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Comparison of FecalSwab and ESwab Devices for Storage and Transportation of Diarrheagenic Bacteria

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Using a collection ($n = 12$) of ATCC and known stock isolates, as well as 328 clinical stool specimens, we evaluated the ESwab and the new FecalSwab liquid-based microbiology (LBM) devices for storing and transporting diarrheagenic bacteria. The stock isolates were stored in these swab devices up to 48 h at refrigeration (4°C) or room (~25°C) temperature and up to 3 months at −20°C or −70°C. With the clinical stool specimens, the performances of the ESwab and FecalSwab were compared to those of routinely used transport systems (Amies gel swabs and dry containers). At a refrigeration temperature, all isolates survived in FecalSwab up to 48 h, while in ESwab, only 10 isolates (83.3%) out of 12 survived. At −70°C, all isolates in FecalSwab were recovered after 3 months of storage, whereas in ESwab, none of the isolates were recovered. At −20°C, neither of the swab devices preserved the viability of stock isolates after 2 weeks of storage, and at room temperature, 7 (58.3%) of the stock isolates were recovered in both transport devices after 48 h. Of the 328 fecal specimens, 44 (13.4%) were positive for one of the common diarrheagenic bacterial species with all transport systems used. Thus, the suitability of the ESwab and FecalSwab devices for culturing fresh stools was at least equal to those of the Amies gel swabs and dry containers. Although the ESwab was shown to be an option for collecting and transporting fecal specimens, the FecalSwab device had clearly better preserving properties under different storage conditions.

Appropriate specimen collection and transport are essential for accurate laboratory diagnosis of bacterial infections. Swab collection has been the most frequently used method in health care settings because swabs are inexpensive and specimens are easy to collect, although it may not be the best approach for detecting, e.g., anaerobic and fastidious organisms (1, 2). Recent improvements in the swab tip material and the transport medium have, however, greatly enhanced the recovery and viability of various microorganisms present in specimens (3–6). The flocked nylon swabs (FLOQSwabs) with liquid transport media have shown to yield greater organism release than cotton swabs in dry containers (6) and rayon- or Dacron-based swabs in Amies gel (4).

In this study, we evaluated the performances of the ESwab (Copan Italia, Brescia, Italy) and the recently launched FecalSwab (Copan Italia) liquid-based microbiology (LBM) devices for maintaining the viability of diarrheagenic bacteria at different temperatures. In addition, the suitability of these swab systems for the recovering enteric pathogens in stool specimens was assessed in comparison with the suitability of the routinely used dry containers and Amies gel swabs (Copan Italia).

(These results were presented in part at the 113th General Meeting of the American Society for Microbiology, 18 to 21 May 2013, Denver, CO [7].)

MATERIALS AND METHODS

The survival of gastrointestinal bacterial pathogens in the ESwab and FecalSwab devices was investigated using four ATCC bacterial strains and eight known clinical bacterial stock isolates. The ATCC strains were *Salmonella enterica* subsp. *enterica* serovar Typhimurium ATCC 14028, *Shigella sonnei* ATCC 9290, *Yersinia enterocolitica* ATCC 23715, and *Campylobacter jejuni* ATCC 33291. The clinical isolates were enterohemorrhagic *Escherichia coli* serotype O157:H7, *S. enterica* subsp. *enterica* serovar Enteritidis, *Shigella flexneri*, *Campylobacter coli*, *Vibrio cholerae*, *Aeromonas hydrophila*, *Plesiomonas shigelloides*, and the *tcdB* gene-positive *Clostridium difficile*. All strains except *Campylobacter* species and *C. difficile* were cultured from −70°C stocks on 5% sheep blood agar (Becton, Dickinson, Sparks, MD, USA) at 35°C for 16 to 24 h. *C. coli* and *C. jejuni* were cultured on *Campylobacter* blood-free selective medium (Oxoid Ltd., Thermo Fisher Scientific, Inc., Basingstoke, Hampshire, United Kingdom) and incubated at 42°C in a microaerophilic atmosphere for 48 h. *C. difficile* was cultured on fastidious anaerobe agar (Lab M Ltd., Lancashire, United Kingdom) at 35°C in an anaerobic atmosphere for 48 h.

The inocula of the stock isolates were prepared in 0.9% NaCl to equal a 0.5 McFarland standard (approximately 1.5×10^8 CFU/ml) using a nephelometer (DensiCHEK Plus; bioMérieux, Inc., Durham, NC, USA). Next, each preparation was serially diluted (10-fold dilutions) in order to get 1.5×10^3 to 1.5×10^4 CFU/ml to be inoculated in duplicate into the ESwab and FecalSwab devices. The amount of inoculum that was added into each device was 100 μl. Each inoculated swab device was vortexed for 15 s and stored for 48 h at room temperature (RT) (~25°C) or a refrigeration temperature (4°C), and for 3 months at −20°C or −70°C. The colony counts in each swab device was determined at storage times of 0, 6, 24, and 48 h (from devices stored at RT and 4°C) or at storage times of 0 h, 2 weeks, 1 month, and 3 months (from devices stored at −20°C or −70°C) by triplicate plating of 10 μl ESwab and FecalSwab medium on either 5% sheep blood agar, *Campylobacter* blood-free selective medium, or fastidious anaerobe agar. The plates for each organism were incubated as mentioned above. In addition, mixtures of *E. coli* strain ATCC 25922, *Enterococcus faecalis* strain ATCC 29212, and *S. enterica* serovar Typhimurium strain ATCC 14028 or *S. sonnei* strain ATCC 9290 were prepared in duplicate and stored at RT and 4°C for 48 h. The simulated mixed specimens were plated

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on cystine lactose electrolyte-deficient (CLED) agar (Oxoid Ltd., Thermo Fisher Scientific, Inc., Basingstoke, Hampshire, United Kingdom) at 0, 6, 24, and 48 h of storage and incubated at 35°C for 16 to 24 h. Fisher's exact test was used to determine the statistical significance of the differences in performance between the ESwab and FecalSwab devices.

Clinical stool specimens were collected from 328 patients presenting with gastroenteritis ($n = 228$) or antibiotic-associated diarrhea ($n = 100$) at Vaasa Central Hospital from December 2012 to February 2013. The specimens from the patients with gastroenteritis were aliquoted into one dry container, one Amies gel swab, two FecalSwab devices, and two ESwab devices and were cultured immediately after receiving them at the hospital laboratory on xylose-lysine-deoxycholate (XLD) agar (Oxoid Ltd., Thermo Fisher Scientific, Inc., Basingstoke, Hampshire, United Kingdom), cefsulodin-irgasan-novobiocin (CIN) agar (Oxoid Ltd., Thermo Fisher Scientific, Inc.), *Campylobacter* blood-free selective agar plates, and in selenite broth (Oxoid Ltd., Thermo Fisher Scientific, Inc.). The specimens from the patients with antibiotic-associated diarrhea were aliquoted into one dry container, two FecalSwab devices, and two ESwab devices and cultured immediately on cycloserine-cefoxitin-egg-yolk (CCEY) agar (Oxoid Ltd., Thermo Fisher Scientific, Inc.). In addition, these specimens were tested directly for *C. difficile* toxins with an immunoassay (IA) targeting toxins A and B (Alere Tox A/B Quik Chek; Tech Lab, Waltham, MA, USA) and with the GenomEra *C. difficile* assay (Abacus Diagnostica, Turku, Finland) targeting the *tcdB* gene. The IA and GenomEra *C. difficile* assay were performed according to each manufacturer's instructions. In addition to the immediate culturing and testing, all clinical specimens in the FecalSwab and ESwab devices were recultured and retested, as mentioned above, after being stored at 4°C and RT for 20 h.

The inoculated plates were incubated either aerobically (CIN and XLD) at 35°C or in a microaerophilic atmosphere at 42°C (*Campylobacter* blood-free selective agar) for 48 h. The selenite broth was incubated at 4°C for 24 h and then subcultured onto an additional XLD plate. The plates for *C. difficile* were incubated anaerobically (in CCEY) at 35°C for 48 h. Biochemical analyses were performed on all suspected colonies, and isolates preliminarily identified as being pathogenic were sent to the bacteriology unit of the National Institute for Health and Welfare (THL) for confirmation and strain typing, excluding *C. difficile*. The presumptive growth of *C. difficile* was confirmed by Gram staining, UV light, and IA (Alere *C. diff* Quik Chek Complete) targeting *C. difficile*-specific glutamate dehydrogenase (GDH). The toxigenic nature of the suspected isolate growing on the culture medium was confirmed by the same above-mentioned IA.

RESULTS

Recovery of stock isolates. In both the ESwab and FecalSwab devices, the number of viable organisms remained stable for up to 6 h at RT storage (Table 1). At 24 and 48 h of storage, however, a clear increase in growth was observed for all other isolates, except for *C. jejuni*, *C. coli*, and *C. difficile*. *Campylobacter* spp. yielded no growth after 24 h of storage at RT. Also, the vegetative growth of *C. difficile* ceased after 24 h of storage. However, in these cases, high numbers of thin curved Gram-negative bacilli (presumably *Campylobacter* spp.) or large poorly Gram staining bacilli with spore-like structures (presumably *C. difficile*) were seen when the preservation media of the devices were Gram stained.

At a refrigeration temperature, the recovery of stock isolates in ESwab and FecalSwab was more stable over time (Table 2) than that at RT. Heavy proliferation was not seen, except with *Salmonella* spp., which began to grow in both swab devices after 24 h of storage. *C. difficile* survived for ≥48 h in FecalSwab at 4°C, while in ESwab, the amount of viable cells began to decline rapidly after inoculation, and no colonies were recovered at 48 h of storage. In contrast to the other species, the viability of *Campylobacter* spp. started to reduce in both swab devices after 6 h of storage, and by 24 h, no growth of *C. coli* in FecalSwab and *C. jejuni* in either of the swab devices were observed. However, at 48 h of storage in FecalSwab, both *Campylobacter* spp. were again recovered, although in concentrations <6% of the initial values. From ESwab, only *C. jejuni* was recovered at 48 h of incubation.

At −70°C, all stock isolates ($n = 12$) survived 3 months of storage in FecalSwab, while with ESwab, the viability of the isolates decreased rapidly after 2 weeks, and only *Salmonella* spp. ($n = 2$) survived up to 1 month. The difference in the survival of the stock isolates at −70°C was significant ($P < 0.0001$). However, for most isolates, the cell concentrations in FecalSwab were clearly lower 3 months after inoculation than at 0 h. While the viability of *E. coli* and *Yersinia* spp. remained stable, with no significant reduction at 3 months of storage, a 1 log reduction in the number of viable *Shigella* and *Salmonella* cells, a 1.5 log reduction in the number of viable *Aeromonas* and *Plesiomonas* cells, and a 2 log reduction in the number of viable *Vibrio*, *Campylobacter*, and *Clostridium* cells were observed in FecalSwab. At −20°C, a notable reduction in viable cells was seen in both swab devices 2 weeks after inoculation, after which time we could not recover the growth of any isolates. Thus, for longer preservation, storage at −70°C maintained the viability of stock isolates significantly better than at −20°C ($P < 0.0001$).

For the mixed specimens of *E. coli*, *E. faecalis*, and *S. enterica* serovar Typhimurium, or *E. coli*, *E. faecalis*, and *S. sonnei*, we observed results similar to those in the survival of the separate enteric pathogens (Tables 3 and 4). At RT, a notable proliferation of all isolates was seen after 24 h of storage in both transport devices. Mixing different microorganisms together did not seem to influence the growth processes of individual organisms. At 4°C, the concentration of mixed isolates remained stable at least up to 48 h.

Performance with clinical stool specimens. Of the 228 gastroenteritis stool specimens, 24 (10.5%) were positive for one of the common enteric bacterial pathogens, *Yersinia* ($n = 2$), *Salmonella* ($n = 17$), or *Campylobacter* ($n = 5$) species, from all four specimen collection systems (ESwab, FecalSwab, Amies gel swab, and dry container) used in this study. However, cell recovery was slightly higher using the semiquantitative culture method from ESwab and FecalSwab devices than from routinely used transport systems. For example, when scanty growth of suspected pathogen was observed from the routinely used transport systems, moderate growth was seen from ESwab and FecalSwab. Moreover, the additional 20 h of storage at RT improved the yields (e.g., from moderate to substantial growth) of *Yersinia* and *Salmonella* spp. from ESwab and FecalSwab, while at 4°C, the cell concentrations remained stable. Any *Campylobacter* spp., on the other hand, were not recovered at 20 h of storage at RT or at 4°C from either of the swab devices.

Of the 100 antibiotic-associated diarrheal specimens, 20 (20%) were positive by toxigenic *C. difficile* culture and by PCR, from both swab devices and routinely used transport systems. A direct IA of *C. difficile* toxins, however, revealed only 6 positive specimens. The results from the FecalSwab and ESwab devices were identical even after 20 h of storage at RT or at 4°C.

DISCUSSION

Here, we describe the performances of the ESwab and FecalSwab devices for maintaining the viability of various diarrheagenic bac-

TABLE 1 Survival of enteric pathogens in FecalSwab and ESwab devices at room temperature

Species and/or isolate ($n = 12$)	Swab device	CFU/ml (%) recovered at:			
		0 h	6 h	24 h	48 h
Aeromonas hydrophila	FecalSwab	1.5×10^4	1.5×10^4 (100)	2.4×10^5 (1,600)	1.6×10^7 (>10^5)
	ESwab	1.4×10^4	1.5×10^4 (107)	2.3×10^5 (1,643)	1.4×10^7 (10^5)
Campylobacter coli	FecalSwab	5.5×10^3	5.3×10^3 (96.4)	—[a]	—
	ESwab	3.8×10^3	3.6×10^3 (94.7)	—	—
C. jejuni ATCC 33291	FecalSwab	3.2×10^3	3.0×10^3 (93.8)	—	—
	ESwab	4.5×10^3	4.5×10^3 (100)	—	—
Clostridium difficile	FecalSwab	1.3×10^3	6.2×10^2 (47.7)	2.2×10^2 (16.9)	*[b]
	ESwab	1.0×10^3	5.4×10^2 (54.0)	2.0×10^2 (20.0)	*
Escherichia coli O157:H7	FecalSwab	1.8×10^4	2.5×10^4 (139)	6.4×10^5 (3,556)	5.6×10^7 (>10^5)
	ESwab	3.5×10^4	3.7×10^4 (106)	1.2×10^6 (3,429)	5.5×10^7 (>10^5)
Plesiomonas shigelloides	FecalSwab	8.8×10^3	1.0×10^4 (114)	1.2×10^5 (1,364)	1.0×10^7 (>10^5)
	ESwab	1.0×10^4	1.1×10^4 (110)	1.3×10^5 (1,300)	1.3×10^7 (>10^5)
Salmonella enterica subsp. *enterica* serovar Enteritidis	FecalSwab	1.2×10^4	1.3×10^4 (108)	2.3×10^5 (1,917)	2.8×10^7 (>10^5)
	ESwab	1.5×10^4	1.5×10^4 (100)	2.9×10^5 (1,933)	2.9×10^7 (>10^5)
S. enterica subsp. *enterica* serovar Typhimurium ATCC 14028	FecalSwab	1.4×10^4	1.4×10^4 (100)	2.5×10^5 (1,786)	1.8×10^7 (>10^5)
	ESwab	2.0×10^4	1.9×10^4 (95.0)	3.5×10^5 (1,750)	2.1×10^7 (>10^5)
Shigella sonnei ATCC 9290	FecalSwab	7.1×10^3	7.7×10^3 (108)	1.2×10^5 (1,690)	9.6×10^6 (>10^5)
	ESwab	1.3×10^4	1.2×10^4 (92.3)	2.1×10^5 (1,615)	1.4×10^7 (>10^5)
S. flexneri	FecalSwab	8.5×10^3	8.5×10^3 (100)	1.4×10^5 (1,647)	1.0×10^7 (>10^5)
	ESwab	1.6×10^4	1.6×10^4 (100)	2.5×10^5 (1,563)	1.7×10^7 (>10^5)
Yersinia enterocolitica ATCC 23715	FecalSwab	9.8×10^3	9.6×10^3 (98.0)	1.3×10^5 (1,327)	1.2×10^7 (>10^5)
	ESwab	1.2×10^4	1.1×10^4 (91.7)	1.6×10^5 (1,333)	1.5×10^7 (>10^5)
Vibrio cholerae	FecalSwab	1.8×10^4	1.8×10^4 (100)	2.7×10^5 (1,500)	2.2×10^7 (>10^5)
	ESwab	2.1×10^4	2.0×10^4 (95.2)	3.1×10^5 (1,476)	2.3×10^7 (>10^5)

[a] —, no growth, but a high number of Gram-negative bacilli was seen when Gram stained from the medium.
[b] *, no growth, but a high number of poorly Gram-staining bacilli with spore-like structures was seen when Gram stained from the medium.

teria. All gastrointestinal stock isolates investigated in our study survived in FecalSwab at least up to 48 h at 4°C, whereas in ESwab, 10 out of 12 stock isolates (83.3%) were present at 48 h of storage. However, this difference was not significant, and the cell concentrations remained mainly stable over time in both swab devices at refrigeration temperature, as has been demonstrated by others, using the ESwab system (3, 5). With *Salmonella* spp., though, some proliferation was seen in both swab devices 48 h after inoculation at 4°C. When the swab devices were stored at room temperature, all isolates except *C. difficile* and *Campylobacter* spp. showed notable growth already after 24 h of storage. Similar results with nonfastidious bacteria using ESwab and other swab systems have also been published (3, 5). This phenomenon is thought to be problematic in that it causes misleading culture results (3, 5). However, we observed that higher yields of, e.g., *Salmonella*, *Shigella*, and *Yersinia* spp. were recovered from simulated mixed samples, as well as from patient stool specimens, when the specimens were stored at room temperature for 20 h prior to culture. This nonselective enrichment in transport medium may be advantageous when pathogens are screened, i.e., with selective chromogenic medium.

Although no significant differences were seen between the ESwab and FecalSwab devices for the storage of various microorganisms at room temperature or 4°C, at −70°C, FecalSwab maintained the viability of stock isolates significantly better than ESwab. In ESwab, the recovery of 10 stock isolates out of 12 ceased during 2 weeks of storage, and only two *Salmonella* spp. survived up to 1 month, while in FecalSwab, all isolates survived at least up to 3 months. Moreover, for longer preservation, storage at −70°C proved to be more reliable, as at −70°C, the reduction of viable cells after 2 weeks was significantly less than that at storage at −20°C.

Compared to the other isolates, *C. difficile* survived better in FecalSwab than in ESwab at lower temperatures, while at room temperature, the recoveries were equal in both swab devices. At 4°C, *C. difficile* remained viable up to 48 h in FecalSwab, even though it was stored aerobically. Similar results have also been demonstrated with other transport systems, such as Amies gel swabs (8). Furthermore, as *C. difficile* cytotoxins have been demonstrated to remain highly stable for up to several months at 4°C (9), we performed a preliminary investigation of the preservation of *C. difficile* toxins and toxin genes in FecalSwab and ESwab for an extended period of time as well (data not shown). After 48 h of

TABLE 2 Survival of enteric pathogens in FecalSwab and ESwab devices at refrigeration temperature

Species and/or isolate ($n = 12$)	Swab device	CFU/ml (%) recovered at:			
		0 h	6 h	24 h	48 h
A. hydrophila	FecalSwab	1.5×10^4	1.5×10^4 (100)	1.7×10^4 (113)	2.1×10^4 (140)
	ESwab	2.4×10^4	2.4×10^4 (100)	2.6×10^4 (108)	3.3×10^4 (138)
Campylobacter coli	FecalSwab	5.8×10^3	5.5×10^3 (94.8)	—[a]	1.6×10^2 (2.8)
	ESwab	2.1×10^3	2.0×10^3 (95.2)	3.5×10^1 (1.8)	—[a]
C. jejuni ATCC 33291	FecalSwab	4.5×10^3	4.4×10^3 (97.8)	—	2.5×10^2 (5.6)
	ESwab	4.1×10^3	4.0×10^3 (97.6)	—	2.0×10^2 (4.9)
C. difficile	FecalSwab	3.5×10^3	3.3×10^3 (94.3)	2.8×10^3 (80.0)	8.2×10^2 (23.4)
	ESwab	2.9×10^3	9.0×10^2 (31.0)	6.0×10^2 (20.6)	*[b]
E. coli O157:H7	FecalSwab	1.5×10^4	1.5×10^4 (100)	2.0×10^4 (133)	2.3×10^4 (153)
	ESwab	3.0×10^4	3.0×10^4(100)	3.4×10^4 (113)	3.8×10^4 (127)
P. shigelloides	FecalSwab	9.2×10^3	9.0×10^3 (97.8)	9.1×10^3 (98.9)	9.0×10^3 (97.8)
	ESwab	4.5×10^4	4.4×10^4 (97.8)	4.4×10^4 (97.8)	4.4×10^4 (97.8)
S. enterica subsp. *enterica* serovar Enteritidis	FecalSwab	1.6×10^4	1.4×10^4 (87.5)	1.5×10^4 (93.8)	1.8×10^5 (1,125)
	ESwab	2.8×10^4	2.6×10^4 (92.9)	2.7×10^4 (96.4)	1.9×10^5 (679)
S. enterica subsp. *enterica* serovar Typhimurium ATCC 14028	FecalSwab	1.9×10^4	1.3×10^4 (68.4)	2.2×10^4 (116)	5.5×10^5 (2,895)
	ESwab	2.2×10^4	1.6×10^4 (72.7)	2.2×10^4 (100)	7.8×10^5 (3,545)
S. sonnei ATCC 9290	FecalSwab	5.2×10^3	5.7×10^3 (110)	4.0×10^3 (76.9)	4.2×10^3 (80.8)
	ESwab	1.1×10^4	7.6×10^3 (69.1)	7.9×10^3 (71.8)	8.1×10^3 (73.6)
S. flexneri	FecalSwab	9.8×10^3	9.8×10^3 (100)	8.1×10^3 (82.7)	8.3×10^3 (84.7)
	ESwab	1.4×10^4	1.4×10^4 (100)	1.0×10^4 (71.4)	1.1×10^4 (78.6)
Y. enterocolitica ATCC 23715	FecalSwab	1.1×10^4	1.3×10^4 (118)	1.3×10^4 (118)	2.5×10^4 (227)
	ESwab	1.4×10^4	1.3×10^4 (92.9)	1.9×10^4 (136)	2.4×10^4 (171)
V. cholerae	FecalSwab	1.5×10^4	1.5×10^4 (100)	1.3×10^4 (86.7)	2.3×10^4 (153)
	ESwab	1.8×10^4	1.6×10^4 (88.9)	1.8×10^4 (100)	2.2×10^4 (122)

[a] —, no growth, but a high number of Gram-negative bacilli was seen when Gram stained from the medium.
[b] *, no growth, but a high number of poorly Gram-staining bacilli with spore-like structures was seen when Gram stained from the medium.

storage at 4°C, all initially *C. difficile* toxin A/B- and *tdcB* gene-positive clinical stool specimens were positive from both the FecalSwab and ESwab devices with the IA and PCR assay used in our study. At 7 days of storage, all PCR-positive and approximately 67% of the IA-positive specimens from both devices were still positive. PCR positivity lasted for up to 1 month, after which time the follow-up ended. Of the IA-positive specimens, 50% remained positive until 1 month.

TABLE 3 Recovery of mixed organisms in FecalSwab and ESwab devices at room temperature

Species and/or isolate	Swab device	CFU/ml (%) recovered at:			
		0 h	6 h	24 h	48 h
S. enterica subsp. *enterica* serovar Typhimurium ATCC 14028	FecalSwab	5.0×10^2	6.5×10^2 (130)	2.3×10^4 (3,538)	3.2×10^6 (>10^5)
E. coli ATCC 25922		2.5×10^2	6.0×10^2 (240)	2.4×10^4 (4,000)	6.3×10^6 (>10^5)
E. faecalis ATCC 29212		5.0×10^1	5.0×10^1 (100)	5.0×10^3 (10^4)	1.2×10^5 (>10^5)
S. enterica subsp. *enterica* serovar Typhimurium ATCC 14028	ESwab	7.5×10^2	1.8×10^3 (240)	2.3×10^4 (3,667)	4.9×10^6 (>10^5)
E. coli ATCC 25922		7.5×10^2	6.0×10^2 (80.0)	3.7×10^4 (4,933)	8.0×10^6 (>10^5)
E. faecalis ATCC 29212		5.0×10^1	3.0×10^2 (600)	5.2×10^3 (>10^4)	1.0×10^5 (>10^5)
S. sonnei ATCC 9290	FecalSwab	5.0×10^1	2.0×10^2 (400)	1.4×10^4 (>10^4)	9.8×10^5 (>10^5)
E. coli ATCC 25922		5.5×10^2	3.0×10^2 (54.5)	2.0×10^4 (3,636)	3.2×10^6 (>10^5)
E. faecalis ATCC 29212		1.0×10^2	5.0×10^1 (50.0)	5.0×10^3 (5,000)	2.1×10^6 (>10^5)
S. sonnei ATCC 9290	ESwab	3.0×10^2	2.0×10^2 (66.7)	1.0×10^4 (3,333)	3.8×10^6 (>10^5)
E. coli ATCC 25922		1.2×10^3	6.0×10^2 (50.0)	2.6×10^4 (2,167)	7.8×10^6 (>10^5)
E. faecalis ATCC 29212		1.0×10^2	2.0×10^2 (200)	5.0×10^3 (5,000)	1.8×10^6 (>10^5)

TABLE 4 Recovery of mixed organisms in FecalSwab and ESwab devices at refrigeration temperature

Species and/or isolate	Swab device	CFU/ml (%) recovered at:			
		0 h	6 h	24 h	48 h
S. enterica subsp. *enterica* serovar Typhimurium ATCC 14028	FecalSwab	2.5×10^2	6.0×10^2 (240)	3.0×10^2 (120)	3.5×10^2 (140)
E. coli ATCC 25922		3.5×10^2	4.0×10^2 (114)	3.5×10^2 (100)	3.5×10^2 (100)
E. faecalis ATCC 29212		5.0×10^1	1.0×10^2 (200)	5.0×10^1 (100)	5.0×10^1 (100)
S. enterica subsp. *enterica* serovar Typhimurium ATCC 14028	ESwab	9.0×10^2	8.5×10^2 (94.4)	8.0×10^2 (88.9)	7.0×10^2 (77.8)
E. coli ATCC 25922		7.5×10^2	6.0×10^2 (80.0)	7.5×10^2 (100)	4.5×10^2 (60.0)
E. faecalis ATCC 29212		1.0×10^2	1.0×10^2 (100)	1.5×10^2 (150)	5.0×10^1 (50.0)
S. sonnei ATCC 9290	FecalSwab	5.0×10^1	1.5×10^2 (300)	3.0×10^2 (600)	2.0×10^2 (400)
E. coli ATCC 25922		1.5×10^2	2.5×10^2 (167)	2.5×10^2 (167)	1.0×10^2 (66.7)
E. faecalis ATCC 29212		5.0×10^1	5.0×10^1 (100)	5.0×10^1 (100)	5.0×10^1 (100)
S. sonnei ATCC 9290	ESwab	1.0×10^2	3.5×10^2 (350)	8.5×10^2 (850)	2.0×10^2 (200)
E. coli ATCC 25922		4.0×10^2	8.5×10^2 (213)	6.5×10^2 (163)	2.0×10^2 (50.0)
E. faecalis ATCC 29212		2.0×10^2	2.5×10^2 (125)	1.0×10^2 (50.0)	1.0×10^2 (50.0)

Most interestingly, the extension of storage time from 20 to 72 h seemed to improve gradually the cell viability of *Campylobacter* spp. at 4°C. Higher recovery rates of *Campylobacter* stock isolates were seen at 72 h of storage than at 48 h, and the storage of clinical stool samples containing *Campylobacter* spp. for >20 h (72 h) at 4°C enabled the recovery of growth on selective culture medium, although no growth was seen at 20 h of storage (data not shown). The frequency of and reason for this odd growth behavior of *Campylobacter* isolates at 4°C in FecalSwab and ESwab devices are not known. We did not find any previous reports with similar findings. It is known, though, that *Campylobacter* organisms may undergo a temporal physiological and morphological transition into a viable but nonculturable stage, whereby they retain basal metabolic activity yet fail to grow or multiply in cultures when translocated from their intestinal niche into an aquatic environment (10). However, at 4°C and, e.g., in Cary-Blair medium, which is the medium base in FecalSwab, *Campylobacter* spp. have been shown to remain culturable for days (11), albeit with reduced CFU counts (10, 11). We observed a nonculturable stage of *Campylobacter* spp. rapidly after 6 h of storage at 4°C, mainly in FecalSwab, which then returned to a culturable stage after 48 h of inoculation. However, aerated specimens, e.g., those prepared with shaking, may demonstrate a more rapid decrease in the recoverability of *Campylobacter* organisms than specimens held in a stationary state (10). In our study, all specimens in the FecalSwab or ESwab devices were vigorously mixed by vortexing at each time point prior to plating onto the culture medium. Accordingly, the detection of *Campylobacter* spp. in fecal specimens with culture may vary depending on the storage time and growth stage of the bacteria.

In conclusion, the ESwab proved to be a well-suited swab device for short-term storage and transportation of enteric pathogens. The FecalSwab, on the other hand, proved to be suitable even for extended storage and transportation of enteric pathogens, enabling successful and reliable microbiological analysis when specimens are sent to either a local laboratory or a more distant reference laboratory. Furthermore, due to a more homogenized form, specimens in both the ESwab and FecalSwab devices are easier to handle and use for various laboratory tests, e.g., IA or PCR assay, than specimens in dry containers. In addition, flocked swabs in liquid media are more suitable for culture automation than the gel swabs (12) or even stools in dry containers.

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