

## Comparison of the Copan ESwab System with Two Amies Agar Swab Transport Systems for Maintenance of Microorganism Viability<sup>▽</sup>

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Swab transport systems are used for a variety of specimen types and must maintain organism viability throughout the transport process. The Copan ESwab is a new nylon-flocked swab designed to optimize specimen collection and to minimize entrapment of the specimen. We used the quantitative elution method with recommended strains, as described in CLSI document M40-A, to evaluate the ESwab for maintenance of viability of aerobic and anaerobic microorganisms for 0, 6, 24, and 48 h during room temperature and refrigerated temperature storage. The Becton Dickinson CultureSwab MaxV swab and the Remel BactiSwab were used as comparators. The ESwab met CLSI acceptance criteria for all aerobic isolates stored at both temperatures and for all anaerobic isolates stored at refrigerated temperature. The ESwab also met CLSI criteria for four of five anaerobic strains at room temperature. *Prevotella melaninogenica* was not recovered after 24 or 48 h of room temperature storage with any of the three swab transport systems tested. Overall, the ESwab was equivalent to the Becton Dickinson CultureSwab MaxV swab in organism recovery but recovered more isolates than Remel BactiSwab.

Appropriate specimen collection and transport are essential for accurate laboratory diagnosis of bacterial infections. Because of their convenience, swab systems with transport media are often used to collect and transport specimens of various types. These systems must maintain organism viability during transit to a laboratory. Given the increasing frequency of transport delays due to cost containment measures, consolidations, and services being shifted to centralized or reference laboratories, robust transport systems are becoming increasingly relevant. The CLSI M40-A method (2) was used recently to evaluate several swab collection and transport devices for maintenance of bacteria viability (3, 4, 6, 7, 8, 9).

Swab tips, which are typically rayon or Dacron, should be prepared with material that collects sufficient specimen material, is nontoxic to microorganisms, maintains viability in conjunction with the transport medium, and releases specimen material efficiently onto agar media. A new nylon-tipped swab (ESwab; Copan Diagnostics, Inc., Corona, CA) prepared by spray-on flocked fiber technology has been developed for transport of bacteria and viruses. This technology provides stronger capillary action and strong hydraulic uptake of liquids, which should result in better specimen collection. This design should also provide more efficient release of specimen material and, therefore, less entrapment of specimen than occurs with typical rayon or Dacron fiber-tipped swabs. The ESwab shaft is scored for ease and consistency of tip breakage into the modified liquid Amies transport medium. A swab capture mechanism in the cap locks the broken swab shaft into the cap when it is fully closed.

In this study, we evaluated the new ESwab with liquid Amies

transport medium for maintenance of viability of aerobic and anaerobic bacteria according to the CLSI M40-A quantitative elution method (2). The Becton Dickinson CultureSwab MaxV swab and Remel BactiSwab (RBS; Remel, Lenexa, KS), both with Amies agar gel, were tested as comparators.

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### MATERIALS AND METHODS

**Organisms.** The 10 strains tested were those recommended by CLSI document M40-A (2), as follows: *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, *Prevotella melaninogenica* ATCC 25845, *Peptostreptococcus anaerobius* ATCC 27337, and *Propionibacterium acnes* ATCC 6919. All strains were freshly subcultured to appropriate media at least twice prior to being tested. Media included 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 five anaerobes (media were obtained from Becton Dickinson, Sparks, MD). All isolates were incubated at 37°C with an atmosphere appropriate for each organism, i.e., non-CO<sub>2</sub> for *P. aeruginosa* and *S. pyogenes*, 5% CO<sub>2</sub> for *H. influenzae*, *N. gonorrhoeae*, and *S. pneumoniae*, and anaerobic (EZ anaerobe container system; Becton Dickinson) for the anaerobic isolates.

**Quantitative elution.** Tests were performed by the CLSI M40-A quantitative elution method (2). An organism suspension from a freshly grown isolate of each strain was prepared in sterile saline to a turbidity equivalent to that of a 0.5 McFarland standard. The suspension was further diluted 1:10 in sterile saline to achieve an inoculum concentration of approximately  $1.5 \times 10^7$  CFU/ml. For growth controls, serial 10-fold dilutions were prepared from the suspension and plated on duplicate plates of the appropriate medium. The plates were then incubated at 37°C in the appropriate atmosphere, and colony counts were obtained to confirm that the inoculum concentration was acceptable ( $10^7$  to  $10^9$  CFU/ml).

For swab testing, 12 100- $\mu$ l aliquots of the  $10^7$ -CFU/ml inoculum of each organism suspension were placed onto the surface of a sterile Petri plate. Each of 12 swabs from one manufacturer was rolled into 1 of the 12 100- $\mu$ l inoculum spots for 15 s to ensure complete absorption. Triplicate swabs of each type were inoculated for each storage time period (0, 6, 24, or 48 h), for a total of 12 swabs of each manufacturer's swab type inoculated for each storage temperature to be tested. All swabs were immediately placed into their respective transport systems

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TABLE 1. Recovery of aerobic microorganisms from three swab systems stored at room temperature

Organism	Swab system	No. (%) of CFU recovered at:			
		0 h	6 h	24 h	48 h
<i>H. influenzae</i>	MaxV	$1.3 \times 10^6$	$1.4 \times 10^6$ (108)	$8.2 \times 10^5$ (63)	$1.7 \times 10^6$ (130)
	ESwab	$7.0 \times 10^6$	$9.2 \times 10^6$ (131)	$2.3 \times 10^7$ (329)	$3.0 \times 10^7$ (429)
	RBS	$1.8 \times 10^6$	$7.1 \times 10^5$ (39)	$1.1 \times 10^3$ (0.0006)	No growth
<i>S. pyogenes</i>	MaxV	$6.2 \times 10^5$	$4.3 \times 10^6$ (694)	$8.0 \times 10^6$ (1,290)	$1.0 \times 10^8$ (16,130)
	ESwab	$7.8 \times 10^6$	$8.8 \times 10^6$ (113)	$1.3 \times 10^8$ (1,667)	$1.3 \times 10^8$ (1,667)
	RBS	$1.5 \times 10^6$	$1.5 \times 10^6$ (100)	$3.7 \times 10^5$ (25)	$3.7 \times 10^5$ (25)
<i>S. pneumoniae</i>	MaxV	$6.6 \times 10^5$	$5.8 \times 10^5$ (88)	$3.4 \times 10^6$ (515)	$9.1 \times 10^6$ (1,378)
	ESwab	$3.3 \times 10^6$	$3.1 \times 10^6$ (94)	$3.7 \times 10^6$ (112)	$7.1 \times 10^6$ (215)
	RBS	$1.5 \times 10^6$	$7.9 \times 10^5$ (52)	$1.5 \times 10^5$ (10)	$1.9 \times 10^3$ (0.1)
<i>P. aeruginosa</i>	MaxV	$4.1 \times 10^5$	$4.0 \times 10^5$ (98)	$6.2 \times 10^8$ (> $10^5$ )	$3.6 \times 10^9$ (> $10^5$ )
	ESwab	$1.6 \times 10^6$	$1.0 \times 10^6$ (63)	$4.7 \times 10^8$ (29,375)	$1.7 \times 10^9$ (> $10^5$ )
	RBS	$6.8 \times 10^5$	$5.8 \times 10^5$ (85)	$3.1 \times 10^7$ (4,559)	$1.9 \times 10^8$ (27,940)
<i>N. gonorrhoeae</i>	MaxV	$3.8 \times 10^6$	$2.8 \times 10^6$ (74)	$1.7 \times 10^5$ (4)	No growth
	ESwab	$3.8 \times 10^7$	$1.5 \times 10^5$ (0.4)	$8.2 \times 10^4$ (0.2)	No growth
	RBS	$2.0 \times 10^6$	$4.3 \times 10^5$ (22)	$1.1 \times 10^5$ (6)	No growth

and stored at a controlled  $23 \pm 1^\circ\text{C}$  for room temperature storage or  $4^\circ\text{C}$  for refrigerated storage for each time period prior to plate inoculation.

After the appropriate storage time, including 0 h (tested within 15 min of inoculation), each of the Amies agar gel swabs (MaxV and RBS) was removed from the transport medium, placed into 0.9 ml sterile saline, and mixed by vortexing for 15 s. The ESwab was mixed in the transport tube by being vortexed for 15 s, and 100  $\mu\text{l}$  of the liquid Amies medium was transferred to 0.9 ml of sterile saline. Serial 10-fold dilutions of each swab system suspension were prepared to obtain suspensions theoretically equivalent to  $10^6$  to  $10^1$  CFU/ml. The ESwab transport tube was considered the  $10^6$ -CFU/ml tube because of the initial 1:10 dilution into the liquid Amies medium. A 100- $\mu\text{l}$  aliquot of each dilution was removed, placed onto the surface of duplicate agar media appropriate for the organism tested, and inoculated over the entire agar surface with a sterile spreader. All plates were incubated at  $37^\circ\text{C}$  under appropriate atmospheric conditions for the organism tested. Incubation of the plates was done for up to 48 h for aerobic organisms and up to 96 h for anaerobic organisms. After incubation, two readers counted colonies on plates with 30 to 300 countable colonies, and the average colony count was used. The average CFU at storage times of 6, 24, and 48 h were compared to the average CFU at 0 h. CLSI M40-A (2) criteria were used for evaluation, as follows: a swab system was considered acceptable for the organism tested if the change in CFU from the 0-h value declined no more than  $3 \log_{10}$  ( $1 \times 10^3$  CFU  $\pm$  10%) for storage at room temperatures and, for storage at refrigerated temperature, no more than a 1-log increase or no more than a 3- $\log_{10}$  ( $1 \times 10^3$  CFU  $\pm$  10%) decline compared to the 0-h CFU. CLSI has established a storage evaluation time of 24 h for *N. gonorrhoeae* and 48 h for all other organisms. There is no CLSI M40-A interpretation for *N. gonorrhoeae* beyond a 24-h storage time. Similarly, no CLSI

standard exists for evaluation of overgrowth in swab transport systems stored at room temperature. Comparison of the swab transport systems was based on average CFU and percent recovery of viable organisms at each incubation time and at each storage temperature.

## RESULTS

**Recovery of aerobic isolates.** Recovery of the aerobic isolates after room temperature storage is summarized in Table 1. The ESwab and MaxV systems yielded acceptable recovery of all five isolates according to CLSI standards; the RBS yielded acceptable recovery results for four isolates, failing to recover *H. influenzae* after 24 h and 48 h of storage. All three swabs yielded acceptable recovery of *N. gonorrhoeae* after 24 h of storage. Heavy overgrowth was observed for *P. aeruginosa* with all three swab systems at 24 and 48 h.

Recovery of the aerobic isolates after refrigerated storage is summarized in Table 2. All three swab systems yielded acceptable recovery of the five isolates. There was no observed overgrowth, and all three swab systems also recovered *N. gonorrhoeae* after 48 h of storage.

**Recovery of anaerobic isolates.** Recovery of the anaerobic isolates after room temperature storage is summarized in Ta-

TABLE 2. Recovery of aerobic microorganisms from three swab systems stored at refrigerated temperature

Organism	Swab system	No. (%) of CFU recovered at:			
		0 h	6 h	24 h	48 h
<i>H. influenzae</i>	MaxV	$3.7 \times 10^7$	$4.6 \times 10^7$ (124)	$6.0 \times 10^6$ (16)	$7.2 \times 10^6$ (19)
	ESwab	$4.0 \times 10^7$	$3.4 \times 10^7$ (85)	$1.7 \times 10^7$ (43)	$1.3 \times 10^7$ (33)
	RBS	$1.6 \times 10^7$	$8.0 \times 10^6$ (50)	$3.2 \times 10^6$ (20)	$1.4 \times 10^6$ (9)
<i>S. pyogenes</i>	MaxV	$3.1 \times 10^6$	$2.9 \times 10^6$ (94)	$4.8 \times 10^6$ (155)	$3.2 \times 10^6$ (103)
	ESwab	$6.1 \times 10^7$	$6.0 \times 10^7$ (98)	$4.7 \times 10^7$ (77)	$4.9 \times 10^7$ (80)
	RBS	$6.4 \times 10^6$	$2.8 \times 10^6$ (43)	$9.1 \times 10^6$ (142)	$5.9 \times 10^6$ (92)
<i>S. pneumoniae</i>	MaxV	$1.4 \times 10^6$	$4.2 \times 10^5$ (30)	$1.6 \times 10^6$ (114)	$1.6 \times 10^6$ (114)
	ESwab	$8.3 \times 10^6$	$9.3 \times 10^6$ (112)	$6.2 \times 10^6$ (75)	$4.3 \times 10^6$ (52)
	RBS	$2.6 \times 10^6$	$3.4 \times 10^5$ (13)	$1.2 \times 10^6$ (46)	$1.3 \times 10^6$ (50)
<i>P. aeruginosa</i>	MaxV	$5.0 \times 10^6$	$3.5 \times 10^6$ (70)	$3.3 \times 10^6$ (66)	$4.8 \times 10^6$ (96)
	ESwab	$2.3 \times 10^7$	$2.9 \times 10^7$ (126)	$2.8 \times 10^7$ (122)	$2.8 \times 10^7$ (122)
	RBS	$7.8 \times 10^6$	$1.0 \times 10^7$ (128)	$9.3 \times 10^6$ (119)	$7.1 \times 10^6$ (91)
<i>N. gonorrhoeae</i>	MaxV	$4.2 \times 10^6$	$5.0 \times 10^5$ (12)	$3.8 \times 10^5$ (9)	$2.9 \times 10^5$ (7)
	ESwab	$1.3 \times 10^7$	$4.9 \times 10^6$ (38)	$4.2 \times 10^6$ (32)	$1.6 \times 10^6$ (12)
	RBS	$2.1 \times 10^6$	$3.2 \times 10^4$ (2)	$2.9 \times 10^4$ (1)	$2.6 \times 10^4$ (1)

TABLE 3. Recovery of anaerobic microorganisms from three swab systems stored at room temperature

Organism	Swab system	No. (%) of CFU recovered at:			
		0 h	6 h	24 h	48 h
<i>P. anaerobius</i>	MaxV	$1.6 \times 10^6$	$8.6 \times 10^5$ (54)	$1.2 \times 10^5$ (8)	$1.1 \times 10^4$ (0.7)
	ESwab	$6.6 \times 10^6$	$3.8 \times 10^6$ (58)	$1.2 \times 10^6$ (18)	$1.2 \times 10^5$ (2)
	RBS	$2.2 \times 10^6$	$9.4 \times 10^4$ (4)	No growth	No growth
<i>B. fragilis</i>	MaxV	$4.3 \times 10^6$	$4.4 \times 10^6$ (102)	$3.9 \times 10^6$ (91)	$2.9 \times 10^6$ (67)
	ESwab	$2.8 \times 10^7$	$1.6 \times 10^7$ (57)	$6.5 \times 10^8$ (2,320)	$1.8 \times 10^7$ (64)
	RBS	$8.4 \times 10^6$	$7.6 \times 10^6$ (91)	$5.8 \times 10^6$ (69)	$3.2 \times 10^6$ (38)
<i>F. nucleatum</i>	MaxV	$1.9 \times 10^6$	$4.7 \times 10^6$ (247)	$2.7 \times 10^5$ (14)	$3.6 \times 10^4$ (2)
	ESwab	$1.5 \times 10^7$	$1.2 \times 10^7$ (80)	$3.7 \times 10^6$ (25)	$8.0 \times 10^4$ (0.5)
	RBS	$6.0 \times 10^6$	$2.5 \times 10^6$ (42)	$2.4 \times 10^5$ (4)	$9.0 \times 10^3$ (0.2)
<i>P. acnes</i>	MaxV	$5.0 \times 10^6$	$1.1 \times 10^6$ (22)	$4.8 \times 10^6$ (96)	$5.6 \times 10^6$ (112)
	ESwab	$7.0 \times 10^6$	$8.9 \times 10^6$ (127)	$8.3 \times 10^6$ (119)	$1.4 \times 10^7$ (200)
	RBS	$1.6 \times 10^6$	$1.1 \times 10^6$ (69)	$3.7 \times 10^6$ (231)	$1.8 \times 10^6$ (113)
<i>P. melaninogenica</i>	MaxV	$6.6 \times 10^5$	$1.0 \times 10^6$ (152)	No growth	No growth
	ESwab	$2.5 \times 10^6$	$2.1 \times 10^6$ (84)	No growth	No growth
	RBS	$1.5 \times 10^6$	$6.2 \times 10^5$ (41)	No growth	No growth

ble 3. The ESwab and MaxV systems yielded acceptable recovery of four of the five isolates, while the RBS recovered only three of the five isolates at 24 and 48 h. No swab system recovered the *P. melaninogenica* isolate after 24 h or 48 h of room temperature storage. The RBS also failed to recover the *P. anaerobius* isolate after 24 and 48 h of incubation. No distinct overgrowth was observed for the anaerobes at room temperature, but the *B. fragilis* isolate showed some apparent growth in the ESwab at 24 h, with decreased counts at 48 h.

Recovery of anaerobic isolates after refrigerated storage is summarized in Table 4. The ESwab and MaxV systems yielded acceptable recovery for all five anaerobic isolates. The RBS yielded acceptable recovery for three isolates, failing to recover *P. anaerobius* and yielding unacceptable recovery of *P. melaninogenica* after 48 h of storage. No overgrowth was observed with the anaerobes stored at refrigerated temperature.

## DISCUSSION

CLSI document M40-A (2) provides standardized methods, for both roll plates and elution, to aid manufacturers and laboratories in determining the performance characteristics of

swab transport devices. The roll plate method more closely resembles routine laboratory practices of mechanical swabbing actions that influence organism release onto the agar plate but provides only semiquantitative approximation of recovery. Although the elution method does not reflect typical laboratory swab practices, we performed the assays by the elution method to provide quantitative comparisons that could more effectively detect performance characteristic differences between the swab transport systems. To our knowledge, this is the first report of an evaluation of the ESwab system utilizing all CLSI-recommended bacterial strains tested at both storage temperatures.

The ESwab met CLSI acceptance criteria at both storage temperatures for all isolates tested except for *P. melaninogenica*. In fact, none of the three swab transport systems maintained *P. melaninogenica* viability after 24 h or 48 h of room temperature storage, possibly because inoculation preparation procedures were performed under aerobic conditions. However, this explanation does not fully account for the unacceptable results in that the same preparation procedures were used for the refrigerated storage studies, which recovered *P. melano-*

TABLE 4. Recovery of anaerobic microorganisms from three swab systems stored at refrigerated temperature

Organism	Swab system	No. (%) of CFU recovered at:			
		0 h	6 h	24 h	48 h
<i>P. anaerobius</i>	MaxV	$3.8 \times 10^5$	$3.1 \times 10^5$ (82)	$2.9 \times 10^5$ (76)	$1.2 \times 10^5$ (32)
	ESwab	$2.5 \times 10^6$	$2.0 \times 10^6$ (80)	$6.7 \times 10^5$ (27)	$8.1 \times 10^5$ (32)
	RBS	$7.7 \times 10^5$	$4.0 \times 10^5$ (52)	$2.5 \times 10^4$ (3)	No growth
<i>B. fragilis</i>	MaxV	$3.4 \times 10^6$	$4.2 \times 10^6$ (124)	$1.8 \times 10^6$ (53)	$1.3 \times 10^6$ (38)
	ESwab	$1.2 \times 10^7$	$9.0 \times 10^6$ (75)	$1.0 \times 10^7$ (83)	$5.5 \times 10^6$ (46)
	RBS	$3.2 \times 10^6$	$3.6 \times 10^6$ (113)	$4.8 \times 10^6$ (150)	$7.8 \times 10^5$ (24)
<i>F. nucleatum</i>	MaxV	$3.0 \times 10^5$	$2.3 \times 10^5$ (77)	$3.3 \times 10^4$ (11)	$1.0 \times 10^4$ (3)
	ESwab	$9.3 \times 10^5$	$1.2 \times 10^6$ (129)	$1.8 \times 10^5$ (19)	$7.5 \times 10^4$ (8)
	RBS	$5.7 \times 10^5$	$7.1 \times 10^5$ (125)	$2.9 \times 10^5$ (51)	$1.2 \times 10^5$ (21)
<i>P. acnes</i>	MaxV	$7.3 \times 10^6$	$5.0 \times 10^6$ (68)	$6.5 \times 10^6$ (89)	$4.9 \times 10^6$ (67)
	ESwab	$2.0 \times 10^7$	$2.3 \times 10^7$ (115)	$1.8 \times 10^7$ (90)	$1.4 \times 10^7$ (70)
	RBS	$1.0 \times 10^7$	$5.0 \times 10^6$ (50)	$6.3 \times 10^6$ (63)	$4.3 \times 10^6$ (43)
<i>P. melaninogenica</i>	MaxV	$6.4 \times 10^5$	$1.2 \times 10^6$ (188)	$1.8 \times 10^5$ (28)	$1.0 \times 10^4$ (2)
	ESwab	$5.9 \times 10^6$	$3.6 \times 10^6$ (61)	$1.1 \times 10^6$ (19)	$1.3 \times 10^5$ (2)
	RBS	$1.7 \times 10^6$	$5.2 \times 10^5$ (31)	$3.7 \times 10^4$ (2)	$1.3 \times 10^2$ (0.008)

*ninogenica* isolates with all three swab transport systems (the RBS gave an unacceptable CFU/ml). Others have also reported difficulty in recovering *P. melaninogenica* from swab transport systems stored at room temperature (5, 6). We are not aware of any published swab studies of *P. melaninogenica* tested at refrigerated storage temperatures.

Overgrowth of an organism in a swab transport system is problematic and would misrepresent the true relative proportions of a mixed infection, therefore yielding misleading culture results. However, there are no CLSI standards for organism overgrowth at room temperature incubation (2). We observed heavy overgrowth ( $>10^5\%$  increase) of *P. aeruginosa* after room temperature storage for the ESwab and the MaxV systems, and the RBS also showed lesser overgrowth. Overgrowth of *P. aeruginosa* has been reported for other swab systems after storage at room temperature (6, 7, 8, 9) and for enterics (1, 8). The ESwab allowed less, but distinct, growth of *S. pyogenes* during room temperature storage ( $>10$ -fold recovery at 24 and 48 h). Growth of *S. pyogenes* in swab systems has been observed previously (7, 8). In the present study, the MaxV swab also yielded *S. pyogenes* growth of  $>100$ -fold. However, this amount of growth would still be within acceptable CLSI limits (2) if refrigerated storage limits were applied. The increase in numbers of the *B. fragilis* isolate with the ESwab system after 24 h of room temperature storage remains unexplained, but this could have been due to initial growth in the ESwab system followed by a decline due to loss of viable cells at 48 h. No swab showed overgrowth at refrigerated temperatures, consistent with previous studies of other swab systems (3, 6, 7).

Overall, the ESwab performed as well as the MaxV and RBS systems for maintenance of aerobic organism viability at refrigerated temperature storage. At room temperature, the ESwab maintained organism viability as well as the MaxV swab and better than the RBS, which failed to recover *H. influenzae*. The ESwab maintained anaerobic organism viability equivalent to that with the MaxV swab at both room and refrigerated temperatures and was considered better than the RBS, which failed to recover *P. anaerobius* at both storage temperatures. No swab system recovered *P. melaninogenica* after 24 h of room temperature storage. The RBS also yielded unacceptable recovery of *P. melaninogenica* after 48 h of refrigerated temperature storage. Only one lot of each swab type was used, and this may have accounted for the failures with the RBS. Further studies that test multiple lots of each swab type might need to

be performed to help determine if lot-to-lot variations exist with regard to this type of evaluation.

In conclusion, the ESwab system is an acceptable swab transport system for both aerobes and anaerobes. This system met CLSI acceptance criteria for all aerobic and anaerobic isolates when it was tested under refrigerated storage conditions and failed to allow growth of only *P. melaninogenica* when it was tested at room temperature (as did both comparator swabs). For the laboratory, caution might be advised in using swab transport devices that are not specifically designed for transport of specimens for anaerobic culture, since our study indicates that very fastidious anaerobes might be missed after longer storage and transport ( $>6$  h), especially if they are maintained under room temperature conditions.

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