MICROBIOLOGY COLLECTION DEVICE AND ISO15189: AN APPROPRIATE SELECTION

Loss of bacterial viability during any significant holding period following sampling compromises subsequent recovery by culture. Here, Jamie Laughlin assesses the performance of a range of swab collection devices.

In light of the emphasis being placed on quality in the clinical laboratory, and given the increased awareness of the importance of the pre-analytical phase of specimen processing, the selection of the most appropriate collection device cannot be overemphasised. While swab systems are considered less optimal than direct plating for culturing purposes, they have become increasingly important in view of the delay of specimen transport necessitated by recent strategies of cost containment and consolidation of laboratory services. If the pre-analytical step is performed with suboptimal quality, even the highest standards of laboratory quality management and/or automation will not compensate for the initial flaws, and this may have a negative effect on the patient care pathway.

Collection and transport of bacterial specimens to the laboratory is a critical component in the success of the diagnostic process. Transport time and temperature are now a major concern as the original concept and design of swab transport devices is 70 years old, and they were developed in a time when the patient was only minutes away from the laboratory. Swabs are a very much used sampling device, and the swab components play a major, but often overlooked, role in sampling. The preservation and viability of organisms must be assured. Transport swabs must be seen as a critical component of the diagnostic pathway. Failure to ensure viability of microorganisms at the pre-analytical stage will have an adverse effect on any relevant clinical information received from the investigation.

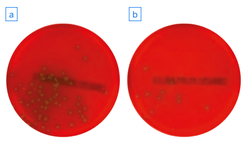

Fig 1. Results for a) MWE and b) Deltalab/Medline swabs with Streptococcus pneumoniae held at RT for 48 h prior to inoculation and incubation on the BD Kiestra TLA system.

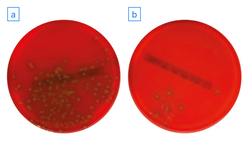

Fig 2. Results for a) MWE and b) Deltalab/Medline swabs with Streptococcus pneumoniae held at 4 ˚C for 48 h prior to inoculation and incubation on the BD Kiestra TLA system.

Tissue biopsy and fluid aspiration methods are preferred for collection of clinical samples; however, swab transport systems are commonly used due to their low cost and practicality (ease of use) and the ability to maintain viability for aerobic, anaerobic and fastidious microorganisms over extended times. [1]

Among the key factors impacting the efficacy of a swab system is its ability to maintain viability of fastidious organisms for sufficient duration.2 The question pertinent to this study is, how certain are laboratories in the ability of their procured swabs to ensure the survival of fastidious organisms over a time period of 24–48 hours? The majority of laboratories have very good quality systems but rarely look at the fundamentals of microbiological survival of important pathogens on the transport swabs. The globalisation of markets has made it possible to procure transport swabs from as far afield as China. A competitive environment is obviously desirable, driving down prices; however, laboratories must take responsibility for the quality of the transport devices.

As part of the ISO 15189 regulations, diagnostic providers must ensure that stated manufacture specifications are true (ISO 15189:2012 5.3.1.4). As such, the laboratory must complete a verification study to demonstrate this accordance. There appears to be surprisingly little information in the literature on the comparative performance of various transport systems, especially to some of the newer brands on the market. The ideal swabs and transport systems are those that maintain viability, allowing good recovery of organisms after a number of hours, yet do not permit overgrowth of either pathogens or commensals.

The recent availability of Clinical and Laboratory Standards Institute (CLSI) procedure M40-A2 (Quality control of microbiological transport systems) for evaluating swab systems has helped tremendously in standardising the methods of evaluating the newly manufactured swab systems. The purpose of this investigation is to demonstrate the efficacy of a number of swab transport systems to maintain the viability of fastidious strains of clinically significant bacteria, and to look at potential overgrowth.

MATERIALS AND METHOD

The following bacterial strains were evaluated for survival after holding at room (21˚C) and refrigerator (4˚C) temperatures using various transport collection devices.

Bacterial strains

- Haemophilus influenzae ATCC 10211 n Streptococcus pneumoniae ATCC 6305 n Neisseria gonorrhoeae ATCC 49226
- Pseudomonas aeruginosa ATCC 27853 These were obtained from Thermo Scientific Culti-Loops as ready-to-use QC organisms.

Transport swab systems

A full range of transport collection devices available throughout the UK was assessed. These included collection devices from Medical Wire & Equipment (MWE) Transwab (product code MW 171), Deltalab/Medline Amies (Product number 300285), Sterilin/Copan both M40 Transport Swab (Product code: 414CST) and Standard Transport Swab (Non-M40 version) (Product code: 18114CST), Technical Service Consultants (TSC) Probact (Product code 5-18) and Sarstedt (Product code: 80.1362.500). All transport systems consist of a sterile peel pouch containing a swab and Amies agar gel medium with charcoal.

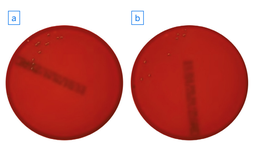

Fig 3. Sterilin/Copan standard (non-M40) collection device: Streptococcus pneumoniae inoculated after a 24-h holding period at a) 4 ˚C and b) 21 ˚C

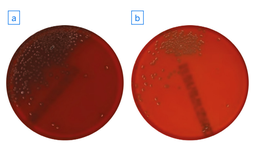

Fig 4. Sterilin/Copan M40 collection device: Streptococcus pneumoniae inoculated after a 24-h holding period at a) 4 ˚C and b) 21 ˚C.

Table 1. Bacterial recovery swabs over 48 h at room temperature and 4˚C, using the roll-plate (qualitative) method.

		Bacterial recovery (CFU) Average of 3		
Bacteria and swab type	**Temperature**	**0 h**	**24 h**	**48 h**
***Streptococcus pneumoniae* ATCC 6305**				
MWE (product number: MW171)	RT	>250	58	54
	4˚C	>250	118	120
Deltalab/Medline (product number: 300285)	RT	>250	9	11
	4˚C	>250	20	8
Sterilin/Copan M40 (product number: 414CST)	RT	>250	88	51
	4˚C	>250	121	119
Sterilin/Copan (product number 18114CST)	RT	>250	8	8
	4˚C	>250	12	6
Sarstedt (product code: 80.1362.500)	RT	>250	50	25
	4˚C	>250	75	65
Probact /TSC (product code: 5-18)	RT	>250	45	30
	4˚C	>250	82	54
***Haemophilus influenzae* ATCC 10211**				
MWE (product number: MW171)	RT	>250	42	31
	4˚C	>250	102	65
Deltalab/Medline (product number: 300285)	RT	>250	4	0
	4˚C	>250	8	0
Sterilin/Copan M40 (product number: 414CST)	RT	>250	52	38
	4˚C	>250	110	95
Sterilin/Copan (product number: 18114CST)	RT	>250	22	5
	4˚C	>250	34	19
Sarstedt (product code: 80.1362.500)	RT	>250	20	11
	4˚C	>250	35	29
Probact/TSC (product code: 5-18)	RT	>250	36	17
	4˚C	>250	67	41
***Neisseria gonorrhoeae* ATCC 49226**				
MWE (product number: MW171)	RT	>250	81	2
	4˚C	>250	250	45
Deltalab/Medline (product number: 300285)	RT	>250	0	0
	4˚C	>250	70	0
Sterilin/Copan M40 (product number: 414CST)	RT	>250	92	0
	4˚C	>250	60	5
Sterilin/Copan (product number: 18114CST)	RT	>250	120	0
	4˚C	>250	31	0
Sarstedt (product code: 80.1362.500)	RT	>250	10	0
	4˚C	>250	200	10
Probact/TSC (product code: 5-18)	RT	>250	0	0
	4˚C	>250	200	0
***Pseudomonas aeruginosa* ATCC 27853**				
MWE (product number: MW171)	RT	>250	>250	>250
	4˚C	>250	>250	>250
Deltalab/Medline (product number: 300285)	RT	>250	>250	>250
	4˚C	>250	>250	89
Sterilin/Copan M40 (product number: 414CST)	RT	>250	>250	>250
	4˚C	>250	>250	>250
Sterilin/Copan (product number: 18114CST)	RT	>250	>250	>250
	4˚C	>250	>250	200
Sarstedt (product code: 80.1362.500)	RT	>250	>250	>250
	4˚C	>250	>250	>250
Probact/TSC (product code: 5-18)	RT	>250	>250	>250
	4˚C	>250	>250	>250

Collection device protocol

A roll-plate protocol methodology was utilised in combination with an automated InoqulA FA (BD Kiestra). A 0.5 McFarland standard (equivalent to 1.5 x 108 colonyforming units [CFU]/mL) standard inoculum of each organism freshly grown at 35˚C for 18–24 hours was prepared in 0.85 physiological saline (pH 6.8–7.2) using a PhoenixSpec nephelometer (Becton Dickinson). Each organism's 0.5 McFarland suspension was diluted to 1 in 10.

In triplicate, 100 µL of each organism suspension was transferred to wells of a microtitre plate using an Eppendorf pipette. Each swab type was rolled into the 100-µL suspension (10 seconds) to completely absorb the inoculum and then placed into the transport device and held for the appropriate time/temperature (0, 24 and 48 hours and 4˚C and 21˚C). For baseline counts (0 hour), three swabs of each organism suspension were removed from the transport device after 15 minutes and spread within the prescribed inoculum area for the InoqulA in manual interactive mode using the roll-plate technique at a predefined area defined by the InoqulA automation. The patented, innovative automated streaking technique of the BD Kiestra InoqulA ensured a consistent streaking of the agar plates.

Media suitable to the ATCC strains was utilised and included Columbia blood agar, cysteine lactose electrolyte-deficient (CLED) agar, and chocolate agar supplied by Oxoid and Chocolate PolyVitex VCA3 (product code: 43611) agar from bioM rieux. Plates were incubated either at 35˚C in 5% CO2 or O2 for 48 hours in the automated BD Kiestra compact incubators, and digital images were taken at time intervals of 24 and 48 hours, respectively, and counts were then performed.

Counts of >250 colonies were approximated and averaged for each of the three swabs for each time point and dilution. The performance of each swab type to maintain the viability of the organism was determined by comparing the average of colony counts at zero time (baseline count) with counts at the 24- and 48-hour holding times. Viability was calculated as percentage of recovery (ie number of organisms recovered as a percentage of the bacterial counts at zero time [baseline counts]). These results were expressed graphically (Figs 5, 6, 12–15). To ensure consistency, the experiments were performed in triplicate (ie three swabs per organism per holding time).

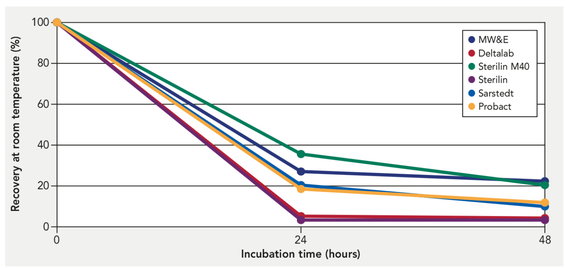

Fig 5. Recovery over time for Streptococcus pneumoniae at room temperature.

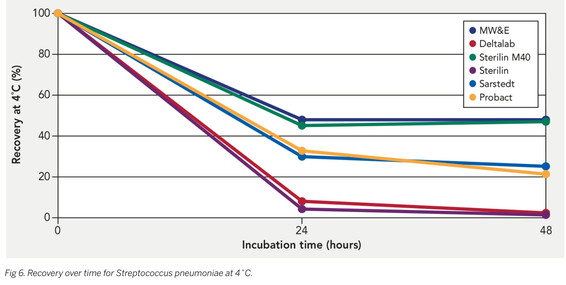

Fig 6. Recovery over time for Streptococcus pneumoniae at 4 ˚C.

RESULTS

Images were acquired automatically by the imaging capability of the BD Kiestra system at time zero, 24- and 48-hour time intervals. This was fully automated and as such is not subject to human error. Withinrun reproducibility (indicative of precision) was demonstrated by low coefficient of variation (CV) values at time zero for the different swab types. Organism dilutions with countable growth within the range 0–250 CFUs were averaged and included for the purpose of comparisons. Estimated CFUs >250 were approximated and included for comparison. The difference in counts between the swab brands were recorded for the various dilutions and specific time points. Figures 1 to 15 and Table 1 summarise the differences in counts between the different transport collection devices.

DISCUSSION AND CONCLUSIONS

This study emphasises the role of preanalytical parameters on the corresponding diagnostic results. Commonly used swabs vary significantly with respect to uptake and release of liquid and bacteria. The benefits of refrigeration have been noted in numerous publications, and again the benefits are brought out in this study.3 The practice of refrigerating swabs if there are any significant delays in processing must be adhered to. Consolidation has resulted in swabs being held at room temperature longer due to transportation to hub sites. This has brought to the fore the viability of particularly fastidious organisms and the varying performance of swabs on the market. This is particularly true when the organisms are present in low numbers.

This study demonstrates that, for the best diagnostic outcome, swabs should be chosen according to their performance profile based on a verification of the manufacturer's parameters. Adherence to the M40-A2 standard for compliance regarding viability is critical, and states that any specimen held at 4˚C or room temperature (RT; 21˚C) should yield no more than a 3-log-unit decrease in CFU between time zero and the end of the specified holding period,4 in this case 24 or 48 hours.

The data presented here clearly demonstrate the superior performance of two brands of collection device (Sterilin/Copan M40-compliant and MWE M40- compliant) in recovery of fastidious organisms such as S. pneumoniae, N. gonorrhoeae and H. influenzae. Overall, all collection devices evaluated appeared to have similar bacterial release at time point zero.

For S. pneumoniae, recovery was negligible for all swab types except for the MWE and Sterilin/Copan M40 devices (Figs 1, 2 and 4), and there were marked difference when the swabs were held at 4˚C between these and the various other brands. 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 diagnosis and treatment. The maintenance of viability in clinical samples when stored in swabs is mandatory and certainly requires the best performance. Morosini et al.5 demonstrated similar poor results with Deltalab/Medline (Spain) for S. pneumoniae recovery, and Barber et al.6 similarly for Sarstedt collection devices.

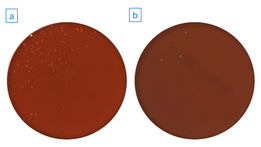

Fig 7.Deltalab/Medline swab: Neisseria gonorrhoeae held for a) 24 h and b) 48 h at 4˚C.

An estimated three to five million deaths occur annually in children under five years of age due to acute respiratory infections, for which S. pneumoniae is the most important pathogen.7 It is essential to ensure that primary swab samples are processed efficiently with optimal recovery of pneumococci and that the transport and storage medium maintains the viability of the swab samples.

In the case of N. gonorrhoeae the percentage recovery for this organism varied tremendously, with the MWE device being the only swab able to maintain the viability of this strain to any significant extent at 4˚C with a holding period of 48 hours (Fig 8). This finding is supported by data from Morosini et al.,[5] although in their study the lack of charcoal was a contributory factor. Furthermore, lessrobust N. gonorrhoeae clinical strains may be more fragile, which highlights the importance of procuring the right collection device.

When considering H. influenzae, performance of the various devices showed limited recovery at RT, with the Sterilin/Copan M40 and MWE devices showing the strongest performance (Fig 14). At 4˚C all but one device (Fig. 15) showed general improvement in recovery. These data are consistent with the previous published findings of Morosini et al.[5]

Clear differences were noted between the M40-compliant Sterilin/Copan collection device and the Sterilin/Copan standard device, with marked improvement in recovery shown by the M40-compliant

device (Figs 3a and 3b, and Figs 4a and 4b). All collection devices showed good recovery of P. aeruginosa at 4˚C and 21˚C, although of concern was overgrowth by the organism at 21˚C if swabs were left for 48 hours. This was observed for all swab types.

Additional concerns have arisen concerning non-viable Gram-negative organisms found in the transport media of Sarstedt and Deltalab/Medline collection devices. This requires further investigation as it has the potential to lead to inappropriate antibiotic treatment when urgent Gram stains are requested for eye swabs.

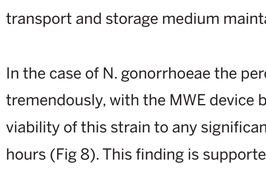

Fig 8. MWE swab: Neisseria gonorrhoeae held for a) 24 h and b) 48 h at 4˚C.

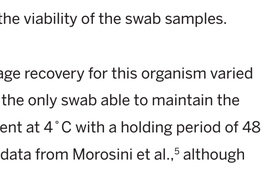

Fig 9. Sterilin/Copan M40 device: Neisseria gonorrhoeae held for a) 24 h and b) 48 h at 4˚C.

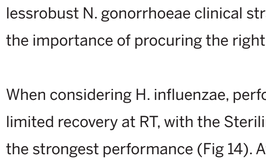

Fig 10. Sterilin/Copan Standard (non-M40): Neisseria gonorrhoeae held for a) 24 h and b) 48 h at 4˚C.

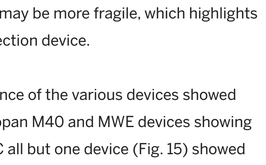

Fig 11. Sarstedt device: Neisseria gonorrhoeae held for a) 24 h and b) 48 h at 4˚C.

Consolidated laboratories where transportation delays the processing of swabs, or where any other significant delays occur, should ensure the use of a swab which maximises the survival of fastidious organisms in significant numbers. More emphasis should be placed on sourcing the best collection device to ensure that laboratories have the best chance of isolating pathogenic bacteria which may be fastidious in nature.

Swabs are often treated as just another hospital commodity purchased in bulk at the lowest possible price without evaluation/verification or approval by the microbiology department. As specimen transport delays increase due to centralisation, acceptable performance of collection devices cannot be assumed, and must be verified. Caution must be exercised in assuming collection devices, currently in use, provide acceptable recovery performance. It will be interesting to see how the new ISO 15189 standard[8] drives changes in the pre-analytical selection processes by ensuring that robust verifications occur for collection devices.

This study illustrates the loss of viability during any significant holding period that compromises recovery. This loss of viability certainly varies significantly between different collection devices, which has been observed in past papers (ie Barber et al.6). This has significant implications in public health as fastidious organisms or less-robust strains may be underrepresented in the sampled population. In such cases, laboratories may need to assess the suitability of molecular methods for detection of these organisms.

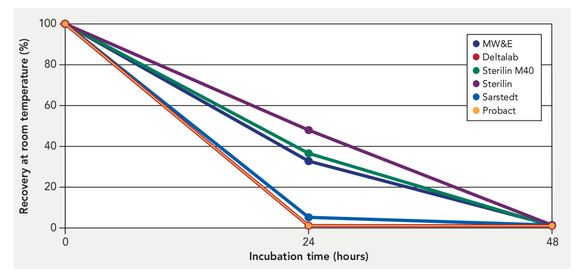

Fig 12. Recovery over time for Neisseria gonorrhoeae at room temperature.

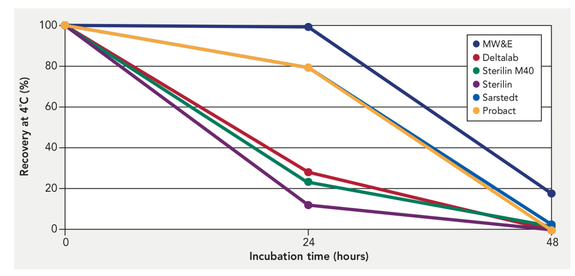

Fig 13. Recovery over time for Neisseria gonorrhoeae at 4 ˚C.

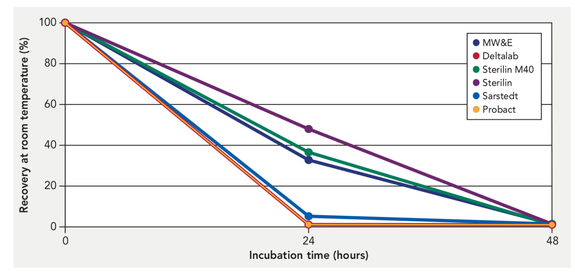

Fig 14. Recovery over time for Haemophilus influenzae at room temperature.

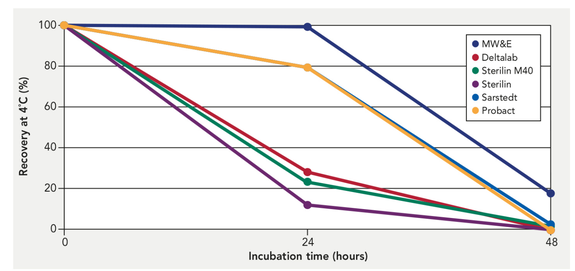

Fig 15. Recovery over time for Haemophilus influenzae at 4 ˚C.

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Jamie Laughlin is Microbiology and Business Development Manager, South West London Pathology, Microbiology, St George's University Hospitals NHS Foundation Trust (jamie.laughlin@stgeorges.nhs.uk)