

How Many Bronchial Alveolar Lavage Specimens Do We Need?

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DOI:10.21276/sjpm.2019.4.7.17

| Received: 15.06.2019 | Accepted: 26.06.2019 | Published: 30.07.2019

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Abstract

Introduction: Bronchoscopy is a valuable diagnostic tool and has a significant clinical impact on the management of pneumonia, especially ventilator-associated pneumonia in ICU patients who fail to respond to standard, guideline-based therapy. The investigation of bronchial alveolar lavage (BAL) specimens involves an extensive laboratory work. Currently, there are very few published studies evaluating the diagnostic benefit of collecting multiple BAL specimens during bronchoscopy. These redundant specimens result in a significant workload increase for the diagnostic microbiology laboratory. **Objective:** To investigate the optimal number of specimens for bacteriology, virology, mycology, *Pneumocystis jirovecii* (PJP) and *Legionella pneumophila* specimens in order to optimize the utility of BAL specimens with the aim of minimizing harm to the patient and optimizing resource utilization for the laboratory. **Method:** BAL specimens collected at an academic institution in Southern Ontario were reviewed retrospectively over a 15 month period for bacteriology, 16 months for virology, one year for mycology, and three years for *L. pneumophila* and *P. jirovecii*. **Results:** One thousand sixty-three BAL specimens were ordered for bacterial culture, yielding positive results in 45.5%. Among them, a concordance rate of 97.1% was found between two or more specimens acquired from different lung lobes. The concordance rate of multiple virology samples was 98.6% among patients in whom 2 specimens were collected per procedure, and 100% among those with 3 specimens per procedure. To study whether one specimen is sufficient for the detection of filamentous fungi, we reviewed 43 BAL samples which grew *Aspergillus fumigatus* between. A concordance rate of only 60% was found between two specimens obtained from different lung lobes. A concordance rate of 100% was found among multiple specimens ordered for *L. pneumophila* and *P. jirovecii* with positivity rates of 0.14% and 0.92% respectively. **Conclusion:** We recommend a single specimen per BAL be sent from the most purulent lung segment for bacteriology and viral PCR. Single specimens may also be appropriate for *L. pneumophila* and *P. jirovecii*, however further study is needed. Multiple specimens should be submitted for mycology investigations. By eliminating duplicate specimens laboratory utilization can be optimized and patient morbidity may be decreased.

Keywords: Bronchial alveolar lavage, ventilator-associated pneumonia Bacteriology, virology, mycology.

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INTRODUCTION

The diagnosis of pneumonia can be challenging sometimes, especially for ventilator-associated pneumonia (VAP) and non-ventilated hospital-acquired pneumonia (HAP) [1]. Empiric antibiotics are usually started when pneumonia is suspected clinically. However, patients may deteriorate despite broad antibiotic therapy. Identifying a microbiologic etiology is valuable in directing appropriate antibiotic choices, allowing timely de-escalation of treatment and minimizing therapy duration. A high-quality specimen from the lower

respiratory tract is essential, ideally before the start of antibiotics. BAL, acquired via flexible fibrotic bronchoscopy (FFB), is commonly recognized as a valuable diagnostic tool in the diagnosis of VAP and persistent HAP [2, 3]. In a study evaluating respiratory specimens acquired via different techniques, BAL was shown to reflect the bacterial burden in lungs most accurately when compared with the postmortem histological results [6]. Patients who underwent BAL have been shown to have a higher antibiotic modification rate and a higher antibiotic de-escalation rate [7, 8]. The commonly-accepted strategy for

processing BAL is a quantitative culture [9, 10]. In a study of 381 patients with 589 VAP episodes, when a load of bacteria in BAL was used as a criterion to discontinue antibiotics, a reduction of 4.8 days on antibiotic treatment per patient was identified without an increase of incidence of VAP [11].

The microbiological investigation of BAL specimen usually involves extensive laboratory work. The diagnostic tests available in our laboratory include bacterial culture and susceptibility, multiplex PCR for respiratory viruses, mycology culture and fluorescent monoclonal antibody staining for *P. jirovecii* (PJP). Culture requests for *Legionella pneumophila* and mycobacteria were referred out to a public health laboratory. The turnaround time (TAT) of these procedures vary from one day to 7 weeks. Microbiology laboratories often set a threshold of reporting depending on the number of bacterial types isolated as well as bacterial load and pathogenicity of each type. A bacterial load above 10⁴ CFU/ml often reflects true infection, while a growth below that is often considered colonization [5]. Thereafter, BAL specimens provide the physician with strong evidence in terms of what to treat and what to omit, even though clinical judgment should never be replaced [7, 8, 9, 11].

BAL poses potential hazards to critically ill patients who are already ventilated with positive ending pressure and several comorbidities [10]. Complications, such as hypoxia (SaO₂<88%), bronchospasm, decrease of PaO₂/FiO₂ ratio, bacteremia, or even hemodynamic instability were not found to be uncommon within first 24 hours post bronchoscopy in a study of 164 ICU ventilated patients [12]. Therefore, it is of clinical value to minimize the BAL specimens number to decrease complications from this procedure.

Currently, there are no guidelines regarding the optimal number of BAL specimens to collect and the practice varies widely among ICU intensivists and respirologists with either one or multiple specimens sent from a single bronchoscopy procedure. Very few studies have been done and the results are controversial [4, 5]. Jonker et al., Group found a higher positivity rate in the bilateral BAL than unilateral BAL specimen in trauma patients suspected of VAP, and a discordance rate of 37.1% was identified in bilateral sites [5]. In contrast, the Zaccard et al., Group suggested a concordance rate of 87.3% between the left and right lung BAL bacterial culture in ICU ventilated patients [5]. All studies have focused on the bacteriology investigation, so far there are no published studies to systemically look at the optimal number of BAL specimen in other microbiological areas. The aim of this study was to investigate the optimal BAL specimen number for bacteriology, mycology, *L. pneumophila* as well as PJP workup, to reduce the burden of specimen load in the diagnostic lab without compromising patients' diagnosis.

METHODS

Study Population

One thousand sixty-three BAL bacterial specimens ordered in Hamilton Health Science, St. Joseph Healthcare, Joseph Brant and Niagara Health System were retrospectively reviewed from 01/03/2015 to 01/06/2016. One hundred and four BAL virology specimens were reviewed from 01/03/2015 to 31/05/2015 as well as twenty-one influenza-positive specimens from 01/09/2014 to 30/03/2015. One hundred and nine mycology specimens were reviewed from 01/01/2015 to 01/07/2015 as well as forty-three *Aspergillus fumigatus* (*A. fumigatus*)-positive specimens from 01/01/2015 to 01/01/2016. PJP and *L. pneumophila*-positive specimens were reviewed from 01/01/2013 to 01/01/2016. This study was reviewed and approved by the institution's research ethics board with consent form waived.

Method

A retrospective review of all BAL specimen results.

Bacteriology Workup

The gram stain of the BAL was first examined under a microscope for the presence and semi-quantitation of pus cells, bacteria, and yeast. Then 1 µL of BAL fluid was inoculated onto the sheep blood (SBA), chocolate (CHOC) and MacConkey's (MAC) agar plates. The MAC plate was finalized at 24 hours and the other plates at 48 hours if no growth. Up to 3 pathogens were worked up from one BAL specimen. Microbiologists were consulted if 4 or more pathogens identified.

Virology Workup

Test was performed in the Virology/Molecular Laboratory of Microbiology, Hamilton Regional Laboratory Medicine Program (HRLMP). 2.0 mL of BAL was added to universal transport media (UTM) and vortexed for 20 seconds. 200µl was used for nucleic acid extraction by easyMAG (bioMérieux). Respiratory viruses (Influenza A and B, Respiratory syncytial virus A and B, Parainfluenza 1, 2 and 3, human Metapneumovirus, Adenovirus and Rhinovirus/Enterovirus) were then detected by Reverse transcription followed by polymerase chain reaction (RT-PCR) on the Rotor-Gene (Corbett Research, Australia).

Fungal Workup

Test was performed in the Regional Mycology Laboratory of Microbiology, HRLMP. BAL smears were stained with KOH-Calcofluor and examined under a fluorescent microscope for fungal elements. BAL were then set up on 3 plates (Mycosel, SABDEX, BHI with 10% sheep blood plus chloramphenicol and gentamycin [CG]) and incubated at 30°C for 4 weeks. Fungal colonies on plates were stained with lactophenol

cotton blue reagent and examined microscopically for fungal spores and other structures. Identification to the genus level was mainly based on the examination of their macroscopic and asexual microscopic characteristics.

PJP Detection

The cysts and trophozoites of PJP in BAL was detected by the MONOFLUO Pneumocystis Immunofluorescent Antibody Test Kit (Bio-Rad) at the Regional Mycology Laboratory of Microbiology, HRLMP. BAL specimen smears were incubated with staining reagent for 30 minutes at 37 °C. Slides were washed and air dried. A drop of anti-quenching mounting medium was added to mount the slides before viewing with a fluorescent microscope. Bright apple-green fluorescing characteristic staining patterns indicate the detection of PJP.

Legionella Detection

BAL specimens were referred to Public Health Ontario Laboratory (PHOL), ON. Legionella specimens were examined by PCR first and all PCR positives are cultured as per the reference lab protocol. Cultures grown are identified to the genus, species and serogroup levels.

RESULTS

Excellent Concordance between Multiple Bacterial Specimens

One thousand sixty-three BAL specimens were ordered for bacteriology culture from 01/03/2015 to 01/06/2016. 16% of these specimens had no growth of any organism, and 31% grew mixed non-pathogenic aerobic organisms which were consistent with oral commensal flora ($\geq 10^6$ CFU/ml). Commonly isolated bacteria were methicillin-sensitive *Staphylococcus aureus* (8%), *Pseudomonas aeruginosa* (7%), methicillin-resistant *Staphylococcus aureus* (5%), *Streptococcus pneumoniae* (5%), *Stenotrophomonas maltophilia* (4%), *E. coli* (3%), *Serratia marcescens* (3%), *Haemophilus influenzae* (1%), *Klebsiella pneumoniae* (1%). 5% were positive for atypical mycobacterium including *Mycobacteria avium complex* (3%) and *Mycobacterium marseillense* (2%). *A. fumigatus* was identified in 1%, and pure *Candida spp.* in 10% (data not shown). As shown in Table 1, the concordance rate between multiple BAL specimens acquired from a single bronchoscopy procedure was 96.5% in the group of 2 specimens per procedure and 97.6% in the group of 3 and more specimens per procedure.

Table-1: The concordance rate of multiple BAL specimens collected per bronchoscopic procedure

BAL # per bronchoscopy	Bacteriology specimen #		Concordance %
	+ (discordance)	-	
1	244	380	N/A
2	204 (14)	194	96.5%
≥ 3	23 (1)	18	97.6%
BAL # per bronchoscopy	Virology specimen #		Concordance %
	+ (discordance)	-	
1	0	28	N/A
2	10 (1)	60	98.6%
≥ 3	0	6	100%
BAL # per bronchoscopy	PJP specimen #		Concordance %
	+	-	
1	7	313	N/A
2	0	60	100%
≥ 3	0	14	100%
BAL # per bronchoscopy	<i>Legionella pneumophila</i> specimen #		Concordance %
	+	-	
1	2	2408	N/A
2	2	340	100%
≥ 3	0	67	100%

Excellent Concordance between Multiple Virology Specimens

One hundred and four BAL specimens were ordered for virology multiplex PCR from 01/03/2015 to 31/05/2015, and 15 were positive for influenza (13.3%), herpes simplex virus (66.7%), cytomegalovirus (13.3%) or rhinovirus (6.7%). The concordance rate of multiple samples is 98.6% among the group of 2 specimens per procedure and 100% among the group of 3 specimens per procedure (Table-1). Influenza was identified only

in 2 specimens, one of which grew both influenza and rhinovirus in the right upper lobe, but only influenza in the left upper lobe. The small number of influenza-positive specimens in this study period has provided us with limited information regarding whether a single specimen would be sufficient to detect this virus, thereby more samples were reviewed for positive influenza results (21 positives out of 2979 specimens) during a typical influenza season from 01/09/2014 to 30/03/2015. 93.8% concordance was found in the group

with 2 specimens per BAL procedure (16 specimens) as shown in Table-2. The one discordant BAL grew Influenza and rhinovirus from the right lower lobe, missing the rhinovirus in the left upper lobe.

Interestingly, 11 out of 21 (52.4%) influenza-positive specimens were shown to be co-infected with

another pathogen. Among them, 5 were co-infected with another virus, including RSV (80%) and rhinovirus (20%); 4 with bacteria, including MRSA (75%) and *E. coli* (25%); 3 with fungus, including *A. nidulans* (33.3%) and *A. fumigatus* (66.7%). One specimen was detected positive for three pathogens including influenza, rhinovirus as well as *A. fumigatus*.

Table-2: The concordance rate of Influenza A/B detected by multiple BAL specimens collected per bronchoscopic procedure (01/09/14-30/03/15)

BAL # per bronchoscopy	Influenza A/B (+) specimen #	Concordance %
1	5	N/A
2	16	93.8%

Excellent concordance between multiple PJP and Legionella specimens

As shown in Table-1, 394 BAL specimens were ordered for PJP from 01/01/2013 to 01/01/2014 with a concordance rate of 100% in 74 paired specimens which were negative for PJP. To understand the effect of specimen number per procedure on the result of PJP detection, twenty six PJP-positive specimens were reviewed from 01/01/2013 to 01/01/2016. 100% concordance was found in the group with two specimens per procedure (data not shown).

Two thousand eight hundred fifty-one specimens were requested for *Legionella pneumophila* culture from 01/01/2013 to 01/01/2016 and referred to public health laboratory, Toronto. Among them, only 2 specimens from same bronchoscopy procedure were *Legionella pneumophila* positive (0.07%). The concordance rate among negative samples was 100%.

Single specimen is not reliable for mycology culture

22 out of 119 mycology specimens from

01/03/2015 to 31/05/2015 grew *Candida.spp* in significant amount ($>10^6$ CFU/ ml) with 100% concordance rate in the multiple mycology specimens (data not shown). A significant growth of *Candida.spp* is considered positive in this study due to the lack of clinical information to evaluate true infection versus colonization. To further study whether one specimen is sufficient for the detection of filamentous fungi, we reviewed 43 BAL samples which grew *A. fumigatus*, a very common pathogenic respiratory fungus, from 3020 BAL mycology specimens (01/01/2015 to 01/01/2016). A concordance rate of only 60% was found between two specimens obtained from different lung lobes (12 out of 20 specimens), which indicates that one specimen may not be reliable when the fungal infection is suspected (Table-3). Interestingly, among the discordant BAL pairs, 4 were co-infected with influenza A/B, 1 with CMV and HSV, and another failed to grow on plates with fungal filaments identified under the microscope. Of note, all these discordant fungal culture results were reported as light growth or one colony.

Table-3: The concordance rate of Aspergillus fumigatus detected by multiple BAL specimens collected per bronchoscopic procedure (01/01/15-01/01/16)

BAL # per bronchoscopy	<i>A. fumigatus</i> (+) Specimen #	Concordance %
1	8	N/A
2	20	60.0%
3	15	53.3%

DISCUSSION

The usefulness of BAL is well-established in both immunocompetent and immunocompromised patients with non-resolving pneumonia [2, 3, 7, 8]. However, the current practice of the utilization of BAL specimens varies widely among physicians, which not only places a costly burden on health care system but also potentially puts patients at increased risk for the complications of bronchoscopy [12]. In this study, we found excellent concordance between multiple specimens for bacteriology and virology, which therefore should be minimized to one specimen per procedure sent from the most purulent lung lobe under bronchoscopy. More interestingly, we identified the necessity of collecting multiple samples for mycology culture to avoid diagnostic failures. So far, there are two

published studies trying to address the issue of using BAL specimens efficiently, however, only focused on bacteriology with conflicting results. To our knowledge, this is the first study examining the efficacy of BAL specimens in all the areas of bacteriology, virology, mycology, PJP and *Legionella pneumophila*.

We have reported excellent concordance rates between multiple specimens in Bacteriology (96.5% for two specimens/per BAL group, 97.6% for three and more specimens/per BAL group). Among the 15 discordant specimens, 10 discordances were due to laboratory errors which failed to identify the bacteria in low amount on agar plates, while 5 failed to grow on the culture plates. Our concordance rates are much higher than the rate reported by the Zaccard *et al.*,

Group [4]. Both studies found the similar overall positive bacterial growth rate and the bacteria profile (types and proportion). The gap of the concordance rate may be attributed to the differences of study design, patient population and sample collection. The specimens in our study were compared between different lung segments, contrastingly, the Zaccard *et al.*, Group compared the difference between BAL samples acquired from both left and right lungs, which might result in a larger discordance if the infection is unilateral. Also, the different patient population composition might play a role. Trauma patients accounted for 69% of the population in the Zaccard *et al.*, study, while we don't have information about patients underlying diseases, but it may be reasonable to presume a relatively higher cancer population in our study due to the involvement of a tertiary cancer center in our system.

In contrast to our study and Zaccard study, Jonker *et al.*, Group reported a high discordance rate (37.1%) in the bilateral BAL pairs and recommended bilateral BAL sampling over unilateral sampling [5]. Similarly, as documented in the Zaccard study, bilateral lung lobes were washed, and comparison was made between left and right sides and our comparison was made between purulent segments as discussed previously. The different methods of collection BAL specimens might also explain the high discordance rate in Jonker study. They aspirated the initial pus after insertion of the bronchoscopy and cultured as a separated sample before washing lung lobes with normal saline, it is difficult to assess whether the excluded pus might contribute to the large difference of bilateral BAL pairs without further information of culture results of pus from their published paper.

In this study, 52.4% of BAL specimens positive for *influenza* were shown to be co-infected with another pathogen, with RSV being the most frequent, followed by MRSA, *A. fumigatus*, rhinovirus, *E. coli*, and *A. nidulans*. This finding is in keeping with the report by the Choi *et.al* group that 51% of influenza-caused severe pneumonia was reported to be co-infected with another pathogen such as *S. aureus*, RSV, *A. fumigatus*, etc [13].

More importantly, we have identified that multiple specimens from purulent segments should be sent for mycology culture, given the high discordance rate of *A. fumigatus* identified. The limitations of a retrospective specimen review prevents us from further studying the underlying mechanism of the discordance. Interestingly, we did observe a fairly high co-infection rate in the discordant group. Four out of 8 were found to be co-infected with Influenza A/B and was reported as 'light' or 'one colony' growth of *A. fumigatus*, which raises the question of whether this low burden of fungi reflects colonization or true infection. Another 2 discordant samples failed to grow on plates even though

fungus filaments were identified via microscopy. More studies are warranted to examine the threshold of reporting *Aspergillus* spp. which may shed light on the differentiation between colonization and real infection.

In conclusion, we recommend collecting a single specimen per BAL procedure to be sent from the most purulent lung segment for bacteriology and viral PCR. Single specimens may also be appropriate for *L. pneumophila* and *P. jirovecii*, however further study is needed. Multiple specimens should be submitted for mycology investigations. By eliminating unnecessary duplicate specimens, laboratory utilization can be optimized, and patient morbidity may be decreased.

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