Interpreting the Results of the Amplified Mycobacterium tuberculosis Direct Test for Detection of M. tuberculosis rRNA

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The Amplified Mycobacterium tuberculosis Direct (AMTD) test detects M. tuberculosis rRNA. By using culture of M. tuberculosis as a gold standard, a number of different diagnostic indices were examined in an attempt to determine the diagnostic performance of the AMTD test and demonstrate how it might usefully be interpreted during the early management of disease.

Recent developments in diagnostic methods have decreased the time needed for detection and identification of Mycobacterium tuberculosis, but the process still requires at least 2 weeks. The Amplified Mycobacterium tuberculosis Direct test (AMTD test; Gen-Probe, Inc., San Diego, Calif.) can be used to detect M. tuberculosis rRNA in respiratory specimens (spu- tum, broncho-alveolar lavage [BAL], and bronchial and tracheal secretions) and can be performed in approximately 3.5 h. With this test specific RNA amplification products are hybrid- ized to complementary acridinium-ester-labeled DNA probes. Subsequent degradation of the acridinium-ester probes results in luminescence that is measured in relative light units (RLU).

According to the manufacturers an RLU reading of >30,000 signifies a positive test, but they recommend repeating the tests at between 30,000 and 100,000 RLU. Sensitivities and speci- ficities of the test at other RLU values, in comparison with a clinical diagnosis of tuberculosis, have been reported (1) with results of around 90% for each. Here we report the perform- ance of the AMTD test with a consecutive series of hospital patients suspected of tuberculous infection and demonstrate how the results might usefully be interpreted during their early management.

From 1994 to 2001 434 sputum and 339 BAL samples from separate patients were received for AMTD testing and myco- bacterial culture. All were processed by a modified Petroff’s method (3) prior to AMTD testing and culture on Löwenstein-Jensen slopes and in Middlebrook 7H12 radiometric broth (Becton Dickinson, Sparks, Md.). Cultured M. tuberculosis was identified by biochemical tests (5) and/or Accu-Probe culture confirmation tests (Gen-Probe, Inc.).

Of the 773 respiratory samples, 178 were smear positive (146 sputum, 32 BAL). Ninety-two cultures grew M. tuberculosis, 66 from sputa (54 smear positive, 12 smear negative) and 26 from BAL (12 smear positive, 14 smear negative). Seventy-five cultures (10%) grew nontuberculous mycobacteria. The median RLU value for specimens with positive cultures for M. tuberculosis was 2,345,744 (range, 3,094 to 3,648,998 RLU), and for those with negative cultures (including nontuberculous myco- bacteria) the RLU value was 7,031 (range, 448 to 3,609,521 RLU). The median RLU value for specimens with only posi- tive cultures for nontuberculous mycobacteria was 12,870 (range, 1,205 to 3,424,688). Figure 1 shows the distribution of RLU readings for AMTD tests on samples with negative cultures or with cultures of either M. tuberculosis or nontuberculous mycobacteria.

Using the culture results as the “gold standard,” we calculated the sensitivities, false-positive rates (100 – % specificity), positive predictive values, and Youden’s indices [(sensitivity + specificity) − 1] of the AMTD test across a range of 10 RLU values. False-positive rates at each value were calculated. Likelihood ratios (positive) were estimated as sensitivity/ false-positive rate and were used to estimate post-AMTD test probabilities for pretest probabilities between 10 and 90%.

Table 1 shows each diagnostic index for the AMTD test across a range of RLU readings. The data are interpreted as if each of the selected RLU readings represents the cut-off be- tween a positive and negative AMTD test. Figure 2 dis- plays posttest probabilities of a patient having a positive culture for tuberculosis plotted against a range of pretest probabilities across the same range of RLU values.

The sensitivity of the AMTD test at a cut-off of 300,000 was 90%, with a specificity of 85% and the highest Youden’s value. These figures are similar to those reported elsewhere (1, 4). One in six test results above this value were not followed by a positive culture for tuberculosis, and false-positive rates of 5% or below were only achieved at RLU readings of 2 million or above. Most clinicians, however, are likely to favor high sensi- tivity at the expense of specificity. Only a small increase in sensitivity was gained at RLU values below 300,000, and a rate of 95% was achieved only at RLU values of 10,000 or less. At this level there was a very high rate (46%) of false-positive results, and the positive predictive value of such a result was just 22%. Test results of 750,000 or above were more likely than not to yield a positive culture for M. tuberculosis.

Some clinicians might favor the use of likelihood ratios,
preferring to interpret clinical situations and test results in terms of pre- and posttest probabilities. Figure 2 may be used to interpret the AMTD test across a range of values before the results of culture are available. Typically for a test that sets out to detect abnormal material (M. tuberculosis rRNA in this case), the highest diagnostic yield for the AMTD test is for low prior probabilities of M. tuberculosis infection. Thus, for a patient with a pretest probability of 20% for respiratory tuberculosis, the posttest probability of disease with an RLU result of around 300,000 is about 35%; with an RLU result of 1 million or over it is at least 80%. Naturally, where the prior probability is high (for example, 80% or over), there is little increase in diagnostic certainty at any test result and very little difference between RLU readings of 500,000 or over.

Many tests used in situations of low disease prevalence have a higher specificity than when they are applied to populations where the disease is more common (6). Our results derive from tests carried out on patients attending a specialist respiratory hospital where the incidence of a positive culture of M. tuberculosis was 12% (95% confidence limits of 9.7 to 14.3%). It is probable that our results will be broadly applicable to other settings with a similar prevalence. The nature of Royal Brom-

![FIG. 1. Distribution of RLU readings for AMTD tests on samples with subsequent positive cultures for M. tuberculosis (filled columns; percentages from 92 samples), positive cultures for nontuberculous mycobacteria (open columns; percentages from 75 samples), and negative cultures (horizontally hatched columns; percentages from 606 samples). K, thousands; mil, millions.](attachment:image1.png)

![FIG. 2. Posttest probabilities across a range of pretest probabilities and for different RLU values for the AMTD test in respiratory tuberculosis. The curves (bottom to top) represent RLU readings of 10,000, 20,000, 30,000, 100,000, 300,000, 500,000, 1 million, 1.5 million, 2 million, 2.6 million, 3 million, and 3.6 million, respectively.](attachment:image2.png)
ton Hospital explains the relatively high incidence of nontuberculous cultures, but there was no important difference in our findings when these samples were excluded from the analysis. In theory neither nontuberculous mycobacteria in the sample nor the immune status of the patient should affect the result of the AMTD test. A further issue may be the use of *M. tuberculosis* culture as a reference in these analyses. Only 85% of cases of respiratory tuberculosis are accompanied by a positive culture result (2); therefore, the proportion of apparently false-positive AMTD tests may have been inflated.

We believe that the information presented here will provide a useful resource for the interpretation of AMTD test results. We suggest that microbiologists and clinicians, when in the early stages of considering a diagnosis of respiratory tuberculosis, report and interpret AMTD test results numerically rather than qualitatively.

### REFERENCES


