Copan ESwab, the first Liquid Based Microbiology device, preserves microbial viability up to 96/120 hours.

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Abstract

Objectives: Although it is recommended that swabs specimens should be processed soon after collection, delays are occurring due to microbiology laboratory consolidation. Specimens transport to centralized laboratories results in long storage times, samples accumulation and increasing workload. Therefore the use of a preservation medium that supports microbial viability for prolonged storage time is necessary. ESwab (ES), a high release flocked swab combined with one ml of liquid Amies medium, is the first liquid based microbiology (LBM) collection and preservation system that provides a homogeneous specimen suspension and allows multiple testing from the same original sample. The objective of the study was to compare the ability of ES to Amies Agar Gel Transystem (TS) to maintain the viability of a panel of microorganisms for an extended period of time.

Method: A panel of ATCC microorganism strains, representative of different body sites infections, was tested. P. anaerobius (PA), S. pneumoniae (SP), B. fragilis (BF), H. influenzae (HI) were selected for the respiratory system; VRE for the gastrointestinal system; S. pyogenes (SPY), P. acnes (PAC) for the skin membrane system; C. albicans (CA), for the genital tract system; MRSA for multi-site infections. Each strain was serially titrated and each swab was loaded with 100uL inoculum, to obtain 300-500CFU from each time zero (T0) plate. After inoculation, the swabs were held at room temperature (RT) for the first 24h, refrigerated for the following 72/96h and plated every 24h.

Results: Colonies count was recorded for each strain for all storage times. HI in ES was viable up to 120h but negative in TS. SP, BF, SPY, PA and PAC were stable at 120h in ES; one log reduction was found for SP at 48h, for BF, SPY and PA at 96h in TS. CA, MRSA and VRE were stable up to 120h in both devices, but TS had one log reduction. Testing of additional 10 microorganisms is in progress and results will be reported later.
In some cases at T0, recovery rate of ES was up to 20% higher than TS.

Conclusion: ES was superior to TS in maintaining bacterial viability for longer time and had a higher colonies count than the TS. The ES with its ability to maintain the original microbial load up to 96/120h can be used for the collection of clinical specimens that require longer processing time, multiple testing and results confirmations.

Methods

Copan ESwab is a new device dedicated to the collection and transport of clinical samples. It consists in a flocked swab treated with a special coating combined with 1 ml of modified liquid Amies. The study is a comparison of the ability of ES than traditional TS in maintaining a panel of organisms for a prolonged storage time. For that, a 0.5 McFarland suspension of each organism listed in Table1 was prepared: it was serially diluted in order to obtain a concentration of 100-500CFU from each T=0 per plate. swabs from each type were spiked with 100 ul inoculum and then transferred in the respective medium. 6 pieces of ES and 6 pieces of TS were inoculated. Two pieces per type were plated immediately as T=0, the rest was hold at RT for the first 24h and then refrigerated for the next 72/96h. TS was plated by direct swabbing, ES was plated by transferring and spreading 100ul of inoculated Amies liquid onto appropriate agar. *N. meningitidis* was also tested at RT until 96h without refrigeration step. The agar plates were incubated in appropriate conditions and colony count was performed.

Table 1: ATCC strains tested

Strain	ATCC
P.anaerobius	27337
S.pneumoniae	6305
B.fragilis	25285
S. agalactiae	12386
S. pyogenes	19615
H.influenzae	10211
E. faecalis (VRE)	51299
P. acnes	11828
B.pertussis	8467
N.meningitidis	13090
S. aureus (MRSA)	43300
C. albicans	10231
A. niger	16404
C. sporogenes	11437
F. solani	36031

Results

Table 2 and Fig. 1: Comparison between ES and TS recovery at T=0

Strains	Abbreviation	ES	TS	Δ Recovery %
P. acnes	PAC	152,5	86,5	76%
P. anaerobius	PA	357	210	70%
S. pneumoniae	SP	378	162	133%
B. fragilis	BF	591,5	384	54%
S. agalactiae	StrepB	184	95,5	93%
S. pyogenes	StrepA	89,5	47	90%
E. faecalis	VRE	111	118	-6%
C. sporogenes	CS	199	186	7%
C. albicans	CA	216	176	23%
B. pertussis	BP	400	278	44%
A. niger	AN	18	8	125%
S. aureus	MRSA	183,5	55	234%
N. meningitidis	NM	145	62	134%
H. influenzae	HI	211	125	69%
AVERAGE				82%

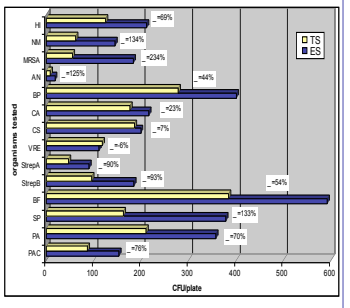


Table 2 and Fig.1 are reporting the actual number of CFU/plate obtained after plating the devices at T=0. Particular attention was given to the difference in recovery between ES and TS and the Δ Recovery % was calculated as per the following formula:

$$\Delta\ \text{Recovery \%} = \frac{\text{(Eswab CFU/plate-Transystem CFU/plate)}}{\text{Transystem CFU/plate}}$$

Table 3: Organisms viability up to 96/120h

	ES			TS		
	T=0	T=24h	T=96h/120h	T=0	T=24h	T=96h/120h
P. acnes	152,5	151	44,5	86,5	27	19
P. anaerobius	357	306	201	210	0	0
S. pneumoniae	378	515	780	162	26,5	2
B. fragilis	591,5	740	600	384	364,5	56
Strep B	184	394	319	95,5	59,5	21
Strep A	89,5	500	614	47	13,5	16,5
VRE	161	515	650	153	358	416
C. sporogenes	199	132	108	186	13,5	2,5
C. albicans	216	222	196,5	176	800	456
B. pertussis	400	252	95	278	165	70
A. niger	18	17	8,5	12,5	10	8
MRSA	183,5	535	665	55	54	24,5
N. meningitidis (RT)	145	84	531	62	18	265
N. meningitidis (RT and 4°C)	145	103,5	0	62	30,5	0
H. influenzae	125	71,5	26	211	17	0

Picture 1: *F. solani* growth

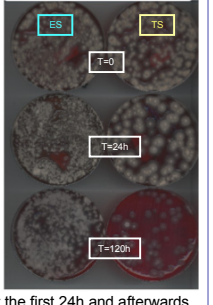
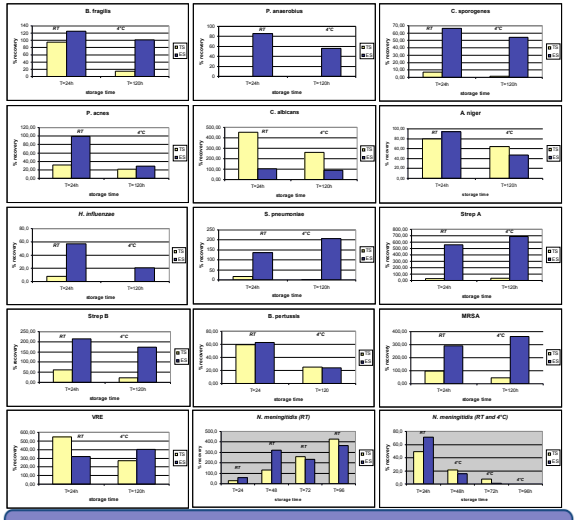


Table 3: reports organisms viability after storage of devices at RT for the first 24h and afterwards transferred to refrigerated conditions. Data are expressed as CFU/plate. Picture 1 shows the growth of *F. solani* in both ES and TS.



Conclusions

•Δ recovery % at T=0 was calculated to quantify the increase of bacterial inoculum that ESwab is able to release as compared to Transystem. Although the two devices were spiked with the same amount of bacteria, in 14/15 strains ESwab shows a significan higher recovery % (on average by 82%) and therefore a better sensitivity.

•After 96h-120h of storage, ESwab was able to maintain viable 15/15 strains,TS 11/15, one strain of which (*P.anaerobius)* was lost after T=24h.

•After 96h-120h of storage, 10/15 strains trasported in ESwab maintained more than the 50% of the starting inoculum, only 4/15 in case of Transystem.

•Some strains transported in Eswab (*StrepA, VRE and MRSA)* showed an increased in the starting bacterial inoculum (never >1log than T=0) after T=24h, the same was observed in TS with *C. albicans.*

•A separate note on *N. meningitidis* whose growth was not enhanced by the refrigeration as for all the other organisms but even inhibited. When stored at RT, NM was maintaned until 96h by both devices.

•In general, ES showed the best ability to maintain a wide panel of bacteria (aerobes, anaerobes and fungi) till 120h in refrigerated conditions. The superior capability to release and maintain the initial inoculum, makes ES a suitable device for prolonged transporting time, multiple testing and results confirmation.