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Accuracy and Precision of Quantitative Calibrated Loops in Transfer of Bronchoalveolar Lavage Fluid

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Quantitative cultures of bronchoalveolar lavage (BAL) fluid are important in the diagnosis of ventilator-associated pneumonia, and calibrated loops are commonly used to set up these cultures. In this study, the performances of calibrated 0.010- and 0.001-ml loops in the transfer of BAL fluid were determined. Five loops of one lot from seven manufacturers were tested. Calibrations were performed by the gravimetric method (0.010-ml loops) and the colorimetric method (0.001-ml loops). Most of the 0.010-ml loops displayed a precision that was less than 10%, but six of them showed very poor accuracies as they transferred a deficiency (nichrome loops) or an excess (disposable loops) of BAL fluid that exceeded ±10%. The mean maximum and minimum BAL fluid volumes delivered by the 0.010-ml loops differed by a factor 3. The 0.001-ml loops displayed acceptable precision. Five of them showed inaccuracies of ≤±10%, and mean maximum and minimum BAL fluid volumes had a range of a factor of 2. For all loops, the volumes of BAL fluid sampled were larger than the volumes of reagent-grade water sampled. Results of the colony counting experiments confirmed these findings and revealed a high intra-assay variability for the 0.001-ml loops. We conclude that, when BAL fluid samples are cultured with calibrated loops, (i) proper verification of the calibration of these loops is mandatory, (ii) calibrations should be performed with BAL fluid as the test solution, and (iii) borderline quantitative culture results should be interpreted with knowledge of the inaccuracy values of these loops.

Quantitative cultures of bronchoalveolar lavage (BAL) fluid are used in the diagnosis of ventilator-associated pneumonia (VAP). As the dilution of the lung secretions in the BAL fluid is 10- to 100-fold, a colony count of $\geq 10^4$ CFU/ml represents a bacterial load of 10^5 to 10^6/ml in the collection site, which is indicative of bacterial pneumonia (3, 15). Conversely, a BAL fluid colony count below the 10^4-CFU/ml threshold points to oropharyngeal contamination. This theoretical concept has been validated in numerous clinical studies, and quantitative culture of BAL fluid specimens is consequently recommended as the reference method for the diagnosis of VAP (6).

For quantitative cultures of BAL fluid, two approaches are used: the serial dilution method and the calibrated loop method (3). In the serial dilution method, 0.100-ml aliquots of the raw BAL fluid and two serial 100-fold dilutions are inoculated onto the agar plate surfaces. After incubation, counts are made from the dilution that contains the greatest number of bacteria without confluence or overcrowding. Many microbiological laboratories perceive this method as too cumbersome and labor-intensive (15). Therefore, they prefer the calibrated loop method, which they are familiar with. Calibrated loops are routinely used to set up quantitative urine cultures (5, 17). Calibrated loops are designed to transfer a well-defined sample volume to agar plates, omitting the need for dilutions. They may be reusable (loops made of nichrome or platinum) or disposable (loops made of plastic). For BAL fluid samples, quantitative calibrated loops designed for the delivery of 0.010 and 0.001 ml are used. After incubation, the colonies are counted on the plates and the number of CFU per milliliter is determined by multiplying the number of colonies by the dilution factor. Calibrated loops are widely used for quantitative BAL fluid cultures in the diagnostic and research settings (4, 7, 8, 11, 12, 19, 20, 21, 23).

In our hospital, quantitative culture of BAL fluid is the standard method for the microbiological diagnosis of VAP (9). We prefer the calibrated loop method and use reusable nichrome loops. By repetition, we observed that colony counts on plates inoculated by use of 0.010-ml loops did not reach a 10-fold range of those obtained by use of 0.001-ml loops. We therefore decided to determine the performance characteristics of different types of calibrated loops.

MATERIALS AND METHODS

Calibrated loops included and transfer procedures. The different quantitative loops that were studied are listed in Table 1. They included both reusable nichrome loops and disposable plastic loops. For each loop type and manufacturer, five loops of one lot were tested. Calibrations were performed by the gravimetric method for the 0.010-ml loops and by the colorimetric method for the 0.001-ml loops (2). Reagent-grade water (Milli-Q plus system; Millipore, Etten-Leur, The Netherlands) and freshly obtained BAL fluid specimens were used as the test solutions.

All procedures were carried out in a standardized way, and the different aliquots were delivered with uniform timing and motion. All containers were widemouthed (diameter, 27 mm), nonadhesive, polypropylene, conical, 50-ml tubes containing 25 ml of test solution (product no. 227.261; Greiner, Alphen aan de Rijn, The Netherlands). The loops were held vertically and were inserted into the test solution just below the liquid's surface, thereby taking care not to immerse the shank. After a check to ensure that no bubbles were within the loops, they were gently lifted straight up and the sample was transferred. Before the first transfer, the nichrome loops were rinsed in a separate flask of reagent-grade water, flamed, and cooled. Together with the loops tested, the volumes delivered by a pipette were determined. For the delivery of a 0.010-ml and a 0.001-ml volume, an adjustable air-displacement pipette (Pipetman P20; Gilson, Villiers-le-Bel, France) was used.

Calibration with reagent-grade water. For the gravimetric method (0.010-ml loops), weight determinations were performed with an analytic balance with a readability of 0.1 mg (AE 166; Mettler-Toledo Ltd., Tiel, The Netherlands). For each 0.010-ml loop, 20 loopfuls of reagent-grade water were successively added to filter paper on the balance pan. All filter papers were handled with forceps, and the weighings were quickly completed in order to avoid any effect of evaporation. The ambient temperature (22 ± 1°C) was checked for each test procedure.

For the colorimetric method (0.001-ml loops), a 0.75% (wt/vol) stock solution of Evan's blue dye (Gurr, Essex, United Kingdom) was prepared in reagent-

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TABLE 1. Quantitative calibrated loops analyzed in the present study

Manufacturer	Type	Vol (ml)	Product no.
Pro-Lab Diagnostics, Merseyside, United Kingdom	Nichrome, reusable	0.010 0.001	PL 100 PL 105
Medical Wire, Corsham, United Kingdom	Nichrome, reusable	0.010 0.001	MW 190 MW 195
Emergo, Landsmeer, The Netherlands	Plastic, disposable	0.010 0.001	10890 10865
Sarstedt, Etten-Leur, The Netherlands	Plastic, disposable	0.010 0.001	86 1562 010 86 1567 010
Greiner, Alphen a.d. Rijn, The Netherlands	Plastic, disposable	0.010 0.001	731175 731165
L.P. Italiana, Milan, Italy	Plastic, disposable	0.010 0.001	LPIT 131058 LPIT 130158
Simport, Beloeil, Quebec, Canada	Plastic, disposable	0.010 0.001	L200-2 L200-1

grade water and was strained through filter paper (product no. 311620; Schleicher & Schuell, Dassel, Germany). The maximal absorbance wavelength was checked with a Kontron Uvikon 680 spectrophotometer (Beun de Ronde Ltd., Abcoude, The Netherlands). Of this stock solution, working dilutions of 0.2:100, 0.4:100, 0.6:100, 0.8:100, 1.0:100, 1.2:100, and 1.4:100 were prepared. Of each working solution, an aliquot of 100 μl was transferred to a flat-bottom microtiter plate (Nunc, Roskilde, Denmark). The absorbances were subsequently read at 600 nm with a microtiter plate reader (SLT 340 ATCC; Beun de Ronde), and a calibration curve was prepared by plotting the absorbance on the vertical scale versus the volume of Evan's blue solution in the working dilutions on the horizontal scale. This curve was checked for linearity and reproducibility. For each 0.001-ml loop, 12 loopfuls of the Evan's blue stock dye solution were successively transferred to 100 μl of reagent-grade water in the flat-bottom microtiter plate. After thorough mixing, absorbances were recorded by the microplate reader, plotted on the calibration curve, and converted into the corresponding volumes.

Calibration with BAL fluid specimens. In addition to reagent-grade water, freshly obtained BAL fluid samples were used as the test liquid by the gravimetric method for the assessment of the 0.010-ml loops. BAL fluid samples were obtained by instillation of four 50-ml aliquots of 0.9% NaCl during bronchoscopy, as described previously (10). The BAL fluid samples were gently mixed on a roller mixer (Coulter Electronics Ltd., Luton, England) before each test. Likewise, 1 ml of a stock solution of 7.5% (wt/vol) Evan's blue in reagent-grade water was diluted in 19 ml of a BAL fluid sample, and this solution was used as the test liquid for the 0.001-ml loops by the colorimetric method.

Description of the test characteristics. For quantification of the performance of the loops, the parameters accuracy and precision were used (2). Accuracy is the closeness of agreement between the stated (expected) volume of the calibrated loop and the mean volume obtained during repeated, controlled deliveries. Accuracy is numerically expressed as inaccuracy, which can be thought of as the difference between the stated volume and the mean measured volume. Inaccuracy is expressed in percent; a positive value and a negative value indicate the delivery of an excess and a deficiency, respectively, compared to the expected volume. Precision expresses the agreement between replicate measurements and can be regarded as intra-assay variability. Precision is numerically expressed as imprecision, which can be considered the coefficient of variation of replicate, controlled measurements.

Statistical analysis. For the gravimetric method, the mean weight was calculated for each loop. This value was converted into the mean volume by using a standard Z value of 1.0032, which corresponds to an air pressure of 720 mm Hg at a temperature of 22°C (2). For the colorimetric method, the mean volume of each loop was calculated from the individual absorbance determinations. For each loop, inaccuracy values were calculated by using the mean volume and the nominal (expected) volume. Imprecision values were calculated by using the individual weights, the corresponding mean weight, and the number of weighings for the gravimetric method and by using the corresponding data on volumes for the colorimetric method. Both inaccuracy and imprecision values were expressed as percentages (2).

Quantitative cultures of BAL fluid. For quantitative cultures, a freshly obtained sterile BAL fluid specimen was used as the test liquid, and *Staphylococcus aureus* ATCC 29123 was selected as the test organism. From an overnight culture, a suspension with a density of a no. 1 McFarland standard was made in 0.9% NaCl. A 10^{-5} dilution from this suspension was made in BAL fluid until a final concentration of approximately 10^4 CFU/ml was achieved, and this solution was sampled with the 0.001- and 0.010-ml loops and pipettes. For three loops of each type, three successive samplings were performed. Between the samplings with the individual loops, the test solution was well mixed on a roller mixer. The aliquots were transferred to cystine lactose electrolyte-deficient agar (Becton Dickinson Microbiology Europe, Meylan Cedex, France) and were spread over the entire surface of the agar. After incubation at 35°C for 24 h, the colonies were counted. Mean ± standard deviation (SD) colony counts were determined for each loop type, and the intra-assay variability for each loop was calculated.

RESULTS

The test characteristics assessed for the 0.010-ml loops in the gravimetric method are listed in Table 2. From the data in

TABLE 2. Test characteristics of the 0.010-ml loops assessed by the gravimetric method with reagent-grade water and BAL fluid as the test solutions[a]

Test characteristics	Test solution	Reusable loops		Disposable loops					Pipetman P20 pipette
		Pro-Lab	Medical Wire	Emergo	Sarstedt	Greiner	Italiana	Simport	
Inaccuracy (%)	Reagent-grade water	−53 ± 3.6	−48 ± 4.6	−8 ± 8.0	0 ± 6.7	−40 ± 13.0	+23 ± 4.8	−26 ± 2.7	−1
	BAL fluid	−48 ± 5.3	−33 ± 2.0	+24 ± 12.6	+49 ± 1.1	+8 ± 7.4	+58 ± 7.3	+18 ± 3.9	0
Imprecision (%)	Reagent-grade water	18 ± 1.4	13 ± 2.1	11 ± 1.3	16 ± 2.2	19 ± 4.8	11 ± 1.1	17 ± 4.0	1
	BAL fluid	10 ± 3.1	9 ± 1.8	11 ± 1.3	7 ± 0.8	8 ± 1.0	8 ± 1.0	8 ± 0.8	2

[a] For five loops of each type tested, mean ± SD values of inaccuracy and imprecision are expressed as percentages; for the pipette, values of inaccuracy and imprecision for 20 consecutive samplings are listed. Positive and negative values express the delivery of an excess or a deficient volume, respectively.

TABLE 3. Test characteristics of the 0.010-ml loops assessed by the gravimetric method with five different BAL fluid samples as the test solutions[a]

BAL fluid characteristics and manufacturer and test characteristics	BAL 1	BAL 2	BAL 3	BAL 4	BAL 5
Total cell count (10^3/ml)	240	78	195	1,000	109
Vol (ml) recovered	155	135	135	71	105
Total protein concn (mg/liter)	167	21	131	646	2,135
Albumin concn (mg/liter)	88	6	30	274	1,030
Urea concn (mmol/liter)	0.3	0.1	0.3	2.3	3.2
Specific gravity	1.006	1.006	1.005	1.005	1.007
Pro-Lab					
Inaccuracy (%)	−47	−45	−50	−44	−37
Imprecision (%)	10	14	9	10	9
Medical Wire					
Inaccuracy (%)	−32	−38	−45	−41	−37
Imprecision (%)	8	7	8	9	9
Greiner					
Inaccuracy (%)	+11	+2	+3	+4	−4
Imprecision (%)	8	6	7	5	9
Simport					
Inaccuracy (%)	+20	+19	+16	+16	+20
Imprecision (%)	8	5	8	8	9

[a] For three loops of each type tested, mean values of inaccuracy and imprecision are expressed as percentages. Positive and negative values express the delivery of an excess or a deficient volume, respectively.

Table 2, it is clear that the transfers of both reagent-grade water and BAL fluid specimens by the adjustable pipette were accurate and precise. The calibrated loops displayed less favorable test characteristics, and both inaccuracy and imprecision varied according to the fluid transferred. For the transfer of reagent-grade water, most calibrated loops delivered a volume that was too small, resulting in negative inaccuracy values. The nichrome loops delivered the smallest volumes; i.e., instead of the expected 10-μl volume, mean volumes of only 4.7 and 5.2 μl were delivered by the Pro-Lab and the Medical Wire loops, respectively. The Italiana loop was the only loop that transferred an excess of reagent-grade water, and the Sarstedt loop delivered exactly the expected volume. With BAL fluid samples as the test fluid, all loops transferred larger volumes than those of reagent-grade water transferred, and the inaccuracies varied accordingly. The difference was most marked for the Greiner loop, which transferred mean volumes of 6.0 and 10.8 μl for reagent-grade water and BAL fluid, respectively. For the transfer of BAL fluid, only one of seven loop types tested (i.e., Greiner) displayed inaccuracy values of $\leq \pm 10\%$. Furthermore, the range between the inaccuracies of the different loops for the transfer of BAL fluid was high. The difference between the mean BAL fluid volume transferred by the Pro-Lab nichrome loop, on the one hand, and the Italiana loop, on the other, was more than 10 μl, or, in other words, the Italiana 0.010-ml loop delivered threefold the volume sampled by the Pro-Lab homologue. The imprecision values for all different types of loops for the transfer of BAL fluid were fairly good relative to our other findings (i.e., precision variation of >15 to 20%), and the imprecision values for BAL fluid transfer were better than those obtained for the transfer of reagent-grade water. To exclude factors related to the BAL fluid specimen applied in the assay as the cause of the differences observed, both nichrome loops and the disposable Greiner and Simport loops were assessed with four additional BAL fluid specimens with different protein contents and total cell counts (Table 3). The results were similar to those obtained with the initial BAL fluid specimen.

The test characteristics for the 0.001-ml loops are listed in Table 4. In line with the results for the 0.010-ml loops, the volume of BAL fluid sampled was larger than the corresponding volume of reagent-grade water. The nichrome loops dis-

TABLE 4. Test characteristics of the 0.001-ml loops assessed by the colorimetric method with reagent-grade water and BAL fluid as the test solutions[a]

Test characteristic	Test solution	Reusable loops		Disposable loops					Pipetman P20 pipette
		Pro-Lab	Medical Wire	Emergo	Sarstedt	Greiner	Italiana	Simport	
Inaccuracy (%)	Reagent-grade water	+7 ± 5.7	0 ± 5.9	−53 ± 10.1	−45 ± 12.9	−46 ± 8.5	−40 ± 6.7	−53 ± 3.6	−1
	BAL fluid	+26 ± 7.7	+16 ± 5.0	+4 ± 3.8	+6 ± 1.6	+2 ± 6.4	+5 ± 5.3	−39 ± 4.6	+3
Imprecision (%)	Reagent-grade water	19 ± 4.3	11 ± 2.5	26 ± 4.1	19 ± 3.0	22 ± 9.0	18 ± 2.2	18 ± 2.3	2
	BAL fluid	6 ± 2.4	4 ± 1.1	11 ± 1.9	8 ± 3.1	9 ± 1.8	7 ± 1.3	12 ± 3.7	3

[a] For five loops of each type tested, mean ± SD values of inaccuracy and imprecision are expressed as percentages; for the pipette, values of inaccuracy and imprecision for 12 consecutive samplings are listed. Positive and negative values express the delivery of an excess or a deficient volume, respectively.

TABLE 5. Colony counts for three loops of each type, tested in triplicate, with a control culture of *S. aureus* in BAL fluid as the test solution

Loop	Mean ± SD CFU/ml (10^4)							
	Reusable loops		Disposable loops					Pipetman P20 pipette
	Pro-Lab	Medical Wire	Emergo	Sarstedt	Greiner	Italiana	Simport	
0.010 ml	0.61 ± 0.15	0.64 ± 0.14	1.3 ± 0.18	1.6 ± 0.29	1.1 ± 0.27	1.7 ± 0.78	1.6 ± 0.73	1.3 ± 0.26
0.001 ml	1.9 ± 0.18	2.1 ± 0.57	3.5 ± 0.75	2.4 ± 0.21	3.6 ± 0.20	2.6 ± 0.33	1.9 ± 0.31	2.3 ± 0.65

played the smallest inaccuracies in the transfer of reagent-grade water, but they performed less well in the transfer of BAL fluid. For the transfer of BAL fluid, better inaccuracy values were found compared to those for the 0.010-ml loops. Three of seven types of loops tested showed inaccuracy values of ≤5%. The highest difference between the inaccuracies of the 0.001-ml loops in the transfer of BAL fluid was found between the Pro-Lab and the Simport loops, and this difference reached 65%; that is, the Pro-Lab loop delivered approximately twofold the volume of BAL fluid transferred by the Simport loop. As for the 0.010-ml loops, the imprecision values for all 0.001-ml loops were satisfactory when BAL fluid was used as the test fluid, and the inaccuracy values were similar for BAL fluid samples with different protein contents and total cell counts (data not shown).

The culture results (mean colony counts) obtained with the 0.010-ml and the 0.001-ml loops are listed in Table 5. For most of the 0.010-ml loops, the differences in mean colony counts reflected the calibrated differences. The nichrome reusable loops consistently generated low colony counts, and three disposable loop types yielded higher colony counts compared to those achieved by use of the pipette. The mean maximum and minimum colony counts delivered by the loops differed by a factor of 2.8. As can be read from the SD values, the variations between the mean colony counts obtained with the individual loops were higher than those recorded by use of volume calibration. In addition, intra-assay variabilities exceeding 10% were observed for the triplicate samplings for 13 of 21 individual loops tested.

Overall, the 0.001-ml loops and the pipette generated higher colony counts compared to those obtained with the 0.010-ml loops. The differences in mean colony counts between the 0.001-ml loops of various manufacturers were higher than those observed by use of the calibrated volumes. The triplicate samplings for the 21 loops tested generated intra-assay variabilities that exceeded 10% for 19 loops and 30% for 6 loops. The mean maximum and minimum colony counts delivered by the loops differed by a factor of 1.9.

DISCUSSION

In the present study, we assessed the performances of both reusable and disposable quantitative calibrated loops in the transfer of reagent-grade water and BAL fluid. The 0.010-ml calibrated loops displayed good precision, but most of them showed poor accuracies and delivered volumes of BAL fluid that were too small (nichrome loops) or delivered an excess volume (disposable loops). The 0.001-ml calibrated loops displayed good precision, and four of seven types tested delivered a BAL fluid volume with an inaccuracy of $<\pm 10\%$. The mean maximum and minimum BAL fluid volumes delivered by the 0.010- and 0.001-ml loops of various manufacturers differed by factors of 3 and 2, respectively. The results of the colony counting experiments confirmed these findings but revealed a high intra-assay variability for replicate samplings for the 0.001-ml loops.

According to leading textbooks, quantitative calibrated loops may be used for various purposes such as preparation of inocula for antimicrobial tests, preparation of serum dilutions, addition of ingredients to media and reagents to test procedures, and setting up of quantitative cultures (2, 22). For the quantitative culture of BAL fluid specimens, the 0.010- and 0.001-ml calibrated loops are very attractive because at final dilutions of 1:100 (0.010-ml loop) and 1:1,000 (0.001-ml loop), a minimum concentration of 10^2 CFU/ml can be detected and a maximum concentration of more than 10^5 CFU/ml can be discerned without piling up of the colonies on the agar plate's surface. In this way, the consensus culture cutoff point of 10^4 CFU/ml for the diagnosis of VAP (13) is within the reach of this technique. The calibrated loop method is more amenable to a routine clinical microbiology laboratory than the serial dilution method, and its feasibility allows performance during off hours. For inoculation of clinical samples in the microbiological laboratory, calibrated loops are preferred over pipettes because the latter are generally slower and may present problems in terms of decontamination, cross-contamination, and infectious aerosol control (5). Calibrated loops are also used for quantitative cultures of other respiratory specimens such as protected specimen brush (PSB) samples (14, 16) and bronchial aspirates (18).

The results of the present study demonstrated poor accuracies for the majority of the 0.010-ml loops. The 0.001-ml loops performed better, and several manufacturers are marketing 0.001-ml loops that displayed excellent accuracy for the transfer of BAL fluid. It should be noted that we tested only loops that belonged to single lots and that we do not have further grounds to prefer one manufacturer's loop over another. Irrespective of the loop that is chosen, we underscore the need for determination of its accuracy before use and at any time that a new lot is used, as recommended by Baron (2). Although not required for calibrated loops (2), we elected to determine their precision. When applied to set up a quantitative BAL fluid culture, calibrated loops (including disposable ones) are repeatedly used for inoculation of several enriched and selective agar plates, and for this reason we were interested in precision as a test characteristic. In the volume calibration experiments, the imprecision values were considered satisfactory, but these values were not reflected by low intra-assay variabilities in the colony counting experiments. Replicate transfers by the 0.001-ml pipette also yielded higher variations in colony counts, and the ranges in colony counts observed for the 0.001-ml loops were higher than those observed for the 0.010-ml loops. Therefore, it is tempting to speculate that sampling error has been the cause of the high intra-assay variability: irregular distribution of organisms and the presence of cellular material, mucus, and debris may have influenced the number of organisms present in the sample transferred. Procedures such as vortexing or filtration may facilitate further homogenization of the BAL fluid specimen, but because they alter the morphologies of the cells in the BAL fluid specimen, we prefer to mix the BAL fluid samples on a roller mixer designed for peripheral blood samples.

For the 0.010-ml loops as well as for the 0.001-ml loops, we found a large range between the mean maximum and minimum volumes transferred by the loops of different manufacturers. From Table 5, it is clear that a single BAL fluid sampled with loops from different manufacturers may yield colony counts spread out on either side of the 10^4-CFU/ml cutoff point for VAP. Therefore, BAL fluid samples with quantitative culture results that approximate the threshold value should not be strictly categorized as VAP positive or VAP negative by exclusive dependence on their colony counts. Borderline quantitative culture results should be interpreted together with information on the patient's clinical condition and on the use of antibiotics prior to bronchoscopy and together with data on the BAL fluid's cytology (3, 9).

Apart from the performance characteristics linked to the calibration of the loop, many other variables may cause sampling errors. Less fluid is sampled when containers with small diameters are used, and a larger volume is sampled when the loop is inserted and withdrawn at a 45° angle or when the shank of the loop is immersed (1, 2). Containers with small diameters may cause the loop to pick up a smaller volume since plastic-liquid (adhesive) forces are greater than liquid-liquid (cohesive) forces (1). When the shank of the loop is wetted by deep immersion into the fluid, excess liquid drains down and enlarges the volume transferred (2). In the present study, all these factors were carefully controlled, but in daily practice, they are additional reasons for a loop's inaccuracy and they may account for the high degree of variability in the volume of BAL fluid sampled.

It is of interest to compare the present results with those of a previous study on the use of calibrated loops in respiratory specimens. With PSB samples immersed in laboratory stock cultures as well as with clinically obtained PSB samples, Middleton and coworkers (15) compared the culture results obtained by both the serial dilution and the calibrated loop methods. When their results are compared with the present findings, several factors need to be considered. First, Middleton and coworkers used platinum loops, and these loops were not included in the present study. To our knowledge, platinum loops are not commonly used because of their high costs (their price approaches that of a pipette). Next, the PSB samples cultured in the previous study were diluted 1:1,000 in lactated Ringer's solution, and this solution may have cohesive and adhesive forces different from those for the BAL fluid specimens in the present study. Furthermore, in the previous study, 6 of 14 PSB samples cultured by the serial dilution method yielded colony counts far above the diagnostic threshold (10^3 CFU/ml) for PSB samples, and 1 sample yielded no growth. The correlation between the serial dilution method and the calibrated loop method for these samples was excellent, although the colony counts obtained with the 0.010-ml loop were lower than those obtained with the 0.001-ml loop and the serial dilution method. If, however, PSB specimens with colony counts obtained by the serial dilution method just above the culture cutoff point were considered, three of six samples did not reach the 10^3-CFU/ml threshold when cultured with the 0.010-ml loop, suggesting a deficiency of the volume transferred by the 0.010-ml platinum loop. In line with the findings of the present study, the 0.001-ml platinum loop in the previous study (15) performed better, with no threshold discrepancy between the culture results obtained with this loop and by the serial dilution method.

In conclusion, the results of the present study indicate that for the quantitative culture of BAL fluid specimens, most of the 0.001-ml loops displayed good accuracy, but the majority of the 0.010-ml calibrated loops did not meet acceptable accuracy limits for the transfer of BAL. If calibrated loops are considered for use in the setup of quantitative BAL fluid cultures, proper verification of their calibration is mandatory. Calibrations should be performed with BAL fluid samples and not with reagent-grade water. Borderline quantitative BAL fluid culture results obtained by the calibrated loop method should be interpreted with knowledge of the inaccuracy values for these loops.

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