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ARTICLE

Comparison of Copan eSwab with the Copan Venturi Transystem for the quantitative survival of *Escherichia coli*, *Streptococcus agalactiae* and *Candida albicans*

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Abstract Swab transport systems should preserve the viability and stability of micro-organisms in clinical specimens throughout transport and storage. eSwab, a nylon-tipped swab in liquid medium, designed for better specimen collection and less micro-organism entrapment, was evaluated for the maintenance of viability and quantitative survival of *Escherichia coli*, *Streptococcus agalactiae* and *Candida albicans*. The quantitative elution method was used to evaluate eSwab in vitro. In vitro, the recovery of the three micro-organisms was higher in eSwab (97–100%) as compared to the Copan Venturi Transystem (CVT) (86–96%) at room temperature (RT) for time point 0 h and remained similar after 6 h. *E. coli* and *C. albicans* proliferated in both transport systems when preserved beyond 6 h. At 4°C, the recovery of eSwab was higher (>94%) compared to CVT (77–94%) for the micro-organisms tested. eSwab did not only meet the Clinical Laboratory and Standards Institute (CLSI) criteria for microbiological transport devices, but as its recovery rate in vitro was higher than that of CVT, it might also enhance the sensitivity of bacterial culture in the future.

Introduction

Swab transport systems should preserve the viability and stability of micro-organisms in clinical specimens throughout the transport and storage process. Most swab collection devices currently on the market are prepared with rayon, Dacron or cotton fibres wound onto the tip of the swab shaft. The flocked swab (Copan Diagnostics, Italy) is a nylon-tipped swab prepared by a spray-on flocked fibre technique, developed for the transport of bacteria, yeast and viruses [1, 2]. This technique provides stronger capillary action and strong hydraulic uptake of liquids, which should result in better specimen collection. This design should also provide more efficient uptake and release of specimen material and, therefore, less entrapment of specimens. The new transport device, eSwab, is the combination of the new nylon-flocked swab with the modified liquid Amies transport medium. Up until now, only two studies describe the comparison of this new flocked transport system with the current systems available for the recovery and maintenance of aerobic and anaerobic bacteria [1, 2]. Both studies describe the performance of eSwab for the strains described in the CLSI document M40-A [3], i.e. fastidious organisms and strains difficult to culture. These data are also indicated on the package insert provided by Copan. On easy-growing aerobic bacteria or yeasts however, no information is available yet. Therefore, in this study, eSwab was compared with an Amies agar swab system for the maintenance of viability and the quantitative survival of *Escherichia coli*, *Streptococcus agalactiae* and *Candida albicans* in vitro.

Materials and methods

Strains The three strains tested were *E. coli* ATCC 25922, *S. agalactiae* ATCC 13813 and *C. albicans* ATCC 90028.

Quantitative elution The quantitative elution method, as described in the CLSI document M40-A [3], was used to

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Table 1 Recovery of single organisms from the Copan Venturi Transystem (CVT) and eSwab at room temperature (RT)

Organism	Swab system	$\log_{10}$ (cfu/ml, %) recovered			
$\log_{10}$ (cfu/mL) growth control		0h	6h	24h	48h
E. coli, 8.4	CVT	8.0 (96)	8.2 (98)	10.4 (124)	11.0 (131)
	eSwab	8.3 (99)	9.0 (108)	9.8 (117)	10.0 (119)
S. agalactiae, 8.3	CVT	7.2 (86)	6.8 (82)	6.8 (81)	6.4 (77)
	eSwab	8.3 (100)	8.3 (100)	8.3 (100)	8.4 (100)
C. albicans, 6.6	CVT	5.9 (90)	5.9 (90)	6.4 (97)	7.8 (119)
	eSwab	6.3 (97)	6.2 (94)	7.0 (107)	8.0 (123)

evaluate the performance of eSwab (480CE, Copan Diagnostics, Italy) and the Copan Venturi Transystem (CVT, 108C.USE, Copan Diagnostics, Italy). For each microorganism, inoculated swabs were preserved at room temperature (RT) and 4°C for 0 (15 min after inoculation), 6, 24 and 48 h. Also, three mixtures of *E. coli* and *S. agalactiae* (900/100 µL, 500/500 µL and 100/900 µL) were tested under the same conditions.

In each experiment, strains were freshly sub-cultured twice onto Columbia agar plates supplemented with 5% sheep blood (COL5%, 221165, BD Diagnostics, US) and incubated in 5% CO_2 at 35°C. In the single-strain experiments, an initial organism suspension of 0.5 McFarland standard (approximately 10^8 cfu/mL) in sterile saline (0.85% NaCl w/v) was prepared and 10-fold-diluted to obtain a suspension of 10^7 cfu/mL (also used as the positive control suspension). For each time point (n=4) and each temperature (n=2), one swab was inoculated with 100 µL of the 10^7-cfu/mL suspension for the three organisms tested and allowed to absorb the fluid completely (~30 s). Subsequently, all swabs (24 CVT and 24 eSwab) were closed in their respective tube, i.e. eSwab or CVT, and stored at the proper conditions. This experiment was performed in triplicate. The swabs for time point zero (T=0) were processed 15 min after inoculation.

The CVT swabs were mixed well into 1 mL of sterile saline in order to obtain ~10^6 cfu/mL, as already present in the eSwab liquid medium. Subsequently, the CVT suspension and eSwab medium were serially 10-fold-diluted. A 100-µL aliquot of each dilution was inoculated to duplicate COL5% and spread over the entire surface using a sterile L-spatula (174CS01, Copan Diagnostics, Italy). Plates were incubated for 18–24 h in 5% CO_2 at 35°C. Colony counts were obtained by reading plates with 30 to 300 countable colonies. A series of 10-fold dilutions of the positive control suspension was used as growth controls.

In the mixed experiments of both *E. coli* and *S. agalactiae*, an initial organism suspension of 0.5 McFarland (approximately 10^8 cfu/mL) in sterile saline was prepared and 10-fold-diluted to obtain a suspension of 10^7 cfu/mL. Of both 10^7-cfu/mL suspensions, a mixture was made with ratios of 900/100 µL, 500/500 µL and 100/900 µL *E. coli*/*S. agalactiae*, respectively. These mixed suspensions were also used as positive growth controls. Swabs were further inoculated and processed with the mixtures as described above for the single-organism suspensions. This experiment was performed in triplicate. After preservation for 0, 6, 24 or 48 h at RT or 4°C, a 100-µL aliquot of the diluted sample was inoculated onto duplicate MacConkey (221270, BD Diagnostics, US) and CNA agar (221353, BD Diagnostics, US) for the enumeration of *E. coli* and *S. agalactiae*, respectively. MacConkey plates were incubated for 18–24 h at 35°C in ambient air, whereas CNA plates where incubated in 5% CO_2 at 35°C. Colony counts were obtained by reading plates with 30 to 300 countable colonies. A series of 10-fold dilutions of the positive control suspension was used as growth controls.

The $\log_{10}$ of the average cfu recovered was determined for the growth controls (100%) and for each swab type, temperature and time combination, and based upon a cfu

Table 2 Recovery of single organisms from CVT and eSwab at 4°C

Organism	Swab system	$\log_{10}$ (cfu/ml, %) recovered			
$\log_{10}$ (cfu/mL) growth control		0h	6h	24h	48h
E. coli, 8.5	CVT	7.9 (93)	7.9 (94)	8.0 (94)	7.8 (92)
	eSwab	8.3 (98)	8.0 (95)	8.1 (95)	8.9 (105)
S. agalactiae, 8.7	CVT	7.4 (84)	7.3 (83)	7.0 (80)	6.7 (77)
	eSwab	8.5 (97)	8.4 (96)	8.6 (99)	8.3 (95)
C. albicans, 6.4	CVT	6.0 (93)	5.9 (92)	6.0 (94)	5.9 (93)
	eSwab	6.4 (100)	6.5 (101)	6.3 (98)	6.4 (100)

Table 3 Recovery of mixed organisms from CVT and eSwab at RT

Mixture	Organism	Swab system	$\log_{10}$ (cfu/ml, %) recovered			
(*E. coli*/*S. agalactiae*)	$\log_{10}$ (cfu/mL) growth control		0h	6h	24h	48h
900/100	*E. coli*, 8.2	CVT	7.6 (93)	7.0 (85)	9.1 (110)	10.8 (131)
		eSwab	7.9 (97)	8.6 (105)	11.3 (137)	11.0 (133)
	S. agalactiae, 7.1	CVT	5.9 (83)	5.7 (79)	5.2 (73)	5.0 (69)
		eSwab	7.3 (102)	7.3 (102)	7.3 (102)	5.4 (75)
500/500	*E. coli*, 8.5	CVT	6.4 (75)	6.5 (77)	9.0 (106)	10.2 (120)
		eSwab	7.4 (87)	7.6 (90)	10.4 (123)	12.5 (147)
	S. agalactiae, 8.2	CVT	6.4 (78)	5.8 (71)	5.8 (71)	4.7 (58)
		eSwab	7.3 (89)	7.2 (88)	6.8 (83)	5.6 (68)
100/900	*E. coli*, 8.3	CVT	6.7 (81)	7.2 (87)	10.1 (123)	11.1 (134)
		eSwab	7.4 (89)	8.2 (99)	10.6 (128)	10.9 (132)
	S. agalactiae, 9.2	CVT	6.6 (72)	6.3 (69)	6.3 (69)	5.8 (64)
		eSwab	8.3 (90)	8.2 (90)	7.1 (78)	6.1 (67)

comparison with the growth controls expressed in % recovery. According to the CLSI guideline M40-A [3], for samples stored at RT, no more than a 3 $\log_{10}$ decline in cfu is accepted. At RT, for overgrowth, no limit for acceptance is defined. For samples stored at 4°C, no more than a 1 $\log_{10}$ increase in cfu and 3 $\log_{10}$ decline in cfu is acceptable.

Results

The recovery of the three micro-organisms separately was higher in eSwab (97–100%) as compared to CVT (86–96%) at RT for time point 0 h and remained at the same level after 6 h of preservation (Table 1). Preservation beyond 6 h resulted for both *E. coli* and *C. albicans* in proliferation of the micro-organism in both transport systems. *S. agalactiae* recovery, on the other hand, remained stable throughout the experiment at RT. The preservation of eSwab and CVT at 4°C prevented the proliferation of *E. coli* and *C. albicans* (Table 2). The recovery after preservation with eSwab was higher (>94%) as compared to CVT (77–94%) throughout the whole experiment (4°C) for the three micro-organisms tested. Both transport systems, however, did stay within the 3 $\log_{10}$ decrease limit defined by the CLSI.

For the mixed culture of *E. coli* and *S. agalactiae*, similar results were found as compared to the recovery of the micro-organisms separately (Tables 3 and 4). At RT, eSwab showed a higher recovery for both micro-organisms after 0 and 6 h preservation as compared to CVT. Preservation beyond 6 h resulted for *E. coli* in proliferation for both transport systems.

Table 4 Recovery of mixed organisms from CVT and eSwab at 4°C

Mixture	Organism	Swab system	$\log_{10}$ (cfu/ml, %) recovered			
(*E. coli*/*S. agalactiae*)	$\log_{10}$ (cfu/mL) growth control		0h	6h	24h	48h
900/100	*E. coli*, 8.3	CVT	7.5 (90)	7.4 (89)	7.4 (89)	7.3 (88)
		eSwab	8.1 (97)	8.1 (97)	8.3 (100)	8.5 (103)
	S. agalactiae, 7.3	CVT	6.8 (93)	6.0 (83)	5.3 (73)	5.2 (72)
		eSwab	7.2 (99)	7.2 (99)	7.1 (98)	7.1 (98)
500/500	*E. coli*, 8.2	CVT	7.0 (85)	7.0 (85)	6.4 (78)	6.8 (82)
		eSwab	7.9 (96)	7.6 (92)	7.6 (93)	8.1 (98)
	S. agalactiae, 8.1	CVT	6.0 (75)	6.3 (77)	5.1 (64)	6.3 (78)
		eSwab	7.6 (94)	7.4 (91)	7.2 (89)	6.8 (84)
100/900	*E. coli*, 8.1	CVT	6.8 (84)	6.5 (80)	5.9 (72)	6.5 (79)
		eSwab	7.4 (91)	7.3 (89)	7.6 (93)	7.4 (91)
	S. agalactiae, 8.9	CVT	6.5 (73)	6.2 (70)	6.3 (71)	5.6 (63)
		eSwab	8.2 (92)	8.0 (91)	8.2 (93)	7.9 (89)

For *S. agalactiae*, a decline in the percentage cfu recovered was found for both transport systems, with eSwab still showing the highest recovery rate. The different concentrations of micro-organisms mixed together did not seem to influence the processes occurring at RT in both transport systems. At 4°C, the recovery of *E. coli* in eSwab ranged between 89–103%, depending on the micro-organism concentration in the experiment. *S. agalactiae* recovery in eSwab fluctuated between 84–99%, independently of its concentration. CVT recovery rates were lower throughout the whole experiment for both micro-organisms tested.

Discussion

Specimen collection and transportation is a cornerstone of good diagnostic performance in the Clinical Microbiology laboratory. Viability must be maintained, as well as the relative proportions of all micro-organisms present in the clinical specimen [4]. Therefore, in this study, we evaluated the performance of the new flocked swab with liquefied Amies transport medium for the quantitative survival of *E. coli*, *S. agalactiae* and *C. albicans* in vitro. Furthermore, we analysed the in vitro survival of *E. coli* and *S. agalactiae* in mixed cultures with different proportions of each bacteria. To our knowledge, this is the first study evaluating the performance of eSwab for the detection and survival of easy-growing aerobic bacterial strains and mixed cultures in vitro.

In vitro, eSwab met the CLSI acceptance criteria at both storage temperatures for the three isolates tested. Although no significant differences were detected between both transport devices, the recovery rate was higher in eSwab as compared to the cotton swab for both the single-micro-organism experiments and the mixed cultures. At refrigerated temperature, the recovery rates were very stable over time for eSwab (recovery of 90% and higher). At RT on the other hand, both for *E. coli* and *C. albicans*, overgrowth could be detected from 6–24 h after inoculation of both transport systems. Van Horn et al. also observed heavy overgrowth of *Pseudomonas aeruginosa* after RT storage for eSwab and other transport devices tested [2]. Although the CLSI does not have criteria on the percentage overgrowth allowed [3], this phenomenon could lead to the misinterpretation of mixed infections and misleading culture results in laboratory practice. As for other swabs, a prompt transport to the laboratory of eSwabs is, therefore, recommended.

One limitation of the in vitro study might be the use of the quantitative elution method as proposed by the CLSI, as this method does not reflect typical laboratory swab practices. However, the advantage of this method over the roll plate method (which resembles more closely routine laboratory practices) is that it provides quantitative data that can more easily detect performance characteristic differences between the swab transport systems [1, 2].

One can conclude that eSwab did not only meet the criteria set by the CLSI for a transport device for microbiological culture, but as its recovery rate in vitro was higher than currently available systems, it might also enhance the sensitivity of bacterial culture in the future.

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