Adequately Washed Bronchoscope Does Not Induce False-Positive Amplification Tests on Bronchial Aspirates in the Diagnosis of Pulmonary Tuberculosis*

Tae Sun Shim, MD; Hyun Sook Chi, MD; Sang Do Lee, MD; Younsuck Koh, MD; Woo Sung Kim, MD; Dong Soon Kim, MD; and Won Dong Kim, MD, FCCP

Study objectives: To investigate the clinical usefulness of amplification (COBAS AMPLICOR; Roche Diagnostics Systems; Branchburg, NJ) on bronchoscopic aspirate specimens in the diagnosis of pulmonary tuberculosis, with particular regard to the possibility of false-positive results in subsequent specimens due to residual Mycobacterium tuberculosis DNA.

Design and setting: A prospective clinical study at a tertiary referral medical center.

Participants and methods: Four hundred fiberoptic bronchoscopic procedures were performed, using seven bronchoscopes on 335 consecutive patients, for therapeutic or diagnostic purposes. Serial bronchial aspirates were collected and tested for M tuberculosis, using COBAS AMPLICOR (CA). Bronchoscopes were cleaned and disinfected automatically, between patient use, by the same endoscope washer. The name of each bronchoscope and the sequence of its use were recorded, together with the sequence of washing. The CA results were compared with the bacteriologic and histologic results for M tuberculosis infection. When there was a suspicion of contamination, outward polymerase chain reaction analysis was performed.

Results: Of 392 specimens (332 subjects), excluding the 8 specimens (4 subjects) in which bacteriologic and histologic analyses were omitted, a smear-positive result for acid-fast bacilli (AFB), culture-positive or biopsy-positive results, and CA-positive results were obtained in 16, 49, and 32 specimens, respectively. In AFB smear-positive subjects, the sensitivity, specificity, positive predictive values (PPVs), and negative predictive values (NPVs) were 92%, 67%, 92%, and 67%, respectively. In AFB smear-negative subjects, the sensitivity, specificity, PPV, and NPV values were 38%, 99%, 74%, and 94%, respectively. The CA test was more sensitive than the AFB smears for the diagnosis of pulmonary tuberculosis (53% vs 27%, respectively; p < 0.05). False-positive CA results were seen in only six specimens. Three of these six subjects received a diagnosis of pulmonary tuberculosis on clinical and radiologic grounds, and none of the six results seemed to be associated with bronchoscopic cross-contamination.

Conclusions: Adequately cleaned and disinfected bronchoscopes did not cause false-positive amplification test results for M tuberculosis on bronchial aspirates by cross-contamination. Furthermore, sensitivity was greater with the CA tests. Therefore, CA tests on bronchial aspirates seem to be useful in the diagnosis of pulmonary tuberculosis. (CHEST 2002; 121:774–781)

Key words: bronchoscopy; direct amplification test; Mycobacterium tuberculosis; pulmonary tuberculosis

Abbreviations: AFB = acid-fast bacilli; APIC = Association for Professionals in Infection Control; CA = COBAS AMPLICOR; DAT = direct amplification test; NPV = negative predictive value; PCR = polymerase chain reaction; PPV = positive predictive value; RFLP = restriction fragment-length polymorphism

Tuberculosis is the principal cause of death due to infection worldwide. Following several decades of decline, the incidence of tuberculosis has recently begun to increase in many countries. Early diagnosis and treatment is important for the control of tuberc-

*From the Departments of Internal Medicine (Drs. Shim, Lee, Koh, W.S. Kim, D.S. Kim, and W.D. Kim) and Clinical Pathology (Dr. Chi), Division of Pulmonary and Critical Care Medicine, University of Ulsan College of Medicine, Asan Medical Center, Seoul, Korea.

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Correspondence to: Tae Sun Shim, MD. Department of Internal Medicine, Division of Pulmonary and Critical Care Medicine, University of Ulsan College of Medicine, Asan Medical Center, 388–1 Pungnap-dong, Songpa-gu, Seoul 138–600, Korea; e-mail: shims@www.amc.seoul.kr
culosis. A direct smear for acid-fast bacilli (AFB) in a sputum specimen is a simple and cheap method for the diagnosis of pulmonary tuberculosis, but has the drawback of limited sensitivity. Since 1989, various direct amplification tests (DATs) have been widely used in the diagnosis of tuberculosis. The main advantages of DATs are their rapidity and high-level sensitivity compared with direct smears for AFB. However, their benefits are compromised by the risk of false-positive results, arising from amplicon carry-over or cross-contamination.

Fiberoptic bronchoscopy has been used successfully in the diagnosis of pulmonary tuberculosis in AFB smear-negative patients. DATs for Mycobacterium tuberculosis have also been applied to bronchial aspirate specimens, and have shown promising results. In contrast, some reports indicate that mycobacterial cross-contamination can occur during bronchoscopic procedures. Because even a few dead bacilli remaining after disinfection procedures can result in an amplification product in a subsequent bronchoscopic specimen, the clinical usefulness of DATs on bronchial aspirate specimens has not yet been defined. Kaul et al. reported that 2 of 55 washing solutions (3.6%) for bronchoscopes contained amplifiable M. tuberculosis DNA. Carricojo et al. reported an experimental case in which an amplification test on the washing fluid from a bronchoscopic channel was positive for M. tuberculosis, even after the bronchoscope was disinfected by exposure to 2% glutaraldehyde for 1 h.

This study was undertaken to investigate the clinical utility of DATs on bronchoscopic aspirates in the diagnosis of pulmonary tuberculosis, with particular regard to the possibility of false-positive results arising from cross-contamination via bronchoscopes. COBAS AMPLICOR (CA) [Roche Diagnostics Systems; Branchburg, NJ] was used as the DAT for the diagnosis of tuberculosis.

**Materials and Methods**

**Bronchoscopic Procedures**

Four hundred fiberoptic bronchoscopic procedures were performed, using seven different bronchoscopes (three model 1T200 bronchoscopes, and one each of model 1T30, model 2T10, model 3C10, and model N20 bronchoscopes; Olympus; Tokyo, Japan) on 335 consecutive patients at the Asan Medical Center from April to June 2000. The Asan Medical Center is a 2,200-bed tertiary referral center, in which seven pulmonologists perform approximately 2,000 bronchoscopic procedures per year. The mean number of bronchoscopic procedures performed in this study was 8.3/d (400 procedures in 48 days; range, 2 to 14/d). Bronchoscopy was performed for therapeutic purposes in 14 patients and for diagnostic purposes in the remaining subjects. In 35 patients, bronchoscopy was performed more than once. All 400 bronchial aspirates were collected and tested for M. tuberculosis using CA, independent of any suspicion of pulmonary tuberculosis. Between patient use, the seven bronchoscopes were cleaned and disinfected automatically by the same bronchoscope washer (EW-20 system; Olympus). With the exception of shortening the disinfection cycle from 20 to 10 min, our cleaning and disinfection protocol was within the guidelines proposed by the Association for Professionals in Infection Control (APIC) and the manufacturer of the bronchoscopes. Immediately following a procedure, the lumen was irrigated with 10 mL of 70% alcohol, after which the bronchoscopic surface was cleaned with saline solution gauge. The bronchoscope was subsequently disassembled into component parts in preparation for disinfection, and the lumen cleaned manually by brushing with povidone-iodine (Betadine; MundiPharma; Seoul, Korea). The parts were cleaned using the standard protocol. A total of 23 min of automated washing was performed between uses, consisting of a cleaning cycle, a disinfection cycle, and a drying cycle. Disinfection was performed using 2% glutaraldehyde (Cidex; Johnson and Johnson K.K.; Tokyo, Japan) for 10 min. After completion of the bronchoscopic procedures for the day, each of the bronchoscopes was washed for 43 min, including a 23-min disinfection cycle with 2% glutaraldehyde. The name of each bronchoscope, the order of bronchoscopic procedures undertaken each day, and the order of washing during this study period (cumulative numbers 1 to 400) were recorded.

All bronchoscopy was performed by experienced bronchoscopists, by a transoral or transnasal route. Patients were usually premedicated with IM atropine and pethidine. Lidocaine was used for topical anesthesia of the larynx and tracheobronchial trees. After examination of the bronchial trees, 5 to 10 mL of normal saline solution was instilled into the orifice of the bronchial segment in which abnormalities were likely to be located, and then aspirated back. In the cases of patients in whom the abnormalities were not confirmed, normal saline solution was instilled into both bronchial trees. In cases where mucosal abnormalities were detected or lung tissue was required, tissue was obtained using a biopsy forceps.

**Sample Preparation**

At least 5 mL of bronchial aspirate and sputum were collected in a sterile conical tube, and then treated with NALC-NaOH. After Ziehl-Neelson staining, 300 fields were examined using light microscopy at high power (×1,000) by experienced personnel. Specimens were regarded as positive for AFB even if only one or two cells per 300 fields were observed. Cultures were grown using egg-based Ogawa medium, and growth was observed for 8 weeks, after which the M. tuberculosis complex was identified using the Accuprobe test (Gen-Probe; San Diego, CA). All bronchial aspirates were stored at −70°C until 400 bronchoscopic procedures were completed, and then the CA tests were performed collectively.

**Detection of the M. tuberculosis Complex in Bronchial Aspirates by CA**

The CA test was performed according to the instructions of the manufacturer. CA uses the polymerase chain reaction (PCR) nucleic acid amplification technique, nucleic acid hybridization, and colorimetric detection of M. tuberculosis. With the benchtop CA analyzer (Roche Diagnostics Systems), the amplification, addition of denaturation agent, and detection of the amplified
product were automated. Briefly, the procedures are as follows. NaOH/NaCl-treated specimens were washed with a respiratory specimen wash solution, after which organisms were lysed by inoculation with a respiratory specimen lysis reagent. Finally, specimens were made ready for amplification by the addition of a respiratory specimen neutralization reagent. After the processed specimens were added to the amplification mixture, which contains an internal control and urasyl-N-glycosylase, PCR was performed. The internal control identifies specimens containing substances that may interfere with PCR amplification. When heated in the first thermal cycling step at the alkaline pH of the amplification mixture, urasyl-N-glycosylase breaks the carry-over ampiclon DNA at deoxyuridine, thereby rendering the DNA nonamplifiable. The required number of cycles is automatically performed by the CA. Following PCR amplification, a suspension of magnetic particles coated with an oligonucleotide probe specific for *M tuberculosis* is added to the ampiclon. After the magnetic particles are washed to remove unbound material, avidin-borseradish peroxidase conjugate binds to the bioin-labeled ampiclon captured by the specific DNA probe. After removing unbound conjugates by washing, substrate is added. Specimens with A$_{660}$ ≥ 0.35 are interpreted as positive for *M tuberculosis*. Specimens with A$_{660}$ < 0.35, and internal controls with A$_{660}$ ≥ 0.35, are interpreted as negative for *M tuberculosis*.

**Data Analysis**

After CA results were obtained, each patient’s clinical and laboratory records were reviewed, together with chest radiography. The diagnosis of tuberculosis was confirmed only when cultures of sputum or bronchial aspirates were positive for *M tuberculosis*, or if lung or bronchial biopsy specimens showed chronic granulomatous inflammation with caseation necrosis. Clinical tuberculosis was defined as radiologic findings compatible with tuberculosis, which improved with antituberculosis drug treatment, without bacteriological or histologic confirmation of tuberculosis. In cases of clinical tuberculosis, with histologic evidence of chronic granulomatous inflammation without caseation necrosis, tuberculosis was considered to be confirmed. The specificity, sensitivity, positive predictive value (PPV), and negative predictive value (NPV) of CA testing were calculated, using bacteriologic or histologic diagnoses as the ultimate diagnostic standard for tuberculosis. In the case of patients with false-positive CA results, the clinical history and radiographic results were investigated. Bacteriologic and CA results of the patients who underwent bronchoscopy using the same bronchoscope immediately before the patient with a false-positive CA result were reviewed to investigate the possibility of contamination via the bronchoscopes. Bacteriologic and CA results of the patients in whom the bronchoscope was washed immediately before the patients who underwent bronchoscopy using the same bronchoscope immediately before the patients with false-positive CA result were reviewed to investigate the possibility of cross-contamination via the bronchoscope washer.

**Outward PCR Analysis**

In one case with a positive CA result in which contamination was suspected through the washing procedure, outward PCR genetic relatedness was tested by molecular fingerprinting employing the IS6110-based outward PCR method. In brief, outward PCR employed the single primer based on the invert-repeat fragment located at the ends of IS6110 whose sequence was 5’-GACIHCCCGGGGCGGTTCA-3’, where ‘I’ was inosine. The 3’ end of the primer was directed outwardly from both sides of the inverted repeats present in IS6110, amplifying the flanking sequences between two copies of IS6110. PCR was carried out with approximately 10 ng of genomic DNA in a final volume of 50 μL, in a reaction buffer containing 0.4 pmol primers, 2 mM MgCl$_2$, 200 μL of deoxyribonucleotide triphosphates, and 2.5 U of Taq polymerase. DNA samples were denatured by incubation for 3 min at 95°C before amplification for 35 cycles at 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min using a thermocycler (GeneAmp PCR System 9600; Perkin Elmer; Foster City, CA). After the last amplification cycle, the samples were incubated for 7 min at 72°C to complete the elongation of the PCR intermediate products. Positive and negative controls were used for each reaction. The positive control was the reference strain *M tuberculosis* H37Rv, and the negative control was the PCR mix without DNA. The PCR products were analyzed by electrophoresis through a 1.2% agarose gel and stained with ethidium bromide. Each sample was tested at least three times in order to verify the reproducibility of the results.

**Statistical Analysis**

Statistical analysis was performed using software (SPSS for Windows version 7.5; SPSS; Chicago, IL). The McNemar test was used to compare the sensitivity of direct AFB smear and CA testing. Data are expressed as mean ± SD, and statistical significance was defined as p < 0.05.

**Results**

Because bacteriologic study of sputum or bronchial aspirate specimens was not performed in 4 patients (8 specimens), a total of 331 patients (392 bronchial aspirate specimens) were included in this study (Table 1). Bacteriologic study of sputum

**Table 1—Clinical Characteristics of the 335 Patients Studied**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>56 ± 16$^*$</td>
</tr>
<tr>
<td>Male/female sex</td>
<td>217/118</td>
</tr>
<tr>
<td>Positive/negative/unknown history of tuberculosis, No.</td>
<td>80/245/10</td>
</tr>
<tr>
<td>Bronchoscopies performed, No.</td>
<td>400 (1–8/person)</td>
</tr>
<tr>
<td>Diagnosis, No.</td>
<td></td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>52</td>
</tr>
<tr>
<td>Confirmed tuberculosis</td>
<td>43</td>
</tr>
<tr>
<td>Clinical tuberculosis</td>
<td>9</td>
</tr>
<tr>
<td>Lung malignancy</td>
<td>115</td>
</tr>
<tr>
<td>Hemoptysis</td>
<td>40</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>30</td>
</tr>
<tr>
<td>Interstitial lung disease</td>
<td>27</td>
</tr>
<tr>
<td>Lung collapse</td>
<td>17</td>
</tr>
<tr>
<td>Airway stenosis (for intervention)</td>
<td>14</td>
</tr>
<tr>
<td>Pleural disease</td>
<td>11</td>
</tr>
<tr>
<td>Aspergilloma</td>
<td>7</td>
</tr>
<tr>
<td>Esophageal disease</td>
<td>5</td>
</tr>
<tr>
<td>Other</td>
<td>17</td>
</tr>
<tr>
<td>Total</td>
<td>335</td>
</tr>
</tbody>
</table>

$^*$Mean ± SD.
and bronchial aspirate specimens was performed in 302 and 321 patients, respectively. Bronchial mucoa or lung biopsies were performed in 116 patients. Of the 331 subjects, 43 patients were confirmed as having pulmonary tuberculosis either bacteriologically,28 histologically,8 or both,9 and 9 patients had clinical tuberculosis. Because the CA tests were performed after all 392 samples were collected, there were no room for the attending physicians to be biased by the CA results at the time of decision whether or not the patients had pulmonary tuberculosis clinically.

Direct smear-positive, culture- or biopsy-confirmed, and CA-positive results were obtained in 16 subjects (16 specimens), 43 subjects (49 specimens), and 29 subjects (32 specimens), respectively (Table 2). Excluding the 3 subjects who had a positive AFB smear but negative culture or histology results for \textit{M} tuberculosis, of the 13 smear-positive tuberculosis subjects, 7 subjects had positive findings only in bronchial aspirates, 3 subjects had positive findings only in sputum, and the remaining 3 subjects had positive findings in both sputum and bronchial aspirates.

For sputum or bronchial aspirate AFB smears, sensitivity was 27% (13 of 49 smears) and specificity was 99% (340 of 343 smears). The sensitivity and specificity of CA tests were 53% (26 of 49 smears) and 98% (337 of 343 smears), respectively. The CA test was more sensitive than the Ziehl-Neelson AFB smear for the diagnosis of pulmonary tuberculosis (53% vs 27%, \( p < 0.05 \)). In AFB smear-positive patients, the sensitivity, specificity, PPV, and NPV of CA tests for the diagnosis of tuberculosis were 92%, 67%, 92%, and 67%, respectively. In AFB smear-negative patients, the sensitivity, specificity, PPV, and NPV of CA tests were 38%, 99%, 74%, and 94%, respectively. Overall, the sensitivity, specificity, PPV, and NPV of CA tests were 53%, 98%, 81%, and 94%, respectively. In nine patients with clinical tuberculosis, one patient had a positive finding in both AFB smear and CA tests, two patients had positive findings only in CA tests, and six patients had negative findings in both AFB smear and CA tests.

With regard to the washing order of the bronchoscopes, there were three episodes of possible contamination via bronchoscope washer. However, of three events of possible contamination, two had only confirmed tuberculosis; one was confirmed by histologic diagnosis, and the other had a positive culture for \textit{M} tuberculosis in another sputum examination. The remaining patient (Fig 1, C) had lung cancer and no evidence of tuberculosis clinically or bacteriologically, so a contamination via bronchoscope washer could be suspected. The patient who underwent bronchoscopy using the same bronchoscope immediately before patient \( C \) had no evidence of tuberculosis, but patient \( B \), in whom bronchoscope was washed immediately before the patient who underwent bronchoscopy using the same bronchoscope immediately before the patient \( C \), had a bacteriologically confirmed tuberculosis. However, in this case, outward PCR analysis revealed that they were different strains (Fig 1).

With regard to the order in which each bronchoscope was used, two consecutive positive CA results were observed twice. In each episode, the second patient had bacteriologically confirmed tuberculosis, independent of the CA results. These results also suggest that it is unlikely that the bronchoscope caused false-positive CA results in subsequent specimens. In summary, in our study, none of the 49 bronchoscopic procedures that were performed on 43 patients with confirmed pulmonary tuberculosis caused the false-positive CA test results in subsequent bronchial aspirate specimens.

### Discrepant Analysis

Six patients had false-positive CA results. However, considering the sequence of bronchoscopy, the order of bronchoscope washing, and the result of outward PCR analysis, there was no evidence of contamination through the bronchoscopes or bronchoscope washer. Of the six patients with false-positive results, three patients had a strong likelihood of tuberculosis based on clinical criteria.

<table>
<thead>
<tr>
<th>Specimens</th>
<th>TB</th>
<th>Non-TB</th>
<th>Sensitivity, ( % )</th>
<th>Specificity, ( % )</th>
<th>PPV, ( % )</th>
<th>NPV, ( % )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smear positive (n = 16)</td>
<td>CA (+)</td>
<td>CA (-)</td>
<td>CA (+)</td>
<td>CA (-)</td>
<td>92</td>
<td>67</td>
</tr>
<tr>
<td>Smear negative (n = 37)</td>
<td>12</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>53</td>
<td>38</td>
</tr>
<tr>
<td>All</td>
<td>39</td>
<td>26</td>
<td>231</td>
<td>61</td>
<td>337</td>
<td>53</td>
</tr>
</tbody>
</table>

\( ^{*} \text{TB = tuberculosis; CA (+) = CA positive; CA (-) = CA negative; tuberculosis was diagnosed bacteriologically or histologically.} \)

\( ^{†} \text{All culture reports showed low colony counts, \( \leq 10 \) colonies.} \)

\( ^{‡} \text{Three of six were clinically highly probable tuberculosis patients.} \)
In the remaining three patients, we could not find any possible causes of false-positive results. Of the 20 patients (23 specimens) with false-negative CA results, all but one were AFB smear-negative. All bacteriologically confirmed positive patients produced a culture of \( \times 10^{2} \) colonies, indicating a low colony count. Three AFB smear-positive patients were not considered to have tuberculosis because cultures were negative for \( M. \) tuberculosis. In two patients who showed an AFB smear of one to two AFB per 300 high-power field (\( \times 1,000 \)), with no clinical or radiologic evidence of tuberculosis, CA gave negative results for \( M. \) tuberculosis. In the other patient, who seemed to have pulmonary tuberculosis on radiologic examination and showed gradual improvement with antituberculosis treatment, CA had positive findings for \( M. \) tuberculosis.

In summary, the CA tests were positive in 53% (26 of 49 cases) of confirmed tuberculosis and 33% (3 of 9 cases) of clinical tuberculosis cases. After combining the confirmed and clinical tuberculosis (58 specimens in 52 tuberculosis patients) cases, the sensitivity, specificity, PPV, and NPV of CA tests were 50% (29 of 58 cases), 99% (331 of 334 cases), 91% (29 of 32 cases), and 92% (331 of 360 cases), respectively.

**Discussion**

In this study, we have shown that automatic bronchoscopic washing, including 10 min of a disinfection cycle using 2% glutaraldehyde, did not cause false-positive results or cross-contamination in amplification techniques or bacteriological tests for \( M. \) tuberculosis. COBAS AMPLICOR, the commercial kit used for the diagnosis of tuberculosis, which uses an amplification-based technique, had the additional benefit of diagnosing pulmonary tuberculosis. These results cannot be applied directly to other laboratories, because careful cleaning before the automated disinfection procedures and well-trained personnel are the most important factor in preventing the transmission of infectious organisms or DNA through bronchoscopes. However, we have demonstrated that bronchoscopic aspirate can be a useful specimen for the amplification of \( M. \) tuberculosis DNA in the diagnosis of pulmonary tuberculosis, if each bronchoscopic laboratory maintains high-quality control monitoring during bronchoscopic disinfection.

In recent years, PCR and other amplification-based techniques have been widely used in the diagnosis of tuberculosis. Most studies have used sputum as the specimen from which \( M. \) tuberculosis DNA has been amplified for the diagnosis of pulmonary tuberculosis. However, even these amplification techniques are relatively insensitive in smear-negative pulmonary tuberculosis. Therefore, because bronchoscopic procedures have increased diagnostic potential, some researchers have used bronchoscopic specimens for the amplification of \( M. \) tuberculosis DNA in the diagnosis of tuberculosis. Endoscopic procedures, however, have the potential for cross-contamination of pathogens. Several investigators have reported false-positive diagnosis of tuberculosis, infection, or the development of clinical tuberculosis (even multidrug-resistant tuberculosis), related to the use of bronchoscopes. In these cases, it has been suggested that the disinfection procedures used were not performed thoroughly before the use of the bronchoscopes on subsequent patients. Carriço et al showed ex-
perimentally that it is possible to obtain positive amplification results for *M tuberculosis* from the washing fluids of a bronchoscopic channel, even after the bronchoscope was disinfected by a 1-h exposure to 2% glutaraldehyde. Michele et al confirmed the transmission of *M tuberculosis* via a fiberoptic bronchoscope, using DNA fingerprinting analysis. Although Wong et al demonstrated an increased sensitivity of PCR on bronchial aspirates in the diagnosis of pulmonary tuberculosis, the false-positive rate was unacceptably high. However, until now, there has been no systematic research into the potential for false-positive results in amplification-based diagnostic techniques for *M tuberculosis* using bronchial aspirate specimens. This is because amplification-based techniques are more sensitive than conventional bacteriological studies, and positive results can arise from the amplification of DNA from even a few dead *M tuberculosis* bacilli.

As bronchoscopes are semicritical devices that come into contact with mucus membranes during use, they should be sterilized or at least undergo high-level disinfection. High-level disinfection is defined as the inactivation of all vegetative bacteria, mycobacteria, fungi, and viruses, but not necessarily all bacterial endospores. To meet the requirement for disinfection of endoscopes, a US Environmental Protection Agency-registered liquid sterilant/disinfec tant, with objective evidence of efficacy, should be used. Of these, 2% glutaraldehyde has been widely used. The APIC recommends disinfection of endoscopes for at least 20 min, to achieve adequate high-level disinfection. Some previous guidelines have recommended a disinfection procedure of 30 to 40 min or more. In addition, careful cleaning before the disinfection procedure is also important in preventing the transmission of infectious organisms via bronchoscopes.

In this study, we used an automatic bronchoscopic washer, with 2% glutaraldehyde as the disinfectant agent. The disinfection time was 10 min between uses, and 23 min after completion of daily use. The disinfection time between uses was somewhat shorter than that recommended in the APIC guidelines. Despite this, cross-contamination of *M tuberculosis* was not a problem in our bronchoscopic procedures, even when we performed the more sensitive amplification procedures for the diagnosis of tuberculosis. One possible explanation is that we employed experienced personnel for bronchoscope washing. Two experts have been working in our bronchoscopic laboratory for the last 5 years, and are well acquainted with bronchoscope washing and maintenance. They are aware of the importance of careful cleaning before automatic disinfection. Immediately following a procedure, all bronchoscopes were aggressively hand-brushed to remove remaining tissue and blood. Disinfectant and rinsing solutions were changed frequently. Routine surveillance cultures have been taken from fiberoptic bronchoscopes every month. There have been no positive culture findings for *M tuberculosis* in the washing fluid from bronchoscopic channels during regular monitoring for several years in our laboratory. In our opinion, regular monitoring procedures to screen for cross-contamination should include techniques that amplify *M tuberculosis* if bronchoscopic specimens are used for the diagnosis of tuberculosis using amplification techniques. If this regular monitoring reveals positive results for *M tuberculosis* DNA in a laboratory, laboratory staff should strengthen their bronchoscopic disinfection procedures.

Although 400 bronchoscopic procedures were included in this study, pulmonary tuberculosis was confirmed in only 43 patients, and usually with a low colony count of *M tuberculosis*. However, because bronchoscopy is usually required in cases of smear-negative, low-colony-count tuberculosis, it seems that the patient population in this study can be compared to actual clinical situations we meet in everyday practice. Of 13 smear-positive tuberculosis patients, 5 patients had positive findings in bronchial aspirates but not in sputum. Of 30 tuberculosis patients with negative results for AFB smears on sputum or bronchial aspirates, 12 patients (40%) had positive findings for *M tuberculosis* on CA tests. These data suggest that AFB smears and CA tests on bronchoscopic aspirates are useful for an earlier diagnosis of sputum smear-negative patients. In the study by Wong et al, the sensitivity of PCR on bronchial aspirate specimens in the diagnosis of smear-negative pulmonary tuberculosis was 97.2%. However, these researchers used in-house PCR assays, and false-positive rates were as high as 27% (22 of 82 cases). They did not mention why the false-positive rate was so high. It may have been due to cross-contamination via bronchoscopes, or to poor quality control. In-house PCR assays usually vary greatly between institutions in both sensitivity and specificity. There is no worldwide standardization, and it may be personnel dependent. For this reason, we selected the CA test to amplify *M tuberculosis* DNA in this study. The CA test, which automates amplification and detection and decreases hands-on labor, was approved by the US Food and Drug Administration. Others have reported the sensitivity of PCR on BAL specimens to be from 40 to 79%. Had we used BAL fluid...
instead of bronchial aspirates, the sensitivity may have not been improved. However, in this study, we do not know whether the CA test on bronchial aspirates has some advantages over the test done on sputum specimens. Our results are comparable with the results of other studies performed on sputum specimens, which showed about 50% sensitivity of DAT in AFB smear-negative patients. Further study is needed to compare the diagnostic efficacy of DATs between sputum and bronchial aspirates, and BAL fluid specimens.

In contrast to a previous study that used restriction fragment-length polymorphism (RFLP) analysis to identify contamination arising via bronchoscopy, we performed outward PCR analysis because the amount of DNA was too small to do RFLP analysis. Although we could not perform outward PCR analysis in all consecutively positive cases, because of PCR failure in some specimens, we could exclude the possibility of contamination through the bronchoscope washer in the most suspicious case. As others suggested, it could be used as a rapid and simple method for typing isolates of M. tuberculosis, especially in cases where the amount of DNA was not enough to do RFLP analysis as in this case.

In conclusion, amplification-based techniques can be used for the diagnosis of pulmonary tuberculosis in bronchial aspirate specimens without the increased risk of cross-contamination, if adequate cleaning and disinfection procedures are adopted. To perform diagnostic tests using amplification techniques, each bronchoscopic laboratory should monitor cleaning and disinfection procedures continuously, until generalized guidelines for the use of amplification techniques in bronchoscopic specimens have been formulated.

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