonary tuberculosis was 90% and the specificity 84%. These results are comparable with those of other reports.<sup>7 9 11 12</sup> Patients with low antibody activity seemed to fall into two categories: one consisted of strongly direct microscopy positive culture positive cases, as reported earlier<sup>12</sup>; and the other immunocompromised patients. Location and extent of the infection, degree of dissemination, and the immunological state of the host must therefore be important factors in determining the antibody response.

The sensitivity of the antigen assay for active tuberculosis was 45% with no false positive reactions. The results suggested a possible inverse relation between antibody activity and antigen detected but not with concentration of antigen. The level of dissemination, or the amount of antigen released, and the rate of formation and removal of immune complexes from

Table 4 Comparison of performance characteristics of both enzyme immunoassays with those of accepted diagnostic criteria

Criteria	Predictive value		
	Positive value	Negative value	Efficiency
Chest x-ray	0.51*	0.87**	0.62
Chest x-ray and clinical signs	0.67	0.79	0.71
Microscopic examination of sample	1.00	0.76	0.84
Culture of sample	1.00	0.84	0.90
ACIA	1.00	0.71	0.77
TB ELISA	0.78	0.89	0.85
ACIA and TB ELISA	0.81	0.98	0.91

Values were calculated from all types of tuberculosis including extrapulmonary:

\*with an abnormal chest x-ray including patients with old tuberculosis foci

\*\*with a normal chest x-ray.

circulation may have influenced this aspect.

Antigen and antibody have been detected in the cerebrospinal fluid of patients with tuberculous meningitis.<sup>30-32</sup> Our own laboratory has also described a method of detection of mycobacterial antigens<sup>17</sup> and measurement of antibodies directed to specific mycobacterial antigens.<sup>12</sup> In the present study we detected antigen and antibody in the serum of patients with active pulmonary and extrapulmonary tuberculosis. The combined results of the two assays gave rise to improved sensitivity for active disease. Comparison of the diagnostic performance of the two assay test with that of existing criteria shows that the efficiency (proportion of tests giving the correct result) of the test was superior to microscopic examination and comparable with culture results (Table 4). A limitation of the test was the false positive rate given by the antibody assay inpatients. Interpretation of a positive antibody only result must therefore be considered together with supporting evidence from other accepted criteria, including prevalence of the disease in a particular area. Specific monoclonal antibodies to *M tuberculosis* antigens have been produced,<sup>33</sup> and the use of such antibodies together with improved sensitivity of the ACIA may permit a change either in the dilution factor or in the baseline of positivity of the antibody assay, eliminating a high proportion of the false positive results.

The test described here is highly specific and sensitive but warrants further investigation to validate its clinical application. There is a long history of attempts to produce serological tests including that previously described by Nicholls and coworkers,<sup>34-35</sup> which subsequently proved not to be clinically useful.<sup>36-38</sup> We believe, however, that our present method is specific enough to be useful and may add considerably to the diagnosis of tuberculosis.

#### References

<sup>1</sup> Khan MA, Kovnat DM, Bachus B, Whitcomb ME, Brody JS, Snider GL. Clinical and roentgenographic spectrum of

- pulmonary tuberculosis in the adult. *Am J Med* 1977;62:31-8.
- <sup>2</sup> Faser LS, Lowell AM, Meoder MP. Extrapulmonary tuberculosis in the United States. *Am J Epidemiol* 1979;109:205-17.
- <sup>3</sup> Boyd JC, Marr JJ. Decreasing reliability of acid-fast smear techniques for detection of tuberculosis. *Ann Intern Med* 1975; 82:489-92.
- <sup>4</sup> Blair EB, Brown GL, Tull AH. Computer files and analysis of laboratory data from tuberculosis patients. II. Analysis of six year's data on sputum specimens. *Am Rev Respir Dis* 1976;113:427-32.
- <sup>5</sup> Glassroth J, Robins AG, Snider DE. Tuberculosis in the 1980's. *N Engl J Med* 1980;302:1441-50.
- <sup>6</sup> Nassau E, Parsons ER, Johnson GD. The detection of antibodies to *Mycobacterium tuberculosis* by microplate enzyme-linked immunosorbent assay (ELISA). *Tubercle* 1976;57:67-70.
- <sup>7</sup> Benjamin RG, Daniel TM. Serodiagnosis of tuberculosis using enzyme linked immunosorbent assay (ELISA) of antibody to *Mycobacterium tuberculosis* antigen 5. *Am Rev Respir Dis* 1982;126:1013-6.
- <sup>8</sup> Stroebel AB, Daniel TM, Lan JHK, Leong JCY, Richardson H. Serological diagnosis of bone and joint tuberculosis by an enzyme linked immunosorbent assay. *J Inf Dis* 1982;146:280-3.
- <sup>9</sup> Kalish SB, Radin RC, Phair JB, Levitz D, Zeiss CR, Metzger E. Use of enzyme-linked immunosorbent assay technique in the differential diagnosis of active pulmonary tuberculosis in humans. *J Infect Dis* 1983;147:523-30.
- <sup>10</sup> Radin RC, Zeiss CR, Phair JP. Antibodies to purified protein derivative in different immunoglobulin classes in the diagnosis of tuberculosis in man. *Arch Allerg Appl Immunol* 1983;70:25-9.
- <sup>11</sup> Benjamin RG, Debanne SM, Daniel TM. Evaluation of mycobacterial antigens in an enzyme-linked immunosorbent assay (ELISA) for the serodiagnosis of tuberculosis. *J Med Microbiol* 1984;18:309-18.
- <sup>12</sup> Krambovitis E. Detection of antibodies to *Mycobacterium tuberculosis* plasma membrane antigen by enzyme-linked immunosorbent assay. *J Med Microbiol* 1986;21:257-64.
- <sup>13</sup> Faulkner JB, Carpenter RL, Patnode RA. Serum protein and immunoglobulin levels in tuberculosis. *Am J Clin Pathol* 1967;48:556-60.
- <sup>14</sup> Nash DR. Serum immunoglobulin abnormalities in patients with respiratory tract disease. *Ann Clin Res* 1979;11:49-52.
- <sup>15</sup> Kaplan MH, Chase MW. Antibodies to mycobacteria in human tuberculosis. I. Development of antibodies before and after antimicrobial therapy. *J Infect Dis* 1980;142:825-34.
- <sup>16</sup> Carr RI, Chakraborty AK, Brunda MJ, et al. Immune complexes and antibodies to BCG in sera from patients with mycobacterial infections. *Clin Exp Immunol* 1980;39:562-9.
- <sup>17</sup> Krambovitis E, McIlmurray MB, Lock PE, Hendrickse W, Holzel H. Rapid diagnosis of tuberculous meningitis by latex particle agglutination. *Lancet* 1984;ii:1229-31.
- <sup>18</sup> Duncan RJS, Weston PD, Wrigglesworth R. A new reagent which

- may be used to introduce sulphhydryl groups into proteins and its use in the preparation of conjugates for immunoassay. *Analyt Biochem* 1983;132:68-73.
- <sup>19</sup> Grange JM, Gibson J, Nassau E, Kardjito T. Enzyme-linked immunosorbent assay (ELISA): a study of antibodies to *Mycobacterium tuberculosis* in the IgG, IgA and IgM classes in tuberculosis, sarcoidosis and Crohn's disease. *Tubercle* 1980;61:145-52.
- <sup>20</sup> Kardjito T, Handoyo T, Grange JM. Diagnosis of active tuberculosis by immunological methods. I. The effect of tuberculin reactivity and previous BCG vaccination on the antibody levels determined by ELISA. *Tubercle* 1982;63:269-74.
- <sup>21</sup> Kardjito T, Grange JM. Diagnosis of active tuberculosis by immunological methods. II. Qualitative differences in the dermal response to tuberculin in patients with active pulmonary disease and healthy tuberculin positive individuals. *Tubercle* 1982;63:275-8.
- <sup>22</sup> Tandon A, Saxena RP, Saxena KC, Jamil Z, Gupta AK. Diagnostic potentialities of enzyme linked immunosorbent assay in tuberculosis using purified tuberculin antigen. *Tubercle* 1980;61:87-9.
- <sup>23</sup> Viljanen MK, Eskola J, Tala E. Enzyme linked immunosorbent assay (ELISA) for antibodies to purified protein derivative of tuberculin (PPD). IgM-IgA-, and IgG- anti-PPD antibodies in active pulmonary tuberculosis. *Eur J Respir Dis* 1982;63:257-62.
- <sup>24</sup> Zeiss GR, Radin RC, Williams JE, Levitz D, Phair JP. Detection of immunoglobulin G antibody to purified protein derivative in patients with tuberculosis by radioimmunoassay and enzyme-linked immunosorbent assay. *J Clin Microbiol* 1982;15:93-6.
- <sup>25</sup> Reggiardo Z, Vazquez E, Schnaper L. ELISA tests for antibodies against mycobacterial glycolipids. *J Immunol Methods* 1980;34:55-60.
- <sup>26</sup> Brostoff J, Lenzini L, Rottoli P, Rottoli L. Immune complexes in the spectrum of tuberculosis. *Tubercle* 1981;62:169-73.
- <sup>27</sup> Lindquist KJ, Coleman RE, Osterland CK. Autoantibodies in chronic pulmonary tuberculosis. *J Chron Dis* 1970;22:717-21.
- <sup>28</sup> Kardjito T, Grange JM. Immunological and clinical features of smear-positive pulmonary tuberculosis in East Java. *Tubercle* 1980;61:231-8.
- <sup>29</sup> Samuel AM, Ashtekar MD, Ganatra RD. Significance of circulating immune complexes in pulmonary tuberculosis. *Clin Exp Immunol* 1984;58:317-24.
- <sup>30</sup> Sada E, Ruiz-Palacios GM, Lopez-Vidal Y, Ponce de Leon S. Detection of mycobacterial antigens in cerebrospinal fluid of patients with tuberculous meningitis by enzyme-linked immunosorbent assay. *Lancet* 1983;ii:651-2.
- <sup>31</sup> Bal V, Kamat RS, Kamat J, Kandoth P. Enzyme linked immunosorbent assay for mycobacterial antigens. *Indian J Med Res* 1983;78:477-83.
- <sup>32</sup> Kalish SB, Radin RC, Levitz D, Zeiss CR, Phair JP. The enzyme-linked immunosorbent assay method for IgG antibody to purified protein derivative in cerebrospinal fluid of patients with tuberculous meningitis. *Ann Intern Med* 1983;99:630-3.
- <sup>33</sup> Coates ARM, Hewitt J, Allen BW, Ivanyi J, Mitchison DA. Antigenic diversity of *Mycobacterium tuberculosis* and *Mycobacterium bovis* detected by means of monoclonal antibody. *Lancet* 1981;ii:167-9.
- <sup>34</sup> Nicholls AC. A serodiagnostic test for tuberculosis. *J Clin Pathol* 1975;28:850-3.
- <sup>35</sup> Nicholls AC, Horsfield K. Serological diagnosis of tuberculosis: a report of 12 months' clinical experience. *Thorax* 1976;31:289-93.
- <sup>36</sup> Geddes D, Emerson P, Lacey B. Assessment of the agglutination test for tuberculosis. *Thorax* 1977;32:349-51.
- <sup>37</sup> Mitchison DA, Aber VR, Ahmad FS, Allen BW, Devi S. Evaluation of a serological test for tuberculosis. *Br Med J* 1977;i:1383-4.
- <sup>38</sup> Riska H, McIlmurray MB, Krambovitis E, Froseth B, Hellstrom P-E. Assessment of the tuberculosis agglutination test. *Eur J Respir Dis* 1980;61:310-14.

Requests for reprints to: Dr Elias Krambovitis, Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS, England.