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Comparison of 3 swab transport systems for direct release and recovery of aerobic and anaerobic bacteria

Kenneth G. Van Horn*, Carol D. Audette, Kelly A. Tucker[1], Denise Sebeck[2]

Focus Diagnostics, Inc., Cypress, CA 90630, USA

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Abstract

The ability to directly release 5 aerobic and 5 anaerobic bacterial strains from 3 swab transport systems was evaluated by a time zero roll-plate method. The Copan ESwab (Copan Diagnostics, Murrieta, CA), a new nylon-flocked swab with Amies liquid medium, yielded greater organism release and recovered approximately 10-fold more microorganisms than the Becton Dickinson (Sparks, MD) CultureSwab MaxV and Remel (Lenexa, KS) BactiSwab.

Keywords: Swab transport; Release; Aerobic; Anaerobic

Swab collection and transport of clinical specimens continues to be a common practice in many healthcare institutions. Swab transport devices should be able to maintain the viability of microorganisms present, and the swab should allow for release of a sufficient representative portion of the specimen material and preserve the integrity of nucleic acids for amplification testing. Limited studies describe organism or specimen release from swabs in transport systems (Drake et al., 2005; Österblad et al., 2003; Roelofsen et al., 1999).

Most swab collection devices currently on the market are prepared with rayon or Dacron fibers wound onto the tip of the swab shaft. The new ESwab (Copan Diagnostics, Murrieta, CA) is a nylon-tipped swab prepared by spray-on flocked fiber technology that potentially results in better specimen collection and a more efficient release of specimen material with less specimen entrapment than woven fiber-tipped swabs. One study showed better nasopharyngeal epithelial cell yield with the flocked swab compared with a rayon-tipped swab (Daley et al., 2006).

We used an elution method for ESwab and a roll-plate method for MaxV (Becton Dickinson, Sparks, MD) and BactiSwab (Remel, Lenexa, KS) to compare direct initial organism release and recovery capabilities of the 3 swab systems with 5 aerobic and 5 anaerobic isolates.

The inoculum concentration and strains tested were those recommended for swab evaluation testing by the Clinical and Laboratory Standards Institute (2003) M40-A, and the strains included *Haemophilus influenzae* ATCC 10211, *Neisseria gonorrhoeae* ATCC 43069, *Pseudomonas aeruginosa* ATCC BAA-427, *Streptococcus pneumoniae* ATCC 6305, *Streptococcus pyogenes* ATCC 19615, *Bacteroides fragilis* ATCC 25285, *Fusobacterium nucleatum* ATCC 25586, *Peptostreptococcus anaerobius* ATCC 27337, *Prevotella melaninogenica* ATCC 25845, and *Propionibacterium acnes* ATCC 6919. Strains were freshly subcultured at least twice before testing to tryptic soy agar with 5% sheep blood for *P. aeruginosa*, *S. pneumoniae*, and *S. pyogenes*, chocolate agar for *H. influenzae* and *N. gonorrhoeae*, and anaerobic *Brucella* agar with 5% sheep blood for all anaerobes. All isolates were incubated at 37 °C in an atmosphere appropriate for each organism: non-CO_2 for

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* Corresponding author. Tel.: +1-714-503-1392; fax: +1-714-484-1296.
E-mail address: kvanhorn@focusdx.com (K.G. Van Horn).
[1] Present address: Agensys, 1545 17th Street, Santa Monica, CA 90404.
[2] Present address: Morphotek, Inc., 210 Welsh Pool Rd., Exton, PA 19341.

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P. aeruginosa and *S. pyogenes*; 5% CO_2 for *H. influenzae*, *N. gonorrhoeae*, and *S. pneumoniae*; and an anaerobic atmosphere (Becton Dickinson EZ Anaerobe Container System) for the anaerobic isolates.

Each swab was tested for its capacity for direct release and recovery of the organisms immediately after swab inoculation. An initial organism suspension of approximately 10^7 colony-forming units (CFU)/mL in sterile 0.85% saline was prepared and serially diluted 2-fold to obtain final organism suspensions of 10^3 to 10^6 CFU/mL. For the 100% release and recovery growth control plates, 100-μL aliquots of each organism suspension were directly inoculated to duplicate agar plates appropriate for the test organism and the suspension spread with a sterile bent rod over the entire surface of the plate. For swab testing, 3 swabs of each type were inoculated with 100 μL of each organism dilution and allowed to absorb for 15 s; the swabs were then placed into the transport medium for 15 min to allow the swab to equilibrate in the transport system medium. MaxV and BactiSwab swabs were then removed from the transport medium and rolled 3 times over the entire surface of an appropriate agar plate to produce a lawn of inoculum, rotating the plate 60° with each inoculation. The ESwab was mixed in 1 mL of liquid Amies transport medium in the transport tube with a vortex mixer for 5 s to release the organisms. A 100-μL aliquot from each ESwab Amies liquid was plated to the appropriate agar and spread over the entire surface with a sterile bent rod as directed by the manufacturer. Plates were incubated at 37 °C in the appropriate atmosphere for up to 48 h for aerobes and for up to 96 h for anaerobes. Colony counts were obtained by reading plates with 30 to 300 countable colonies. The average CFU recovered was determined for the 100% recovery growth controls and for each swab type, and a percent recovery based on CFU comparison with the 100% growth controls was obtained for each swab system.

The data for recovery of isolates directly from each swab system are shown in Table 1. There was an approximately 10-fold or greater recovery of organisms directly from the ESwab liquid than from the rayon-tipped MaxV and BactiSwab swabs. Recovery from the ESwab liquid ranged from 60.5% to 85.5% for aerobes and 45.9% to 87.0% for anaerobes. The MaxV recovery for aerobes ranged from 4.2% to 7.5% and the BactiSwab recovery ranged from 4.6% to 14.6%. The MaxV recovery for anaerobes ranged from 4.2% to 9.8% and the BactiSwab recovery ranged from 7.0% to 13.6%. The high level of recovery with ESwab was most likely due to the more complete release of organisms from the flocked swab into the liquid as claimed by the manufacturer. Initial recovery of organisms from the MaxV and BactiSwab were essentially equivalent.

The roll-plate method was used in this study to simulate actual direct swab plating procedures performed in many laboratories. However, laboratory plating methods frequently use only a small portion or quadrant of an agar plate and may use multiple plates on which to roll the specimen swab. Thus, our percent release values might actually be higher than what would be seen in the clinical laboratory for these types of swab systems. Recovery from the ESwab was considered to more closely resemble actual laboratory practices, where 100 μL of the liquid Amies would be added to each plate. A swab system that provides increased organism release would theoretically yield a greater number of all organisms including pathogens and commensals if present. This would increase the potential for more pathogens to be detected in mixed cultures, particularly if selective media are used. Also, infections due to low numbers of organisms might be more efficiently detected on culture if collected with a swab system that has enhanced release capability. Our testing was performed only at the time of swab inoculation and did not include extended swab incubation times, which would have introduced the variable of each swab system's capability to maintain organism viability. We speculate that initial release capabilities would correlate to extended swab incubation times. The data should be used for comparison purposes of each swab system and may not reflect actual recovery rates if clinical specimens were to be tested.

In a previous study, a Copan swab with liquid Stuart's was reported to release more organisms than a Starplex liquid Stuart's swab, based on consistently higher time zero

Table 1
Direct release and recovery of bacteria with 3 swab transport systems

Organism (100% CFU)	Swab type	Recovery (CFU)	% Recovery
S. pyogenes (7.6×10^6)	ESwab[a]	4.6×10^6	60.5
	MaxV	5.7×10^5	7.5
	BactiSwab	5.2×10^5	6.8
S. pneumoniae (4.1×10^6)	ESwab	3.2×10^6	78.0
	MaxV	2.3×10^5	5.6
	BactiSwab	6.0×10^5	14.6
N. gonorrhoeae (1.1×10^7)	ESwab	9.4×10^6	85.5
	MaxV	5.7×10^5	5.2
	BactiSwab	6.2×10^5	5.6
H. influenzae (9.8×10^6)	ESwab	7.9×10^6	80.6
	MaxV	6.5×10^5	6.6
	BactiSwab	8.0×10^5	8.2
P. aeruginosa (1.6×10^7)	ESwab	1.1×10^7	68.8
	MaxV	6.7×10^5	4.2
	BactiSwab	1.5×10^6	9.4
B. fragilis (2.2×10^7)	ESwab	1.9×10^7	86.4
	MaxV	1.3×10^6	5.9
	BactiSwab	2.1×10^6	9.5
F. nucleatum (4.0×10^7)	ESwab	3.1×10^7	77.5
	MaxV	3.9×10^6	9.8
	BactiSwab	5.3×10^6	13.3
P. anaerobius (9.1×10^6)	ESwab	7.0×10^6	76.9
	MaxV	5.9×10^5	6.5
	BactiSwab	9.2×10^5	10.1
P. acnes (2.3×10^7)	ESwab	2.0×10^7	87.0
	MaxV	1.7×10^6	7.4
	BactiSwab	2.1×10^6	9.1
P. melaninogenica (7.4×10^6)	ESwab	3.4×10^6	45.9
	MaxV	3.1×10^5	4.2
	BactiSwab	5.2×10^5	7.0

[a] ESwab is 100 μL of Amies liquid from the ESwab transport tube.

CFU recovered (Drake et al., 2005). However, there was no indication as to how this correlated with the actual number of organisms released. Release and recovery of organisms at time zero has been reported to be low when swabs are placed into agar transport media, with only 8 of 44 recovery data points showing >25% relative recovery compared with the initial inoculum (Roelofsen et al., 1999). Another study tested the weight of the swab after agar plating compared with the weight before plating and reported low amounts of liquid released during the plating process (Österblad et al., 2003). That report did not indicate the number of organisms released and recovered compared with the original inoculum; therefore, organisms might still have been entrapped in the swab material. This study did not assess the ability of any swab system to maintain organism viability over time; however, the ESwab has been shown to be an acceptable swab transport system for maintenance of viability of aerobic and anaerobic organisms (Van Horn et al., 2008).

Because molecular detection of microorganisms has increased in recent years, a study defining the acceptability of the ESwab and flocked swab technology to preserve the nucleic acid of organisms might also be warranted. When compared against 3 nucleic acid amplification kit swabs, the nylon-flocked swab enhanced the analytical sensitivity of molecular detection of both *Chlamydia trachomatis* and *N. gonorrhoeae* (Chernesky et al., 2006). The authors presumed this to be due to enhanced initial trapping of the specimen, better stability in the transport system, and enhanced release of the amplified target. The data from our study indicate that molecular detection of organisms might be improved because of increased organism release from the swab into the transport liquid.

In conclusion, the ESwab appears to release microorganisms more efficiently than the 2 rayon-tipped swabs with Amies agar gel that were tested. Our testing included a high inoculum, and additional testing with low numbers of organisms and clinical samples for organism release may be necessary to fully evaluate the release capabilities of swab systems. Further studies to assess maintenance of nucleic acids for molecular testing may be warranted.

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References

Chernesky M, Castriciano S, Jang D, Smieja M (2006) Use of flocked swabs and a universal transport medium to enhance molecular detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. *J Clin Microbiol* 44:1084–1086.

Clinical and Laboratory Standards Institute (2003) Quality Control of Microbiological Transport Systems. Approved Standard M40-A. Wayne, PA: CLSI; 2003.

Daley P, Castriciano S, Chernesky M, Smieja M (2006) Comparison of flocked and rayon swabs for collection of respiratory epithelial cells from uninfected volunteers and symptomatic patients. *J Clin Microbiol* 44:2265–2267.

Drake C, Barenfanger J, Lawhorn J, Verhulst S (2005) Comparison of Easy-Flow Copan liquid Stuart's and Starplex swab transport systems for recovery of fastidious aerobic bacteria. *J Clin Microbiol* 43: 1301–1303.

Österblad M, Järvinen H, Lönnqvist K, Huikko S, Laippala P, Viljanto J, Arvilommi H, Huovinen P (2003) Evaluation of a new cellulose sponge-tipped swab for microbiological sampling: a laboratory and clinical investigation. *J Clin Microbiol* 41:1894–1900.

Roelofsen E, van Leeuwen M, Meijer-Severs GJ, Wilkinson MHF, Degener JE (1999) Evaluation of the effects of storage in two different swab fabrics and under three different transport conditions on recovery of aerobic and anaerobic bacteria. *J Clin Microbiol* 37:3041–3043.

Van Horn KG, Audette CD, Sebeck D, Tucker KA (2008) Comparison of the Copan ESwab system with two Amies agar swab transport systems for maintenance of microorganism viability. *J Clin Microbiol* 46: 1655–1658.