# New Direct Quantitative Method for Comparative Evaluation of Swab Collection and Transport Systems.

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## ABSTRACT

Comparative evaluations of swab collection/transport systems have been based on qualitative 4 quadrant streak methods or on more complex quantitative elution/dilution methods. We evaluated the BBL CultureSwab Plus (BBL) and the Starplex Starswab II (STAR) by a new, direct roll-plate quantitative method and compared results to a standard quantitative elution/dilution method. A total of 10 organisms were tested by both swab systems using both quantitative methods. For the roll-plate method, 100 *u*l of a $10^5$ and of a $10^6$ CFU/ml suspension of each organism was added to the swabs. Duplicate swabs of each type were cultured after incubation at room temperature of 0, 4, 8, 24, and 48 h to appropriate media by rolling approximately $120^o$ of the swab over the surface of the agar plate. This inoculation was performed twice more by rotating the plate $60^o$ and rotating the swab $120^o$ to ensure contact of the entire swab to the entire agar surface. For the elution/dilution method, 100 *u*l of a $10^5$ or $10^6$ CFU/ml organism suspension was added to BBL and STAR swabs. After incubation at room temperature for 0, 4, 8, 24, and 48 h, swabs were placed into 0.9 ml sterile saline and mixed well to elute the organism from the swab. Two further 1:10 dilutions in sterile saline were prepared and 100 *u*l of each of the 3 dilutions were plated to appropriate media. All media were incubated at $35^oC$ for up to 48 h. Colony counts were obtained for each incubation period and compared to the 0 h colony count to determine % recovery of each organism by each test method. The % recovery was considered to be in agreement (± one-$\log_{10}$ in % recovery) for 39 of the 40 (97.5%) STAR comparative determinations and for 38 of the 40 (95%) BBL determinations. The 3 discrepancies included *H. influenzae* recovered at 0.2 % with the BBL elution/dilution method and at 5% with the roll-plate method after 24 h, and at 48 h, the *S. pneumoniae* in STAR recovered at 0.03% and the *H. influenzae* in BBL recovered at 0.2% while the organisms were not recovered by the corresponding elution/dilution methods. The new roll-plate method is an accurate and easy-to-perform method for comparing swab collection/transport systems.


## INTRODUCTION

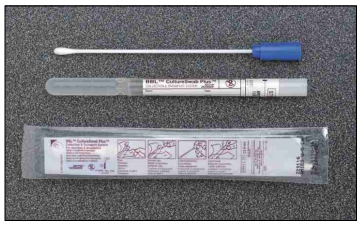
In many clinical microbiology laboratories, specimens are frequently received on swabs. The swab and transport medium are considered to be very important in organism recovery upon culture. Organism survival over extended periods of time in the swab transport system may be affected by the composition of the swab fibers and the swab shaft as well as the pH and medium composition of the transport system. Since there are differences in swab transport systems, several studies have focused on evaluating the various systems for maintenance of viability of both aerobic and anaerobic microorganisms.

Several methods have been employed to test swab transport systems for maintenance of viability after specified incubation times. Two common methods are the qualitative (semi-quantitative) roll/streak method and the quantitative elution/dilution method. For the roll/streak method, the swab is rolled over one quadrant of an agar plate and the plate is streaked in a consistent, standard pattern. Growth is evaluated and semi-quantitation is based on which quadrant(s) grew. For the elution/dilution method, the swab is placed into a sterile fluid (usually saline), mixed with a vortex to elute the organisms into the fluid enhancing the recovery of organisms trapped in the swab tip fibers, the inoculum fluid further diluted, and known quantities of the diluted inoculum fluids plated. Colony counts are then obtained and compared to the 0 h colony counts. Since many laboratories do not vortex swabs in fluid prior to media inoculation, an accurate quantitative roll plate method might be desirable.

In this study, we compared a modified roll plate method as a quantitative method to an elution/dilution (reference) method. For the roll plate method, the swab was systematically rolled over the entire surface of the agar plate and colony counts compared to those at the 0 h reading. Two Amies agar without charcoal swab transport systems, BBL CultureSwab Plus and Starplex Starswab II, were used to minimize swab transport system bias.

Starplex Starswab


BBL CultureSwab



## METHODS

**Organisms Tested:** Freshly grown recent clinical isolates of each of the following: *M. catarrhalis*; *P. aeruginosa*; *E. coli*; *P. multocida*; *S. aureus*; *S. pyogenes*; *S. pneumoniae*; *S. agalactiae*; *H. influenzae*; *E. faecalis*.

**Swab Systems:** BBL CultureSwab Plus (BD Biosciences, Cockeysville, Md) and Starswab II (Starplex Scientific, Etobicoke, Ontario, Canada). Both swab systems have Amies transport medium without charcoal.

**Inoculum preparation, swab inoculation and incubation:** A suspension of each organism was prepared in sterile saline to achieve a turbidity equivalent to a 0.5 McFarland standard (approximately $5x10^8$ org/ml). Each suspension was further diluted to obtain final inoculum concentrations of approximately $10^6$ and $10^5$ org/ml. Duplicate swabs for each incubation time (0, 4, 8, 24, and 48 hr at room temperature) were inoculated with 100 µl of $10^6$ org/ml and/or 100 µl of $10^5$ org/ml as described below.

### Quantitative Swab Test Methods:

1. Elution/Dilution Method (reference)
   a. Swabs were inoculated with $10^5$ org/ml for *E. coli*, *P. aeruginosa*, *E. faecalis*, and *S. aureus* and with $10^6$ org/ml for the other organisms.
   b. Swabs were removed from the transport tube and placed into 0.9 ml of sterile saline, mixed well, and two further 1:10 dilutions prepared (final = undiluted, 1:10, 1:100).
   b. 100 µl of each dilution was inoculated to appropriate media in duplicate, spread over the entire surface of the plate with a sterile spreader, and the plates incubated at 35°C (appropriate atmosphere) for up to 48 hr.
   c. Colony counts were obtained for each incubation time and dilution and compared to the 0 hr colony counts to obtain a % recovery.

2. Direct-Roll Plate Method:
   a. Two swabs were inoculated with $10^5$ org/ml and two with $10^6$ org/ml for each incubation time.
   b. Each swab was removed from the transport system and approximately 120° of the swab surface was rolled over the entire surface of an appropriate medium, Figure 1.
   c. Plates were rotated 60°, the swab rotated 120°, and the swab rolled again through another 120° over the entire surface of the plate.
   d. Step 3 was repeated with the final 120° of the swab surface.
   e. All plates were incubated at 35°C in an appropriate atmosphere for up to 48 hr.
   f. Colony counts were obtained for each incubation time and dilution and compared to the 0 hr colony counts to obtain a % recovery.

Methods were considered in agreement if the % recovery was within ± 1-$\log_{10}$ in percent.

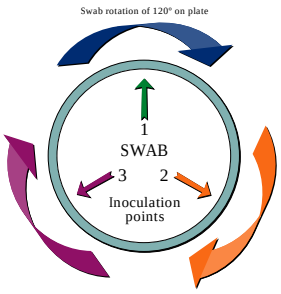



Figure 1. View of tip of swab and contact points with agar plate

## RESULTS

1. The Direct Roll method when compared to the Elution/Dilution method with the BBL CultureSwab system showed recovery agreement of 95%, 38 of the 40 comparative determinations, Table 1.
   a. The *H. influenzae* tested after 24 h room temperature incubation was recovered at 0.2% (5 col, 24h – 2300 col, 0h) by the Elution/Dilution method and 5% (250 col, 24h – 4800 col, 0h) recovered by the Direct Roll method.
   b. The *H. influenzae* tested after 48 h room temperature incubation was not recovered by the Elution/Dilution method with 0.2% (10 col, 48h – 4800 col, 0h) recovered by the Direct Roll method.

2. The Direct Roll method when compared to the Elution/Dilution method with the Starplex Starswab system showed recovery agreement of 97.5%, 39 of the 40 comparative determinations, Table 2.
   a. The *S. pneumoniae* tested after 48 h room temperature incubation was not recovered by the Elution/Dilution method with 0.03% (1 col @$10^6$, 48h – 400 col @ $10^5$, 0h) recovered by the Direct Roll method.

## SUMMARY AND CONCLUSIONS

1. The Direct Roll method described in this study appears to be a reliable method for comparison of swab transport systems when compared to the Elution/Dilution reference method.
   a. Based on the 3 discrepancies the Direct Roll method (all with higher % recovery) may be somewhat more sensitive than the Elution/Dilution method to detect low levels of organisms remaining viable in swab transport systems.
2. The purpose of this study did not include a comparison of swab transport systems, therefore, this data was not evaluated and reported.
   a. Two swab systems were used only as comparators and to help remove any possible bias due to the individual swab transport system for the two recovery methods tested.
3. Organisms that appeared to actually grow within the swab systems were difficult to evaluate at the end points and were considered equivalent if both were determined to be "unreadable" or "greater than" evaluable numbers.

| | | BBL CultureSwab % recovery | | | |
|---|---|---|---|---|---|
| Organism | Method | 4 h | 8 h | 24 h | 48 h |
| M. catarrhalis | Elution/Dilution | 33 | 40 | 31 | 19 |
| | Direct swab roll | 124 | 85 | 70 | 69 |
| P. aeruginosa | Elution/Dilution | 78 | 112 | 1950 | 4000 |
| | Direct swab roll | 38 | 102 | 723 | $>10^4$ |
| E. coli | Elution/Dilution | 287 | 1600 | 1970 | 607 |
| | Direct swab roll | 243 | 386 | 2500 | 2680 |
| P. multocida | Elution/Dilution | 115 | 64 | 42 | 12 |
| | Direct swab roll | 110 | 90 | 24 | 11 |
| H. influenzae | Elution/Dilution | 19 | 6.7 | 0.2 | 0 |
| | Direct swab roll | 28 | 19 | 5 | 0.2 |
| S. aureus | Elution/Dilution | 100 | 50 | 125 | 50 |
| | Direct swab roll | 108 | 108 | 125 | 108 |
| S. agalactiae | Elution/Dilution | 83 | 81 | 85 | 58 |
| | Direct swab roll | 88 | 103 | 140 | 72 |
| S.pyogenes | Elution/Dilution | 100 | 160 | 89 | 57 |
| | Direct swab roll | 89 | 73 | 41 | 32 |
| S. pneumoniae | Elution/Dilution | 3 | 1 | 0 | 0 |
| | Direct swab roll | 5 | 0.2 | 0 | 0 |
| E. faecalis | Elution/Dilution | 500 | 4000 | >10000 | >10000 |
| | Direct swab roll | 95 | 500 | >10000 | >10000 |

Table 1

| | | Starplex % recovery | | | |
|---|---|---|---|---|---|
| Organism | Method | 4 h | 8 h | 24 h | 48 h |
| M. catarrhalis | Elution/Dilution | 32 | 83 | 5 | 0.2 |
| | Direct swab roll | 45 | 25 | 1.7 | 0.6 |
| P. aeruginosa | Elution/Dilution | 77 | 126 | $>10^4$ | $>10^4$ |
| | Direct swab roll | 100 | 133 | $>10^4$ | $>10^4$ |
| E. coli | Elution/Dilution | 200 | 450 | $>10^4$ | $>10^4$ |
| | Direct swab roll | 98 | 250 | $>10^4$ | $>10^4$ |
| P. multocida | Elution/Dilution | 91 | 58 | 0.4 | 0 |
| | Direct swab roll | 300 | 20 | 0.4 | 0 |
| H. influenzae | Elution/Dilution | 0.25 | 0 | 0 | 0 |
| | Direct swab roll | 0.2 | 0 | 0 | 0 |
| S. aureus | Elution/Dilution | 93 | 57 | 57 | 100 |
| | Direct swab roll | 107 | 123 | 58 | 68 |
| S. agalactiae | Elution/Dilution | 95 | 114 | 24 | 9 |
| | Direct swab roll | 100 | 100 | 46 | 13 |
| S.pyogenes | Elution/Dilution | 45 | 96 | 35 | 32 |
| | Direct swab roll | 68 | 100 | 33 | 11 |
| S. pneumoniae | Elution/Dilution | 13 | 7 | 0.1 | 0 |
| | Direct swab roll | 25 | 14 | 0.1 | 0.03 |
| E. faecalis | Elution/Dilution | 74 | 27 | 112 | 26 |
| | Direct swab roll | 63 | 50 | 83 | 36 |

Table 2