For smear-positive cases, at least two sputum specimens should be based on the following criteria:

Criteria of smear-positive and smear-negative pulmonary TB were obtained for microscopic examination. The diagnostic algorithm advised for the diagnosis of TB recommends examination of three consecutive sputum specimens from TB suspects for the presence of AFB. In the present study, we evaluated the contribution of each specimen to the final detection of TB suspect patients with culture-proven disease. The collection and analysis of retrospective data on patients with culture-proven pulmonary TB, from June 2002 to August 2006 at Dokuz Eylul University Hospital, Turkey, have enabled us to assess the value of examining two sputum specimens in diagnosing this disease. AFB were detected from one or more sputum specimens with direct microscopy in 42% of the cases. An analysis of results of smear examination showed that 97% of AFB were detected from the first specimen and only 3% were obtained from the second smear. The third specimen did not have any additional diagnostic value for the detection of AFB by microscopy. As a conclusion, the present study shows that examining two sputum smears is sufficient for the early detection of AFB in our laboratory.

INTRODUCTION

The detection of acid-fast bacilli (AFB) in smears has great value and remains the most widely used rapid diagnostic test for tuberculosis (TB) (1). In countries where the resources are limited, sputum smear microscopy is the main route of diagnosing pulmonary TB (2). Although AFB smears are less sensitive than cultures, the procedure for collecting and examining samples is relatively simple, rapid, and inexpensive. The World Health Organization (WHO) and the International Union Against Tuberculosis and Lung Disease (IUATLD) have endorsed an algorithm for the diagnosis of TB, and they recommend the examination of three sputum specimens to look for AFB (3,4). This recommendation is not always possible in practice for reasons such as heavy workload of technicians and patient-based irregularities (5).

TB is a great concern in Turkey. As a result of a great fight against TB during the 1950s the incidence had declined, but since that period the disease had been on the rise (27/100,000 in 2002) (6,7). The diagnosis and treatment of TB in the national TB control program of Turkey are performed according to national TB control guidelines. Patients with an unexplained productive cough lasting 3 weeks and capable of producing sputum, suspected of having pulmonary TB, should have at least two, and preferably three, sputum specimens obtained for microscopic examination. The diagnostic criteria of smear-positive and smear-negative pulmonary TB should be based on the following criteria:

(i) For smear-positive cases, at least two sputum specimens by microscopy or one positive smear for AFB and clinical and radiological findings or one smear and culture positive specimens.

(ii) For smear-negative cases, two negative sputum smears (with a week between smears) in addition to positive chest radiography findings consistent with TB and lack of response to a trial of broad spectrum antimicrobial agents in a 1-week period and culture-positive sputum specimens (6).

Rapid and cheap methods for the determination of TB are required in TB control programs. In this study, we analyzed the contribution of each of the three consecutive sputum specimens for the detection of AFB. Several earlier studies have discussed how many specimens are necessary to make the diagnosis of pulmonary TB. They examined this question particularly in countries with a high prevalence of TB and in populations with high human immunodeficiency virus prevalence (2,5,8,9).

In the present study, our goal was to assess the number of AFB smears that are necessary to diagnose of pulmonary TB at a university hospital in the west region of Turkey.

MATERIALS AND METHODS

Study institution and design of the experiment: Dokuz Eylul University Hospital (DEUH) is an 800-bed tertiary care, teaching hospital located in Izmir, Turkey. The hospital averages 44,000 patient admissions and almost 480,000 outpatient clinic and emergency room visits annually. In this study, we examined the AFB smear and culture results of all sputum specimens received in the laboratory, for which a definitive diagnosis of pulmonary TB had been made during a 4-year period.

Culture and microscopy procedures: At DEUH expectorated sputum specimens for AFB examination are collected as often as every 24 h (preferably early in the morning). All sputum specimens were decontaminated and concentrated before examination by using N-acetyl-L-cysteine-sodium hydroxide. 
**Microscopy:** AFB was detected microscopically in sputum concentrates, prepared by conventional centrifugation, with Kinyoun stain. Three types of staining procedures are used in the Turkish laboratory for AFB: Ziehl-Neelsen and Kinyoun (generally) and fluorochrome stain (rarely). We prefer Kinyoun stain because it is similar to Ziehl-Neelsen stain but without the necessity to heat specimens and consequently less risk for laboratory personnel.

In the study, only 100 fields had to be examined to declare a smear to be negative, but 300 fields had to be examined for quantification of positive smears.

**Culture:** Sputum sediments were inoculated both into a BACTEC 12B bottle (Becton-Dickinson Microbiology Systems, Sparks, Md., USA) and two separate Lowenstein-Jensen media. Culture media were incubated at 37°C in a 5% CO₂ incubator for 6 weeks.

**Organism identification:** From June 2002 to August 2006, isolated organisms were identified as Mycobacterium tuberculosis complex, on the basis of inhibition by p-nitro-α-acetylamino-β-hydroxypropiophenone (NAP) to organism growth in the BACTEC radiometric culture system (10).

**RESULTS**

The Clinical Microbiology Laboratory at DEUH received 6,097 specimens for AFB smearing and culturing from June 2002 to August 2006. Of these specimens, 165 were positive for mycobacteria.

AFB was detected from one or more sputum specimens by direct microscopy in 42% (70 of 165) of these specimens. Analyzing the results of smear examination of 70 patients showed that 68 (97%) were detected from the first smear and 2 (3%) were obtained from the second smear. The flow chart for the results of the smear examinations is shown in Fig. 1.

The third specimen did not have any additional diagnostic value for the detection of AFB by microscopy. We did note that pulmonary TB was diagnosed by the third culture when the first and second cultures were negative but the second smear was positive in one patient. Of 165 sputum specimens, 155 (94%) appeared as growth on the culture medium by the first specimen, a further 9 (5%) appeared in the second specimen, and 1 (1%) appeared in the third specimen.

![Flow chart for the results of the smear examinations](image)

**DISCUSSION**

TB is a global matter and is a great concern in Turkey. The most probable causes are defects in the TB control program and the migration of people with TB from neighboring countries such as the ex-Soviet Union where the prevalence of TB is high (6). The main requirement for control is the rapid and accurate identification of infected individuals (11). For the detection of TB, microscopic examination of sputum is the first step and can provide quick information to the clinicians (8,12).

In the present study, we evaluated the contribution of each sputum specimen for the detection of AFB by microscopy in our hospital. Our examination of the records shows that under routine conditions, screening TB suspects with two sputum smears was as effective as with three sputum smears and is associated with less laboratory work and thus reductions in the service provider’s and patient’s costs. In addition to those advantages, this strategy could allow more time for examining each slide.

*M. tuberculosis* was recovered from the first specimen with relatively high frequency which was also shown by other investigators (2,5,13). The third specimen did not have an additional value for microscopic examination in smear-negative patients, although it may be beneficial for culture.

The optimum number of sputum specimens to establish a diagnosis has been examined in a number of studies. One guideline reports that using a culture as the reference standard, the mean incremental yield in sensitivity of the second smear was 9% and that of the third smear was 4% (14). In addition to this document, a recent study involving 42 laboratories in four high-burden countries showed that the incremental yield from a third sequential smear ranged from 0.7 to 7.2% (15). The examination of more than one sputum specimen is necessary to maximize the sensitivity of cultures for *M. tuberculosis*. Our data show that the collection of two sputum specimens is almost always adequate to make a diagnosis (100%), irrespective of the quality of the specimens obtained. Obviously, high quality of the collected sputum specimens at our institution may have affected our results. Thus it appears that in a diagnostic evaluation for TB, at least two specimens should be obtained. Accordingly, a third specimen may be useful for a particularly culture (in our study 1%), but examination of more than two specimens had no effect on the smear results in our laboratory specimens.

Cost effectiveness is very important for developing and underdeveloped countries. By reducing the number of smears needed, patients could be moved from isolation earlier and more efficiently, and the cost of hospital stays could be reduced (12,16).

In conclusion, the results of the present study show that examining two sputum smears was sufficient for the detection of AFB in our laboratory. However, these results are based on data from a mycobacteriology laboratory in the west region of Turkey. These results should be reassessed as part of a study involving different laboratories from all of the regions of Turkey.

**REFERENCES**


