EVALUATION of FOUR SWAB TRANSPORT SYSTEMS for the RECOVERY of ATCC and CLINICAL STRAINS with CHARACTERIZED RESISTANCE MECHANISMS

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INTRODUCTION

Recovery of bacteria from clinical specimens after collection and transport or storage with minimum loss of viability is a main goal in clinical microbiology laboratories. Due to the ease of using swabs, swab transport systems (STS) are an acceptable method for transportation particularly when specimens are expected to be processed without significant delay. Isolates gathered at different laboratories are also frequently sent to distant locations where consistent recovery is also of utmost importance particularly in resistance surveillance studies. Moreover, STS have clearly economical advantages when compared with frozen transport systems.

A transport device must assure capability of preserving the widest spectrum organisms. If this does not occur, the most labile organisms and the less metabolically robust strains should be underrepresented not only in clinical sample populations but also in epidemiological surveys and statistics. Moreover, the probable influence of storage and/or transportation on STS on the viability of organisms with resistance mechanisms has not been particularly investigated.

OBJECTIVE

The aim of the present study was to compare the performance of four commercially available STS against different quality control ATCC strains and antibiotic resistant clinical isolates to ascertain bacterial viability after a set holding time.

MATERIALS and GROWTH CONDITIONS

Swab transport devices: the non charcoal-containing swab (clear Amies) transport systems (STS) tested were manufactured by:

<table>
<thead>
<tr>
<th>Swab</th>
<th>Manufacturer/Brand</th>
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<tr>
<td>1 (S1)</td>
<td>Copan Italy, (M40 Transystem)</td>
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<tr>
<td>2 (S2)</td>
<td>MEUS s.r.l, Italy (UNI-TER)</td>
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<tr>
<td>3 (S3)</td>
<td>LAB SERVICE S.p.a Italy (Euromed)</td>
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<tr>
<td>4 (S4)</td>
<td>I.A.S.A. (Deltalab), Spain (Eurotubo®)</td>
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Strains:

- Control strains used for viability studies were:
  - Streptococcus pneumoniae (ATCC 6305)
  - Streptococcus pyogenes (ATCC 19615)
  - Staphylococcus aureus (ATCC 43300)
  - Haemophilus influenzae (ATCC 10211)
  - Neisseria gonorrhoeae (ATCC 43069)
  - Neisseria meningitidis (ATCC 13090)
  - Peptostreptococcus anaerobius (ATCC 27337)
  - Fusobacterium nucleatum (ATCC 25586)

- Clinical isolates included in this study were:
  - Enterococcus faecium (vanA);
  - Klebsiella pneumoniae (ESBL producer)
  - Pseudomonas aeruginosa (VIM-2 producer)
  - Stenotrophomonas maltophilia (intrinsinc metallo-β-lactamase producer)
  - Acinetobacter baumannii (imipenem and meropenem resistant)

Plate media: 5% sheep blood agar and chocolate agar supplemented with PolyVitex (bioMérieux, Marcy l’Etoile, France) and Brucella anaerobic agar (Biomedics, Madrid, Spain) were used for each organism when appropriate.

Incubation temperature and atmosphere: plates were incubated at 35-37 °C in aerobic, 5% CO2, or anaerobic conditions depending on each organism’s requirement.
METHODS

The roll-plate method outlined by M40-A NCCLS guideline (Quality Control of Microbiological Transport Systems; Approved Standard. Draft, 2003) was followed to evaluate the 4 transport devices. Briefly, 0.5 MacFarland standard (ca 1.5 x 10^6 CFU/ml) of each organism freshly grown at 35°C for 18-24 hours was prepared. Successive ten fold dilutions of this inoculum were performed to provide final working concentrations ranging from approximately 1.5 x 10^7 CFU/ml to 1.5 x 10^4 CFU/ml. An aliquot of 100 μl of those dilutions that rendered accurate ranges for total colony counting (most closely approaching 300 CFU in zero-time plates) was used to inoculate swabs using wells of a microdilution plate, as described in M40-A NCCLS guideline. Swabs were held at room temperature (20-25°C) and subsequently sub-cultured by rolling over the entire surface of appropriate agar plates. The dilution that yielded a plate count closest to 300 CFU at time zero was the one selected for subsequent counts.

Plating hours were: 0 (zero time), 6 (only for fastidious and anaerobic organisms), 24, 48, and 72. A count at the 7th day after inoculation was also included when testing clinical isolates with antibiotic resistance mechanisms. Colonies were counted after incubation of the plates for 24-48 hrs at 35-37°C in the correspondent atmosphere.

Viability was calculated as percentage of recovery, meaning number of organisms recovered as a percentage of the bacterial counts at time zero (baseline counts). To ensure consistency, experiments were performed in triplicate (three swabs per set of organism per holding condition).

RESULTS

Figures 1 to 13 represent percentage of organisms’ recovery respect to the baseline counts (counts at time zero) for each STS at each holding time. All values express the average for triplicate experiments.

\[ATCC\text{ strains:}\]

Fig. 1. *S. aureus* ATCC 43300 (methicillin-resistant strain). All swabs performed satisfactorily in supporting the viability of this strain, although with different performances between them. At 24 hours, percentages of recovery were of 217%, 194%, 65%, and 73% for S1, S2, S3, and S4, respectively. In the case of S1 and S2, overgrowth was sustained unless up to the 7th day of storage (1,374% and 390% of recovery each).

Fig. 2. *S. pneumoniae* ATCC 6305. This strain showed poor viability with all swab systems except with S1 that was able to support its growth for 48 hours, although with a 38% of recovery at this holding time. *S. pneumoniae* transportation is a worrying aspect as this organism is frequently isolated in the clinical setting causing complicated infections that require accurate and rapid diagnostic. The maintenance of viability in clinical samples when stored in swabs is mandatory and certainly requires the best performance.

Fig. 3. *S. pyogenes* ATCC 19615. All the 4 swabs performed similarly with regard to maintaining the viability of this strain for the first 6 hours. After this time period, S2, S3, and S4 exhibited a comparable reduction in recovery, although with positive values even at the 7th day of storage (17%, 20% and 20% of recovery, respectively). At this time, S1 has an overgrowth rate of more than 2,000% expressed as percentage of recovery.

Fig. 4. *H. influenzae* ATCC 10211. After a holding time of 6 hours, only S1 maintained the viability of this strain with a 38% of recovery at 72 hours of storage. S2, S3, and S4 (7%, 50%, and 5.4% of recovery at 6 hour-storage period) failed to support the growth of this fastidious organism beyond this holding time.

Fig. 5. *N. meningitidis* ATCC 13090. After 24 hours of incubation (104% of recovery), only S1 maintained the viability of this strain. The number of viable organisms was maintained up through the 7th day with 73% of recovery.

Fig. 6. *N. gonorrhoeae* ATCC 43069. For this strain, percentages of recovery were dramatically low for all STS tested, being S1 the only device able to support its growth but only along the first 6 hours (46% of recovery). The frequent auxotypes found among clinical isolates of this species make this organism particularly susceptible to storage conditions and STS’ performance may be limited due to these requirements. The exiguous preservation
observed in this study may be attributed, unless in part, to the lack of charcoal in the transport media of the tested STS (clear Amies-containing swabs). Less robust *N. gonorrhoeae* clinical strains may be particularly susceptible to this respect.

**Fig. 7.** *F. nucleatum* ATCC 25586. The percentage of recovery of this anaerobe was poor with all swabs, particularly with S4 (no survivors at 6-hour-storage). Only S1 was able to support the growth for 24 hours although with a marked reduction of viability (18% of recovery).

**Fig. 8.** *P. anaerobius* ATCC 27337. All 4 swabs failed to support an adequate growth of this anaerobe. Only S1, but with a dramatic reduction (7% of recovery) maintained viable counts for 6 hours.

 lạ Clinical isolates with known antibiotic resistance mechanisms:

**Fig. 9.** *E. faecium* (van A). All swabs supported the growth of this strain even at the 7th day of storage. In the case of S1 and S3, significant overgrowth was observed at this holding time (6,138% and 1,078%, respectively) while S2 and S4 were able to maintain growth with lower percentages of recovery (8.7% and 30%, respectively).

**Figs 10-13.** Aerobic Gram negative bacilli. In the case of this group of organisms, either the *Enterobacteriaceae K. pneumoniae* strain carrying CTX-M-9 extended-spectrum β-lactamase or the nonfermentative organisms (*P. aeruginosa* carrying VIM-2 β-lactamase; *A. baumannii* resistant to carbapenems; and *S. maltophilia* L2 metallo-β-lactamase producer) were equally well supported by all the 4 STS. A different but marked overgrowth, even at the 7th day of storage, was observed for all these strains.

In summary, the **Copan M40 Transystem** (S1) gave improved recovery of the 8 ATCC strains, yielding the best recovery of even fastidious and anaerobic organisms. All STS except Copan M40 failed to support the recovery of ATCC *S. pneumoniae*, *N. gonorrhoeae*, and *P. anaerobius* at a 6 hour-storage period. In the case of *F. nucleatum*, only the S1 device supported its growth up to 24 hours of storage while the S4 swab failed to support its growth even at 6 hours of storage. Copan M40 Transystem performed satisfactorily with respect to *H. influenzae* and *N. meningitidis* in maintaining their viability for at least 72 h. Overgrowth was observed with the 4 STS for all clinical Gram negative bacilli with characterized resistance mechanisms. To note that the Copan M40 Transystem specimen absorption was much better than the other three swabs in all experiments (not shown).

**CONCLUSIONS**

According to the results of the present study we can summarize:

- Overall, the **Copan M40 Transystem** outperformed the three other swab systems evaluated. Apart from other improvements in design, the nitrogen gas-containing atmosphere of this device strongly improves the maintenance of viability, particularly for fastidious organisms and anaerobes.

- This study indicates that the **Copan M40 Transystem** swab has a good potential to be used for transport of both aerobic and anaerobic microorganisms.

- The presence of resistance mechanisms in clinical isolates and in some quality control ATCC strains seems not to affect the performance of all tested STS.

- On the other hand, the roll-plate method, due to the ease of performance and to a less processing cost, appears to be well suited for the Clinical Microbiology Laboratory setting and it is already being used in most routine quality control protocols.
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