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Efficacy of two commercially available liquid transport collection devices

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Introduction:

Microbiology laboratory diagnosis relies completely on the recovery of bacterial isolates from clinical specimens (Gizzie and Adukwu; 2016). In the context of this statement, and given the increased awareness of the importance of the pre-analytical phase of specimen processing, the importance of selecting the most appropriate collection device is paramount. Failure can lead to patient misdiagnosis and inappropriate treatment.

The Microbiology workflow can be divided into three distinct phase's i.e. (i) pre-analytical, (ii) analysis, and (iii) post-analytical. Optimum performance of the later steps is directly linked to the performance of the prior stages. Hence, if the pre-analytical step is performed with sub-optimal quality, even the highest standards of laboratory quality management and/or automation will not compensate for the initial flaws and this might lead to inadequate treatment of the patient.

The seemingly simple process of sample collection by swab techniques is actually very complex. It is influenced by a vast array of parameters which could largely change the outcome of microbiological investigation and more importantly its sensitivity.

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. Collection and transport of bacterial specimens to the laboratory is a critical component in the success of the diagnostic process. Transport time and temperature is now a major concern as the original concept and design of swab transport devices is 30-40 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 type of sampling device, and the swab material plays 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.

A transport system that will maintain viability of the organism for 24 to 48 hr. is a necessity, as the need to transport these specimens a greater distance becomes a reality with increase consolidation and the creation of "super labs". 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 ease of use and the ability to maintain viability for aerobic, anaerobic, and fastidious microorganisms over extended times (Gizzie, N *et al.,* 2016).

Amongst the key factors impacting the efficacy of a swab system is its ability to maintain viability of fastidious organisms for sufficient duration (Farhat *et al*., 2001). The majority of laboratories have very good quality systems within the laboratory but rarely look at the fundamentals of microbiological survival of important pathogens on the collection devices. In addition, a competitive environment has driven down prices of particular collection devices; 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 place. The ideal swab 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 CLSI procedures M40-A2 (quality control of microbiological transport systems) for evaluating swab systems has tremendously helped in standardizing the methods of evaluating the newly manufactured swab systems The purpose of this investigation was to evaluate the performance of two of the most widely utilized collection devices and highest quality products available in the UK with regards to the efficacy of the transport system to maintain the viability of fastidious strains of clinically significant bacteria and to look at potential overgrowth with Pseudomonas over time.

Method:

The following bacterial strains were evaluated for survival after incubation at room (21^{0}C) and refrigerator (4^{0}C) temperatı

Bacterial strains:

1. *Streptococcus pyogenes* ATCC 19615
2. *Pseudomonas aeruginosa* ATCC 27853
3. *Streptococcus pneumoniae* ATCC 6305
4. *Neisseria gonorrhoea* ATCC 49226
5. *Staphylococcus aureus* ATCC 25923
6. Mixed culture: *Streptococcus pneumoniae* and *Pseudomonas aeruginosa*

Obtained from Thermo Scientific™ Culti-Loops™ as ready-to-use QC organisms

Fig 1: Copan Diagnostics 480C eSwab®

Fig 2: ∑- Transwab® MW176S

Transport swab systems:

Two commercially available transport devices namely the Copan ESwab™ and the Medical Wire & Equipment Sigma Transwab® were utilised in this study.

Recovery studies

A roll plate protocol methodology was utilized in combination with an automated InoqulA FA (BD Kiestra)

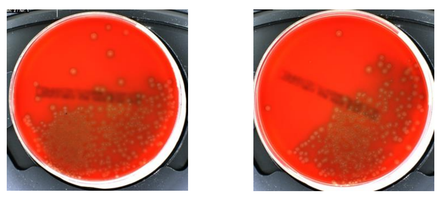
1. For each organism, a 0.5 McFarland standard (1.5 x 10^8 CFU/mL) was prepared in 0.85 physiological saline (pH 6.8 – 7.2) from 18-24 hr. cultures using a Phoenix-Spec Nephrometer (Becton Dickinson).
2. An 0.5 McFarland suspension was diluted to 1:100 to ensure colony counts of 25 to 250 on a 90-mm for statistical validity. Note for *N. gonorrhoeae* a 1:10 dilution was utilised.
3. 100µl of each organism suspension was transferred into wells of a microtiter plate using a Eppendorf pipette;
4. Each swab type was rolled into the 100µl suspension (10 seconds) to completely absorb the inoculum and then placed into the transport device and held for the appropriate time/temperature (21^{0}C and 4^{0}C for 0, 24 and 48 hours);
5. For baseline counts (0hr), three swabs of each organism/dilution 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;
6. The patented innovative automated streaking technique of the BD KIESTRA InoqulA ensured a consistent streaking of the agar plates. The streaking pattern in this study represented what the laboratory utilises on a day to day basis.
7. Agar suitable to the ATCC strain was utilised. The following agar was utilised: Columbia blood agar, Cystine-Lactose-Electrolyte Deficient (CLED) and chocolate agar supplied by Oxoid and Chocolate PolyVitex VCA3 (product code: 43611) agar from Biomerieux.
8. Plates were incubated either at 35^{0}C in 5% CO_2 or 0_2 for 48 hours in the automated BD KIESTRA compact incubators and digital images taken at time intervals of 24 and 48 hrs, respectively.
9. Counts were then performed
10. Colony counts of >250 colonies were approximated and averaged for each of the 3 swabs for each time point and dilution.

Results

Images were automatically acquired by the imaging capability of the BD Kiestra system at 0 hrs, 24 and 48 hrs. time intervals. This was fully automated and as such is not subject to human error. Organism dilutions with countable growth within the range of 20-250 CFU's were averaged and included for the purpose of comparisons. Estimated CFU's that were greater than 250 were approximated and included for comparison. The difference in counts between the two brands were recorded for the various dilutions and specific time points.

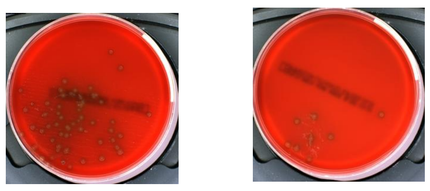
The two liquid transport systems differed in their recovery rate of *Streptococcus pneumoniae* and *Neisseria gonorrhoeae*. The Sigma Transwab® demonstrated enhanced recovery rates at the various parameters. In addition, the Copan eSwab® showed significant overgrowth of *Pseudomonas aeruginosa* as a factor of time and showed complete overgrowth in a mixed culture with *Streptococcus pneumoniae*.

∑ -Transwab® **eSwab®**

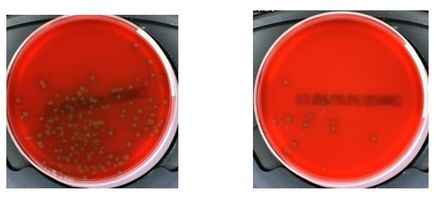


***Streptococcus pneumoniae* at time zero**

***Streptococcus pneumoniae* 4^{0}C for 24 hrs**

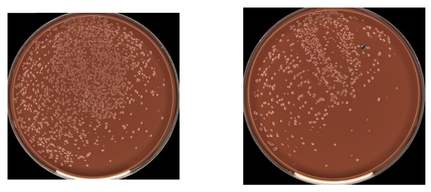


***Streptococcus pneumoniae* RT for 48hrs**

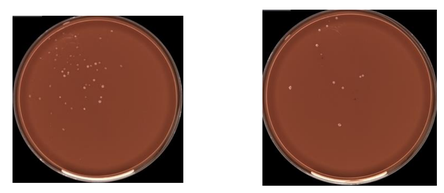


***Streptococcus pneumoniae* 4^{0}C for 48hrs**

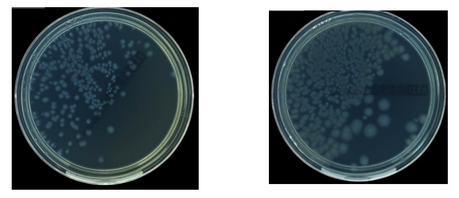
∑ -Transwab® **eSwab®**



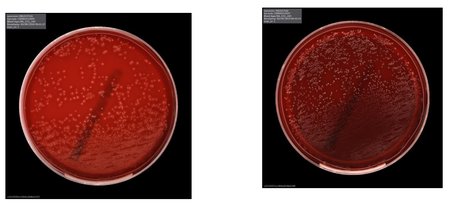
***Neisseria gonorrhoeae* 4^{0}C for 24 hrs**



***Neisseria gonorrhoeae* 4^{0}C for 48 hrs**



***Pseudomonas aeruginosa* RT for 24hrs**



***Streptococcus pyogenes* at time zero**

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

Bacteria and swab type		Bacterial recovery (CFU) average of 3			
	Temperature	0 hr.	24hr	48hr	Compliant
***Streptococcus pyogenes* ATCC 19615**					
Sigma Transwab® MW176S	RT	>250	48	54	Yes
	4^{0}C	>250	108	120	
Copan Diagnostics 480C eSwab®	RT	>250	40	51	Yes
	4^{0}C	>250	111	125	
***Streptococcus pneumoniae* ATCC 6305**					
Sigma Transwab® MW176S	RT	>250	58	48	Yes
	4^{0}C	>250	82	74	
Copan Diagnostics 480C eSwab®	RT	>250	16	7	Yes
	4^{0}C	>250	21	11	
***Neisseria gonorrhoeae* ATCC 49226**					
Sigma Transwab® MW176S	RT	>250	81	2	Yes
	4^{0}C	>250	>250	45	
Copan Diagnostics 480C eSwab®	RT	>250	92	0	?
	4^{0}C	>250	60	5	
***Pseudomonas aeruginosa* ATCC 27853**					
Sigma Transwab® MW176S	RT	>250	>230	>250	Yes
	4^{0}C	>250	>210	>180	
Copan Diagnostics 480C eSwab®	RT	>250	>250	>250	Overgrowth?
	4^{0}C	>250	>250	>250	
***Staphylococcus aureus* ATCC 25923**					
Sigma Transwab® MW176S	RT	>250	139	78	Yes
	4^{0}C	>250	197	112	
Copan Diagnostics 480C eSwab®	RT	>250	162	62	Yes
	4^{0}C	>250	173	52	
Mixed culture: ***Streptococcus pneumoniae & Pseudomonas aeruginosa***					
Sigma Transwab® MW176S (***Strep. pneumoniae*** single colonies)	RT	20	10	Non-visible	
	4^{0}C				
Copan Diagnostics 480C eSwab® (***Strep. pneumoniae*** single colonies)	RT	Non-visible	Non-visible	Non-visible	Overgrowth
	4^{0}C				

Discussion

Microbiology has seen huge transformation in the analytical and post analytical steps of the process improving both sensitivity and specificity. Sample collection quality is crucial for the quality of the subsequent analytical steps and therefore, any improvements of this first step will benefit the whole diagnostic process.

Although leading to inferior diagnostic quality, sample collection utilizing swabs is the preferred technique for most clinicians because of its performance ease and swiftness. Thus, if the general approach for sample collection cannot be changed, it appears prudent to optimize swabs and swabbing techniques especially with respect to test sensitivity. As a prerequisite for optimization, test sensitivities of presently available swabs should be quantified under conditions close to natural circumstances.

Conclusion

The ability of the transport device to ensure viability of fastidious organisms over an extended time period has often been overlooked or undervalued; even though this is an essential prerequisite. Within the United Kingdom the two most common liquid collection devices are supplied by Copan and MWE, respectively. The study aim was to determine if there were any significant differences between the two collection devices in their performance as described above.

The data demonstrates differences in the recovery rate of *S. pneumoniae* and *N. gonorrhoeae* between the Sigma Transwab® and Copan eSwab®. In addition, the Copan ESwab™ exhibited issues around overgrowth in the presence of *P. aeruginosa*.

With the consolidation of Microbiology laboratories were transport delays processing of swabs can amount to holding periods of >48hrs the use of a collection device which is capable of ensuring the survival of fastidious organisms in significant numbers and minimizing the risk of overgrowth is paramount. Certainly 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.

References:

1. Gizzie N, Adukwu E (2016). Evaluation of Liquid-Based Swab Transport Systems against the New Approved CLSI M40-A2 Standard; Journal of Clinical Microbiology, Volume 54 Number 4: 1152-1156.
2. Clinical and Laboratory Standards Institute. 2014. Quality control of microbiological transport systems; approved standard— 2nd ed. CLSI document M40-A2. Clinical and Laboratory Standards Institute, Wayne, PA.
3. Farhat, SE, Thibault, M and Devlin, R (2001) Efficacy of a Swab Transport System in Maintaining Viability of *Neisseria gonorrhoeae* and *Streptococcus pneumoniae.* J Clin. Microbiol. 39(8): 2958–2960.

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