Is Protected Specimen Brush a Reproducible Method to Diagnose ICU-Acquired Pneumonia?

Jean-François Timsit, M.D.; Benoît Missel, M.D.; Serge Francoual, M.D.; Fred William Goldstein, M.D.; Philippe Vaury, M.D.; and Jean Carlet, M.D.

Protected specimen brush (PSB) is considered to be one of the standard methods for the diagnosis of ventilator-associated pneumonia, but to our knowledge, intraindividual variability in results has not been reported previously. 

Purpose: To compare the results of two PSB performed in the same subsegment on patients with suspected ICU-acquired pneumonia (IAP).

Study design: Between October 1991 and April 1992, each mechanically ventilated patient with suspected IAP underwent bronchoscopy with two successive PSB in the lung segment identified as abnormal on radiographs. Results of the two PSB cultures were compared using 10^7 cfu/ml cutoff for a positive result. Four definite diagnoses were established during the follow up: definite pneumonia, probable pneumonia, excluded pneumonia, and uncertain pneumonia.

Population: Forty-two episodes in 26 patients were studied; 60 percent of patients received prior antibiotic therapy. Thirty-two microorganisms were isolated from 24 pairs of PSB. Definite diagnosis was definite pneumonia in 7, probable pneumonia in 8, excluded pneumonia in 17, and uncertain pneumonia in 10 cases.

Results: The PSB recovered the same microorganisms and argued for a good qualitative reproducibility. The distinction of positive and negative results on the basis of the 10^7 cfu/ml classic threshold was less reproducible. For 24 percent of the microorganisms recovered and in 16.7 percent of episodes of suspected IAP, the two consecutive samples gave results spread out on each side of the 10^7 cfu/ml cutoff. Discordance was higher when definite diagnosis was certain or probable than when diagnosis was excluded (p = 0.015). There was no statistical effect of the order of samples between the two specimens for bacterial index and microorganism concentrations.

Conclusion: These findings argue for the poor repeatability of PSB in suspected IAP and question the yield of the 10^7 cfu/ml threshold. In attempting to diagnose IAP, the results of PSB must be interpreted with caution considering the intraindividual variability. (Chest 1993; 104:104-08)

The protected specimen brush (PSB) is considered to be one of the standard methods for diagnosing ICU-acquired pneumonia (IAP).

Fagon et al1 found that a PSB bacterial culture with >10^7 cfu/ml correlated well with the presence of bacterial pneumonia, with a 100 percent negative predictive value and a 75 percent positive predictive value. Several other authors have studied the diagnostic value of PSB with widely variable results. Sensitivity varied from 56 up to 100 percent and specificity from 86 to 100 percent.2-4 These discordances, partly due to differences between reference tests, suggest a poor reproducibility between centers and perhaps also a poor reproducibility of the PSB sample result itself. Therefore, we conducted a prospective study to assess the intraindividual variability of PSB.

METHODS

The study was designed to compare the results of two PSB procedures performed in the same lung subsegment on mechanically ventilated patients with suspected IAP to assess the accuracy of this method for routine diagnosis in clinics.

Preliminary Consideration and In Vitro Study

A major problem in designing the study was to decide how to obtain the PSB samples so that the first test would not affect the result of the second. One method would be to perform two consecutive brushes in the same lung subsegment without withdrawing the bronchoscope. This process could lead to the contamination of the inner channel of the bronchoscope and could force secretions from the inner channel of the bronchoscope into distal airways of the concerned segment. For this reason, we chose to withdraw the bronchoscope between the two brushes to make the inner channel of the bronchoscope as uncontaminated as possible by flushing it with sterile saline solution. The potential risk of this method is that very proximal secretions may be mobilized toward the involved lung segment as the bronchoscope is advanced.

Before deciding to use the latter method, we performed a preliminary study to evaluate the contamination of the inner channel after the PSB procedure and to assess a method to prevent it.

After a bronchoscopic procedure attempting to diagnose IAP, we evaluated the contamination of the inner channel by flushing the inner channel of the bronchoscope with a 5-ml sterile saline solution and collecting the rinsing for bacteriologic study. To eliminate contamination or to at least decrease it, we rinsed the inner channel of the bronchoscope with a 20-ml sterile saline solution and dried it, for 1 min, with a sterile compressed air spray.

To validate if the fast decontamination procedure was effective, we performed another 5-ml rinsing for bacteriologic cultures. Ten preliminary bronchoscopies were evaluated. Bacteriologic cultures went from 2.8 x 10^3 cfu/ml (minimum, 0 to maximum, 10^7) before

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to $1.2 \times 10^6$ cfu/ml (minimum, 0 to maximum, $9 \times 10^6$) ($p=0.02$ 
Wilcoxon paired test) after rinsing procedure and allowed us to
confirm that this method avoids the major contamination of the
inner channel of the bronchoscope.

**Study Population**

Between December 1991 and March 1992, every patient hospi-
talized and mechanically ventilated for more than 24 h in our ICU
was prospectively included in the study when IAP was suspected.
The clinical suspicion of IAP was based on recent and persistent
pulmonary infiltrates on the chest radiograph and at least two of the
following published clinical criteria: fever >38.5°C or hypo-
thermia <36.5°C; leukocytosis ($>10^9/\text{L}$) or neutropenia
(<4$\times$10$^9$/$\text{L}$), and purulent tracheal secretions.

Patients with poor oxygenation (ie, $\text{PaO}_2/\text{FiO}_2 <100 \text{ mm Hg}$) or
unstable hemodynamic condition (systolic BP <90 mm Hg despite
inotropic support) who could suffer from a prolonged bronchoscopic
procedure were excluded from the study. The study protocol was
in accord with the ethical committee of our institution. Informed
consent was obtained from the patients or their close relatives.

**Study Design**

Fiberoptic bronchoscopy was performed for each patient within
12 h of inclusion in the study. All the bronchoscopies were done by
the same experienced pulmonologist (J. F. T.). Patients were
premedicated with phenoperidine, midazolam, and panceuronium
bromide. Topical anesthetics were never used. Each patient was
monitored with a pulse oximeter, and ventilated on 100 percent
$\text{FiO}_2$ during the time of bronchoscopy and for 1 h after the end of
the procedure. Chest radiograph was systematically performed
after bronchoscopy.

Immediately after endotracheal aspiration via a sterile tube, the
bronchoscope was introduced through a special adapter (Bodai,
suction safe, Y.Sontek Medical, Lexington, Mass) and advanced,
without suction, to the bronchial orifice of the lung segment
identified radiographically as containing the new infiltrate. The first
PSB (PSB1) was then inserted into the inner suction channel and
advanced to a 3-cm peripheral position before dislodging. The first
sample was then removed and placed on a sterile operative field.

The bronchoscope was then withdrawn from the endotracheal
tube and the inner channel was flushed with 20 ml of sterile saline
solution. The bronchoscope was dried with compressed air infus-
ated during 1 min into the proximal orifice of the inner channel.
The instrument was then reintroduced and wedged into the same
subsegment, the second PSB (PSB2) was performed using the same
method. The time between the two PSB samples was less than 2
min.

We chose to perform only two brushes to minimize anatomic and
bacteriologic changes in the lung subsegment.

When bronchoscopy was finished (approximately 5 min after the
second brushing), the two specimens were then separately prepared
as follows. Using strict aseptic conditions, the outer surface of the
distal inner cannula was cleaned with a 70 percent alcohol sponge
and dried with sterile compresses. The inner cannula was then
transacted with sterile scissors, distal to the brush so that the brush
would not come into contact with the possibly contaminated distal
portion of the inner cannula. The brush was then advanced and
severed with sterile scissors into numbered screw-capped glass vials
containing 1.0 ml of sterile Ringer's lactate solution. The two
containers were then sealed and sent to the laboratory for immediate
processing. Microbiologic procedures were performed by experi-
enced technicians, blindly for the two specimens, according to the
protocol previously described. After 48 h, the results for each PSB
were available.

**Diagnostic Categories**

Four diagnostic categories were established before initiating the
study: certain bacterial pneumonia, excluded bacterial pneumonia,
probable bacterial pneumonia, and uncertain pneumonia.

Bacterial pneumonia was certain if patients fulfilled one of the
following criteria: positive pleural fluid culture, rapid cavitation
of the lung infiltrate, or histopathologic proof. A histopathologic
diagnosis required the presence of consolidation with intense
polymorphonuclear leukocyte accumulation in bronchioles and
adjacent alveoli involving several adjacent low-power microscopic
fields in autopsies performed within 8 days of bronchoscopy.

Bacterial pneumonia was considered excluded if at least one of
the following criteria was fulfilled: full recovery without antimicro-
bial therapy associated with the diagnosis of another disease of the
chest accounting for the chest radiograph abnormality or absence
of bacterial pneumonia at autopsy performed within 8 days of the
bronchoscopic procedure in patients who had not received antibi-
otics after the results of the bronchoscopic procedure. Probable
pneumonia was defined as a complete recovery after antimicrobial
antibiotic therapy without treatment of another infectious site, and
no other disease of the chest diagnosed during the follow up. When
no diagnostic definition was available, patients were classified as
having uncertain pneumonia.

**Statistical Analysis**

The number of organisms recovered from the cultures of the PSB
specimens was expressed as colony-forming units per milliliter.
Bacterial index was computed as the sum of the log$_{10}$
concentrations of each microorganism found, and we defined positive and negative
PSB using the classic cutoff point of 10$^4$ cfu/ml.  

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>PSB1, cfu/ml</th>
<th>PSB2, cfu/ml</th>
<th>Definite Pneumonia</th>
</tr>
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<tr>
<td>A. baumannii</td>
<td>$3 \times 10^6$</td>
<td>$1.6 \times 10^6$</td>
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</tr>
<tr>
<td>A. baumannii</td>
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<td>$10^6$</td>
<td>Certain</td>
</tr>
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<td>S. pneumoniae</td>
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<td>$10^6$</td>
<td>Certain</td>
</tr>
<tr>
<td>M. catarrhalis</td>
<td>$10^6$</td>
<td>$10^6$</td>
<td>Certain</td>
</tr>
<tr>
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<td>$10^6$</td>
<td>$10^6$</td>
<td>Certain</td>
</tr>
<tr>
<td>E. coli</td>
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<td>$4.5 \times 10^6$</td>
<td>Certain</td>
</tr>
<tr>
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<td>$4.5 \times 10^6$</td>
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</tr>
<tr>
<td>P. morganii</td>
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</tr>
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<td>S. aureus</td>
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<td>10</td>
<td></td>
</tr>
<tr>
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</tr>
<tr>
<td>M. catarrhalis</td>
<td>$10^6$</td>
<td>$2.6 \times 10^6$</td>
<td>Probable</td>
</tr>
<tr>
<td>Streptococcus species</td>
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<td>$10^6$</td>
<td>Probable</td>
</tr>
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</tr>
<tr>
<td>Streptococcus species</td>
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<td>$4 \times 10^6$</td>
<td></td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>$3 \times 10^6$</td>
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<tr>
<td>H. influenzae</td>
<td>$10^6$</td>
<td>$1.2 \times 10^6$</td>
<td>Probable</td>
</tr>
<tr>
<td>P. aeruginosa</td>
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<td>$2.3 \times 10^6$</td>
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<tr>
<td>H. influenzae</td>
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<td>S. epidermidis</td>
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<td>C. albicans</td>
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<td>$10^6$</td>
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</tr>
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<td>A. baumannii</td>
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</tr>
<tr>
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<td>$3 \times 10^6$</td>
<td>Excluded</td>
</tr>
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<td>P. aeruginosa</td>
<td>$3 \times 10^6$</td>
<td>$10^6$</td>
<td></td>
</tr>
<tr>
<td>E. cloacae</td>
<td>0</td>
<td>$6 \times 10^6$</td>
<td>Excluded</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>$4 \times 10^6$</td>
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<td>Excluded</td>
</tr>
<tr>
<td>Streptococcus species</td>
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</tr>
<tr>
<td>S. aureus</td>
<td>$6.1 \times 10^6$</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>A. baumannii</td>
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<td>$4 \times 10^6$</td>
<td>Uncertain</td>
</tr>
<tr>
<td>S. aureus</td>
<td>$3 \times 10^6$</td>
<td>0</td>
<td>Uncertain</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>$4 \times 10^6$</td>
<td>0</td>
<td>Uncertain</td>
</tr>
</tbody>
</table>

*Patients with two sterile protected specimen brush (PSB) are not
mentioned.

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We calculated the sensitivity and specificity of each PSB considering "certain pneumonia" and "probable pneumonia" as definite IAP.

We assessed the accuracy and the intra-individual variability by correlation test for bacterial index, and for log10 concentration of every microorganism we found.

As we were not sure if the first and the second PSB specimen procedures were absolutely identical, we tested the effect of the rank of the PSB by comparing with zero the difference of the results of PSB1 and PSB2 using Wilcoxon matched pairs rank test.

Assessment of Outcome

Therapeutic decisions were left to the discretion of the attending physicians and discussed daily with the medical staff. All patients were monitored until their discharge from the hospital. Subsequent changes in the clinical and therapeutic course were recorded. Postmortem histopathologic investigations were performed as often as possible, especially when the diagnostic category remained uncertain.

RESULTS

Forty-two episodes of suspected IAP in 26 patients were analyzed. Table 1 indicates the results of PSB1 and PSB2 for each patient (type and number of growing bacteria) and the final diagnosis. Before the new septic signs associated with a clinical suspicion of nosocomial pneumonia, 15 patients (24 episodes) received antibiotic therapy for other reasons (patients with COPD with bronchial superinfection [n = 7], peritonitis [n = 8], catheter infection [n = 2], antibiotic prophylaxis for digestive surgery [n = 1], endocarditis [n = 1], and bacteremia of unknown cause [n = 5]). In 7 cases, this antibiotic therapy was stopped at the time or 24 h before the results of the bronchoscopic procedure because the initial septic focus was considered cured. The previous antibiotic therapy was effective on a presumably responsive microorganism in only one case of probable pneumonia. Diagnosis of pneumonia was definitely established in 7 cases (1 by cavitation, 6 by histologic confirmation) and definitely excluded in 17 cases (6 histologic examinations, 3 atelectasis, 8 regressions without antibiotic therapy). The diagnosis was probable in eight cases and uncertain in ten because antibiotic therapy was initiated to treat an associated extrapulmonary infected site (nine cases) or because histologic confirmation was not possible after the patient's death (one case).

Adverse Side Effects

There were no deaths during bronchoscopic procedure. Follow up chest radiographs demonstrated no barotraumas. No patient showed decreased oxygen saturation below 95 percent during the procedure. There were no episodes of bronchial hemorrhage requiring local or intravenous therapy.

Results of the PSB Cultures

The results of PSB sample cultures and the calculation of false-negative, false-positive results, sensitivity, and specificity are plotted on Table 2. Thirty-two microorganisms were isolated from 24 pairs of PSB. The two PSB cultures always recovered the same microorganisms indicating a 100 percent qualitative reproducibility.

The PSB1 and PSB2 gave discordant results with regard to the 10^6 cfu/ml threshold in 16.7 percent cases. Results were not different in the subgroup of patients receiving previous antibiotic therapy (p = 1, x^2). The percentage of discrepancies was significantly higher on confirmed pneumonia (certain and probable) than on excluded pneumonia (p<0.015, x^2) (Table 3).

Table 3—Percentage of Discordant Results Using the 10^6 cfu/ml Threshold Expressed as a Function of the Final Diagnosis and of the Previous Antibiotherapy

<table>
<thead>
<tr>
<th></th>
<th>Discordant Results, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Definite diagnosis</td>
<td></td>
</tr>
<tr>
<td>Pneumonia (certain and probable) [N = 15]</td>
<td>40</td>
</tr>
<tr>
<td>No pneumonia [N = 17]</td>
<td>0</td>
</tr>
<tr>
<td>Uncertain status [N = 10]</td>
<td>10</td>
</tr>
<tr>
<td>Previous antibiotic therapy</td>
<td></td>
</tr>
<tr>
<td>Yes [N = 24]</td>
<td>17</td>
</tr>
<tr>
<td>No [N = 18]</td>
<td>17</td>
</tr>
</tbody>
</table>

*Certain and probable pneumonias were considered as definite pneumonia.
†Discordant results of definite pneumonia (certain and probable pneumonia) were compared with the number of discordant results of excluded pneumonia by Yates-Fischer x^2.

Protected Specimen Brush in Diagnosis of ICU-acquired Pneumonia (Timsit et al)
Surprisingly, the percentage of discordant results seems to be independent of previous antibiotic therapy that has been reported as a major factor affecting the accuracy of the technique.\textsuperscript{4,9} In our study, 57 percent patients received antibiotic therapy for a septic episode unrelated to the septic episode corresponding to the suspicion of nosocomial pneumonia. This antibiotic therapy was always ineffective on presumably responsible microorganism except in one case of probable pneumonia. No patient received probabilist antibiotic therapy before the bronchoscopic procedure. The effect of previous antibiotic therapy is probably much higher when it is effective \textit{in vitro} on microorganisms responsible for the pneumonia. Moreover, the fact that this type of antibiotic therapy did not affect the repeatability of the PSB technique did not mean that previous antibiotic therapy did not affect the diagnostic yield of the procedure.

Despite a strong correlation between the two PSB samples, we have some reservation about the diagnostic value of PSB. If we considered each isolated microorganism, the correlation between the two PSB samples was $r = 0.63$ ($p < 10^{-9}$). This very significant correlation should be well accepted in case of comparison of two different methods for diagnosis, but it is very poor in case of comparison of the same diagnostic method performed twice. Differences of the quantitative culture values of the two PSB appeared commonly greater than 2 log$_{10}$ (Table 1 and Fig 1). For 24 percent of the microorganisms recovered and in 16.7 percent of episodes of suspected IAP, the two consecutive samples gave results spread out on each side of the 10$^8$ cfu/ml cutoff. We think, therefore that the previously adopted threshold must be interpreted with caution. One sample growing between 10$^8$ and 10$^9$ cfu/ml is not enough to definitely exclude the diagnosis of pneumonia and must encourage, if antibiotic therapy has not been already started, the performance of a second PSB procedure when suspicion of IAP persists.

These conflicting results are then probably multifactorial. (1) Great differences between two adjoining areas with pneumonia have been previously reported in baboons with microorganism concentrations in the infected lung varying from 10$^6$ to 10$^9$ cfu/ml and can explain our findings of 2 log$_{10}$ variations. (2) The bacteriologic count can differ up to 50-fold in a lung area with pneumonia compared with a noninvolved lung area.\textsuperscript{10} Moreover, despite methodologic care, the first PSB sample may have led to anatomic and bacteriologic modifications and may have modified the results of the second one. We chose to withdraw the bronchoscope between the two samples which might avoid the major part of contamination of the inner channel. Despite our rinsing procedure, it is possible that the small residual contamination of the inner

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**FIGURE 2.** Mean quantitative culture value (PSB1 + PSB2/2) and difference between quantitative culture of the first and the second protected specimen brushes (PSB1 - PSB2), for each recovered microorganism are represented using a logarithmic scale. Patients with two sterile PSB are not mentioned.

For 24 percent of the microorganisms recovered, the two consecutive samples spread out on each side of the 10$^8$ cfu/ml cutoff.

Bacterial index for PSB1 and PSB2 correlated ($r = 0.61$, $p < 10^{-4}$) as well as log$_{10}$ of each microorganism found ($r = 0.63$, $p < 10^{-9}$). Figure 2 represents the difference between the two PSB cultures expressed as a function of the mean quantitative culture value and disclosed major discrepancies between quantitative culture results (if the repeatability of the PSB had been perfect, each point would be right of the X axis). Differences between the two PSB specimens were greater than 10$^8$ cfu/ml for eight isolated microorganisms and the average difference between the two samples was 1.04 $\pm$ 1.2 log$_{10}$ cfu/ml for each isolated microorganisms.

There were no differences due to the order of sampling considering bacterial index ($p = 0.59$) and number of recovered microorganisms ($p = 0.6$).

**DISCUSSION**

Protected specimen brush is considered to be one of the main diagnostic methods for diagnosing IAP. The exactness of the classic diagnostic threshold of 10$^8$ cfu/ml has been established comparing PSB culture results with histologic and bacteriologic lung cultures.\textsuperscript{8} Utilization, in routine practice, of this precise threshold assumes a correct \textit{in vitro} reliability of the PSB culture which is demonstrated \textit{in vitro}.\textsuperscript{9}

Our results indicate that even with maximal care to carry out the PSB procedures with a standardized method, there were great quantitative culture differences between results of two brushes performed in the same lung subsegment for suspected IAP, independent of the order of the sampling. Using the 10$^8$ cfu/ml threshold, the two samples gave discordant results for 24 percent of the microorganisms.
channel of the bronchoscope might interfere with the
results of the second PSB procedure. Moreover, we
evaluated the bacterial growth due to the rinsing of
the bronchoscope in a prior control group with sus-
ppected IAP but not in the present patients. So, even
if PSB1 culture was higher than PSB2 in several
patients, the contamination might have been higher
than reported.

Using this study design, the bronchoscope might
have mobilized upper bronchial secretions and might
have interfered with the results of samples even if we
had previously performed the bronchoscopic pro-
dure, endotracheal aspiration.

In addition, it is possible that some bronchoscopists
are better able to obtain a consistent sample from the
lower respiratory tract. The bronchoscopic procedure,
which is similar to those of most studies, has been
scrupulously applied by an experienced bronchosco-
pist. Whoever the bronchoscopist may be, we think
that the lack of repeatability, demonstrated in our
study, exists probably with various levels.

In two cases of suspected pneumonia, one PSB was
sterile and the other was positive, which resulted
probably from contamination of the PSB by upper
airway secretions. Previous large aspiration with the
bronchoscope might be helpful in avoiding this cause
of variability between the two results and should be
proposed in case of purulent and abundant tracheal
secretions. The PSB sample might be obtained after
withdrawing the bronchoscope from the endotracheal
tube flushing the inner channel with sterile saline
solution and drying it.

These results tend to indicate an unperfect reliabil-
ity of PSB in suspected IAP. In attempting to diagnose
IAP, the results of PSB should be interpreted with
cautious, particularly regarding the standard cutoff of
10^6 cfu/ml.

ACKNOWLEDGMENT: We are indebted to Dr. I. Cunningham

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