Liquid based microbiological transport systems: Conformity assessment of two commercial devices

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We compared two types of liquid-based microbiology devices for microorganism viability according to standardized quantitative elution method CLSI M40-A2. The eSwab® met CLSI acceptance criteria of viability maintenance for all microorganisms tested. The Σ-Transswab® failed to meet CLSI acceptance criteria for Peptostreptococcus anaerobius, Prevotella melaninogenica, Fusobacterium nucleatum and Haemophilus influenzae.

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Transport systems and devices are essential components of the process of microbiology laboratory testing. It is recognized that the early steps in the total testing processing are critical to the production of clinically relevant information. A variety of microbiological transport systems and devices exist. It is imperative that users systematically evaluate systems for performance effectiveness, ensure standards of performances, and to allow for internal validation of product effectiveness, thus selecting the best for the needs of the physician and the patient (CLSI M40-A2, 2014). There are multiple variables involved in the manufacture of a transport device, as transport medium, collection device, packaging and environment. It is fundamental that the assessment of the device be based on measurable performance characteristics for the device (CLSI M40-A2, 2014).

Recent studies have established that simulated transport performance at cold temperature yields superior results compared to that at room temperature. These data support the current CLSI recommendation that room temperature transport does not represent the optimal holding temperature for maximum preservation of microbiological samples (Nys et al., 2010; Van Horn et al., 2008a,b; Buchan et al., 2014; Stoner et at., 2008; Arbique et al., 2000). The CLSI document M40-A2 (2014) also recommends that if the conditions of the end user differ from those indicated, the actual transport condition should be tested in order to evaluate viability of microorganisms.

Recently, two modified Liquid Amies based swab collection devices were manufactured to enhance specimen collection and release: the eSwabs® (Copan Italia SpA, Brescia, Italy) incorporates a nylon flocked transport system and the Σ-Transswabs® (Medical Wire & Equipment, UK) includes a soft polyurethane foam bud.

The aim of this study was to compare the eSwab and the Σ-Transswab liquid-based microbiology (LBM™) devices for recovery and viability at controlled-room temperature (RT) of the following ATCC organisms: Haemophilus influenzae (10211), Neisseria gonorrhoeae (43069), Streptococcus pneumoniae (6305), Streptococcus pyogenes (19615), Bacteroides fragilis (25285), Fusobacterium nucleatum (25586), Prevotella melaninogenica (25845), Peptostreptococcus anaerobius (27337), and Propionibacterium acnes (6919). The ATCC microorganisms listed are the minimum that must be included in a test battery to evaluate a transport device (CLSI M40-A2, 2014).

The CLSI document M40-A2 (2014) was followed to evaluate the two transport swabs. Briefly, a 0.5 McFarland (about 1.5 × 10⁸ CFU/ml) standard of each organism freshly grown at 37 °C for 18–24 h was prepared in 0.85% saline. For the flocked fiber swabs with an uptake volume of 100 μl (eSwab), the suspension was further diluted 1:10 to achieve a concentration of about 1.5 × 10⁶ CFU/ml and the inoculum was 100 μl. For the foam swab device with an uptake volume of 50 μl (Σ-Transwab), the suspension of 0.5 McFarland was further diluted 1:5 and the inoculum was 50 μl, according to CLSI guidelines. TriPLICATE swabs were inoculated into their respective transport system and stored at designed controlled-room temperature 20–25 °C for 0, 24 and 48 h. After the appropriate storage time, including 0 h (tested within 15 min from inoculation) serial 1:10 dilutions of each swab system were prepared to obtain suspensions equivalent to about 10⁵–10⁶ CFU/ml. In duplicate, 100 μl samples were used to quantify the organisms in each of the dilutions on TSA with 5% sheep blood agar for aerobic and anaerobic and Chocolate agar with Vitox for fastidious organisms respectively. The organisms were spread over the agar surface with a plate spreader, and the plates were

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incubated at 37 °C in 5% CO2 incubator, or in anaerobic atmosphere. To meet the CLSI M40-A2 criteria, no more than 3 log10 decline in CFU count should be observed. The initial inoculum (about 1.5 × 10^7 CFU/ml) was verified by serial 1:10 dilutions plated on duplicate in appropriate medium and incubated at 37 °C in appropriate atmosphere. Colony counts were obtained to confirm that the inoculum size was acceptable (about 1.5 × 10^7–1.5 × 10^9 CFU/ml) (CLSI M40-A2, 2014).

Bacterial recovery was determined by counting the colonies recovered from each dilution. The number of the organisms recovered is

![Image of Table 1](image-url)

**Table 1:** Organism recovery (log change of CFU counts) from Copan eSwab and Σ-Transwab over different incubation time (T = 0, 24, 48 h) at controlled room-temperature storage (acceptability CLSI criteria: Δ LOG no more than 3 log10).

![Image of Fig. 1](image-url)

**Fig. 1.** Changes in CFU counts over 48 h period. Aerobes, anaerobes and fastidious organism plated after different incubation times (T = 0, 24, 48 h) in eSwabs (A) and Σ-Transwabs (B) devices. The mean values and the standard deviations are reported.
expressed as an average for triplicate samples evaluated and as a percentage of the baseline counts (counts at time zero). CLSI M40-A2 criteria were used for evaluation as follows: a swab system was considered acceptable for the tested bacteria if the change in CFU from the 0 h time point declined no more than 3 log (ΔLOG). CLSI M40-A2 establishes a storage evaluation time of 24 h for N. gonorrhoeae and 48 h for all other organisms. 

Mechanically, there was a clear advantage for the eSwab since it smoothly absorbed the loading inoculum, while the Σ-Transwab inoculum adsorption was much less efficient, averaging 50 μl. As shown in Table 1, the different CFU count values at 0 h time-point reflect the critically different absorbance capabilities of the two swabs. The eSwab fulfilled the CLSI acceptance criteria of viability maintenance for all microorganisms tested at T0, T24 and T48 timepoints. The Σ-Transwab failed to meet CLSI acceptance criteria after 24 h of storage for P. anaerobius and P. melaninogenica, and after 48 h for F. nucleatum and H. influenzae (Table 1). In contrast with Van Horn et al. (2008a,b) experiments, in our study the eSwab met CLSI acceptance criteria also for P. melaninogenica after 24 h and 48 h of room temperature storage. Most interestingly, as observed for Campylobacter spp. in FecalSwab and eSwab by Hirvonen and Kaukoranta (2014), the extension of storage up to 24 h at room temperature improved the cell viability and recovery of S. pneumoniae (Table 1).

In this study we aimed to evaluate the conformity assessment and performances of two commercial liquid-base microbiology devices at controlled-RT. The choice of testing only this temperature of storage was to simulate the actual transport conditions in our routine setting, in line with CLSI M40-A2 (2014) recommendation. Under the storage condition evaluated, the Copan eSwab has shown a better recovery capability than Σ-Transwab device, meeting CLSI acceptance criteria of viability maintenance for all microorganisms tested. Σ-Transwab showed less efficient inoculum adsorption and failed to meet CLSI acceptance criteria for P. anaerobius, P. melaninogenica, F. nucleatum and H. influenzae.

Conflict of interest

None of the authors has a potential conflict of interest to declare.

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