Pathology (April 2014) **46(3)**, pp. 230–233

MICROBIOLOGY

Evaluation of bacterial recovery and viability from three different swab transport systems

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Summary

This study evaluated three types of swab transport systems for organism recovery. Swabs with transport media were further assessed for organism viability over 24 hours over a range of different storage temperatures. Test organisms consisted of aerobes, fastidious aerobes and anaerobes. Swabs were tested according to the standardised quantitative elution method published by the Clinical Laboratory Standards Institute (CLSI; M-40A). There were substantial differences in primary organism recovery, with recovery rates from different swabs ranging from <0.1% to 78% for *Streptococcus pyogenes*. Similar differences were noted for other test organisms. In general, the flocked swab (ESwab) demonstrated highest rates of recovery for aerobic organisms, while higher rates of recovery of *Fusobacterium nucleatum* were demonstrated from a standard swab (Transwab). When considering organism viability, no single swab fulfilled all the criteria stipulated by the M-40A standard for all organism/temperature combinations. Organism viability was marginally better for the gel-based swab transport systems as compared to the liquid-media based ESwab. Significant differences between swab transport systems were demonstrated, including differences for primary organism recovery and viability. The ESwab showed the best recovery of organisms, while gel-based media demonstrated marginally better bacterial viability for most tested retention times and temperatures.

Key words: Amies media, CLSI M40, flocked swab.

Received 20 July, revised 11 October, accepted 15 October 2013

INTRODUCTION

Swab samples represent one of the most commonly received samples received for bacteriological analysis. The performance characteristics of swabs may vary depending on the material used (cotton, dacron or rayon), the presence or absence of transport media, and the method of swab construction. Gel-based media for swabs are widely available, while liquid-based swab media have been introduced to facilitate the use of swab transport systems with automated sample processing technology. The introduction of flocked swab technology is claimed to provide enhanced performance compared to conventional fibre-wrapped swabs. The Clinical Laboratory Standards Institute (CLSI) M40-A standard has allowed uniform protocols for the evaluation of the performance of different swab transport systems.[1] Under this standard, specimens held at 20–25°C must demonstrate a fall of less than $3 \log_{10}$ between the zero-time counts and the stored swabs. Specimens held at 4–8°C must demonstrate a fall of less than $3 \log_{10}$ and an increase of less than $1 \log_{10}$ between zero time count and the stored swabs. This study was performed to compare organism recovery and viability from different swab types, using the CLSI M40-A guidelines.

The swabs chosen for evaluation were: dry swab with regular rayon tips (cat no. 155C; Copan, Italy), dry swab with regular dacron tips (cat no. 300263; Deltalab, Spain), Transwab with Amies agar gel (M40-A compliant, cat no. MW170; MWE, United Kingdom), M40 Transystem Amies agar gel (M40-A compliant, cat no. 408C; Copan) and ESwab (M40-A compliant, cat no. 480CE; Copan). The swabs were chosen to compare the performance of existing dry swabs used in our institution and M40 compliant swabs which were available in Singapore. All testing materials were purchased from the respective manufacturers. All tested swabs comprised standard rayon or Dacron-spun tips in semi-solid Amies media (where stated) except for ESwabs, which comprised flocked swabs and liquid Amies media.

METHODS

Dry swabs were only evaluated for immediate recovery of organisms following inoculation. Swabs with transport media were evaluated for immediate recovery of organisms, and recovery and viability of organisms after retaining inoculated swabs for 6 h and 24 h at three different temperatures (4°C, 25°C and 30°C). The additional temperature of 30°C was chosen to better represent room temperature in tropical climates.

Organism recovery from dry swabs was evaluated using *Streptococcus pyogenes* ATCC 19615 and *Streptococcus pneumoniae* ATCC 6305, while organism recovery and viability from swabs with transport media was evaluated with *S. pyogenes* ATCC 19615, *Haemophilus influenzae* ATCC 10211, *Neisseria gonorrhoeae* ATCC 43069, *S. pneumoniae* ATCC 6305, and *Fusobacterium nucleatum* ATCC 25586. Organisms were chosen from the CLSI recommended test panel, and selected to represent the spectrum of aerobic, fastidious and anaerobic bacteria that may be encountered in a clinical setting.

Testing was performed using the CLSI M40-A quantitative elution method. In brief, a 100 μL suspension containing approximately 1×10^7 colony forming units (CFU)/mL of each test organism was inoculated onto each tested swab. The initial test inoculum was verified by plating serial ten-fold dilutions of the test suspension to trypticase soy agar with 5% sheep blood agar for aerobes, chocolate agar plates for *H. influenza* and *N. gonorrhoeae,* and pre-reduced brain-heart infusion agar plates for *F. nucleatum* (all media obtained from Becton Dickinson, USA). Testing for every organism/time combination was performed in triplicate. Inoculated swabs were placed into their respective transport tubes, and then held for the intended time period at

Print ISSN 0031-3025/Online ISSN 1465-3931
DOI: 10.1097/PAT.0000000000000074

the stipulated temperature. Elution of test swabs was performed in 1 mL of 0.85% saline except for the ESwab, which elutes directly into 1 mL of modified liquid Amies medium. Serial ten-fold dilutions were performed from aliquots of the eluates, and 100 μL of each dilution was plated on to the appropriate media. Plates were incubated for 48 h in appropriate atmosphere, and colony enumeration was performed for plates with 30–300 CFU. Colony counts were derived from an average of the counts from test cultures performed in triplicate. Recovery of colony counts at time zero (T_0), 6 (T_6) and 24 h (T_{24}) was compared against the original inoculum (expressed as % change). Organism viability was assessed by comparing colony counts from samples retained for duration of 6 (T_6) and 24 h (T_{24}) against the colony counts at T_0 (expressed as $\log_{10}$ change).

RESULTS

Organism recovery

At T_0, there was significant variability in the recovery of organisms from different swab transport systems, with the lowest organism recovery from the two brands of dry swabs. For *S. pyogenes*, less than 6% of the inoculated organism was recovered from either of the dry swabs, while recovery from the other three swab types ranged from 35% to 78% (Table 1).

The recovery of *S. pneumoniae* from both dry swabs was less than 0.1%, compared to 10% recovery from Transwab and M40 swabs, and 55% from ESwab (Table 2). The improved performance of the ESwab was similarly demonstrated for *N. gonorrhoeae* and *H. influenzae*, where the ESwab was the only transport system to show a recovery rate exceeding 50% for both organisms.

For aerobic organisms, the ESwab generally demonstrated better rates of organism recovery for most temperature/holding time points. At the holding temperature of 30°C, there was some evidence of bacterial overgrowth for *S. pyogenes* in the M40 swab, resulting in higher recovery rates. The M40 swab also performed better for recovery of *S. pneumoniae*. For *F. nucleatum*, the best organism recovery was demonstrated from the Transwab.

Organism viability

No single swab passed the CLSI criteria for all organism/temperature combinations. A >4-log fall in viability of *F. nucleatum* was noted for both M40 and ESwab swabs after 24 h incubation at 25°C. A reduction of >6 log was noted for *N. gonorrhoeae* in the Transwab after 24 h incubation at 30°C.

In general, the gel-based swabs performed marginally better for organism viability when compared to the liquid-based ESwab. Bacterial overgrowth for *S. pyogenes* was more likely in the M40 swab (and to a lesser extent, the ESwab) when incubated at temperatures of 25°C and above. For anaerobes (*F. nucleatum*), the gel-based swab transport devices generally provided better viability for most storage conditions than the liquid Amies medium of the ESwab, with the Transwab demonstrating the best anaerobic viability (Table 2).

For the three swab transport devices, higher incubation temperatures increased the likelihood of bacterial overgrowth for *S. pyogenes*, but conversely, resulted in lower recovery for fastidious organisms (*S. pneumoniae*, *H. influenzae*, *N. gonorrhoeae*), including anaerobes (*F. nucleatum*).

DISCUSSION

This study demonstrated that performance varies significantly between different swab transport systems. Although this is not addressed by the CLSI M40-A standard, the recovery of organisms from the initial inoculum at T_0 varies tremendously. For example, there was a 6-log difference in primary organism recovery between the worst and best performing swabs for *S. pyogenes*. The differences in organism retrieval were less marked between M40 compliant swabs, but the flocked swab (part of the ESwab system) still generally outperformed conventional fibre-wrapped swabs for primary organism recovery. Performance for organism viability was more evenly distributed between the three swab transport systems, with marginally superior overall results from the gel-based M40 swab. Anaerobic viability (as assessed by *F. nucleatum*) was better in the gel-based media than the liquid-based medium.

The cost of swabs may be an important consideration. For the swabs evaluated in this study, the lowest cost was for the dry swabs without media (US$0.15 per swab), followed by the swabs with gel-based media (US$0.45–0.65 per swab). The most expensive was the ESwab (US$1.50 per swab). However, prices listed here are only provided for comparison purposes as

Table 1 Organism recovery from different swab systems

Organism	Swab type (Brand)	Inoculum (CFU)	Recovery T_0 (CFU)	% Recovery T_0	Log change in CFU
S. pyogenes	Dry (Copan)	5.4×10^6	4.7×10^1	0.0%	−5.1
	Dry (Deltalab)	8.4×10^6	4.7×10^5	5.5%	−1.3
	Rayon, Amies Gel (Transwab)	5.4×10^6	3.3×10^6	62.0%	−0.2
	Rayon, Amies Gel (M40)	5.4×10^6	1.9×10^6	34.8%	−0.5
	Flocked, Liquid Amies (ESwab)	9.0×10^6	7.0×10^6	77.6%	−0.1
S. pneumoniae	Dry (Copan)	8.6×10^6	2.4×10^2	0.0%	−4.6
	Dry (Deltalab)	4.7×10^6	3.4×10^3	0.1%	−3.1
	Rayon, Amies Gel (Transwab)	8.6×10^6	9.1×10^5	10.6%	−1.0
	Rayon, Amies Gel (M40)	1.7×10^6	1.6×10^5	9.6%	−1.0
	Flocked, Liquid Amies (ESwab)	1.7×10^6	9.2×10^5	55.7%	−0.3
N. gonorrhoeae	Rayon, Amies Gel (Transwab)	1.3×10^7	4.1×10^6	31.2%	−0.5
	Rayon, Amies Gel (M40)	1.0×10^7	8.9×10^5	8.9%	−1.0
	Flocked, Liquid Amies (ESwab)	8.8×10^6	7.6×10^6	86.3%	−0.1
H. influenzae	Rayon, Amies Gel (Transwab)	1.9×10^7	1.7×10^6	9.2%	−1.0
	Rayon, Amies Gel (M40)	9.9×10^6	1.1×10^6	11.2%	−1.0
	Flocked, Liquid Amies (ESwab)	1.9×10^7	1.6×10^7	84.7%	−0.1
F. nucleatum	Rayon, Amies Gel (Transwab)	2.7×10^5	1.0×10^5	37.8%	−0.4
	Rayon, Amies Gel (M40)	1.7×10^6	1.2×10^5	7.5%	−1.1
	Flocked, Liquid Amies (ESwab)	9.0×10^5	9.5×10^4	10.6%	−1.0

CFU, colony forming units; T_0, time zero after inoculation of swab.