Maintaining Viability of Aerobic and Anaerobic Bacteria from Wounds Using the New Sigma-Swab Transport System.

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Abstract

Background: A crucial step for effective laboratory diagnosis of infection is adequate collection and transport of specimens. Three quantifiable parameters influence the performance of specimen transport: time, temperature and quality of transport medium. In this study, these parameters were evaluated for a novel dry specimen transport system, Sigma-swab (Medical Wire). Additionally, the standard M40-A method (CLSI) was compared with a modified method that assessed the effect of nutrients and mixtures of bacteria on their recovery which would reflect a clinical situation. **Methods:** Viability of common pathogens isolated from wounds; *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacteroides fragilis* and their mixtures were evaluated using the Sigma swab (Medical Wire, UK). Known numbers of bacteria were added to the swab and quantified using serial dilution at 0h, 24h and 48h, after storage at 4 ºC and RT, in the presence and absence of nutrients in pure culture and in mixtures. **Results:** All bacteria were recovered from the Sigma-Swab for up to 48h of incubation at RT and 4 ºC in the presence and absence of nutrients. There was a 1.15 log and 0.5 log increase in numbers of *S. aureus* at RT in the presence and absence of nutrients (respectively). With *E. coli* there was a similar increase seen for the same conditions (1.0 log and 0.7 log increase). No difference in numbers was observed at 24h at RT or at 4 ºC . The numbers of *P. aeruginosa* and *B. fragilis* remained stable for 48h in all conditions. Mixtures of organisms were recovered for up to 48h of incubation in all conditions without significant effect on viability. **Conclusion:** Many laboratories use transport medium to maintain the viability of the bacteria from clinical samples whilst swab is transported. This is especially problematic when bacteria are present in low numbers. The Sigma-Swab met acceptance criteria at both storage temperatures for all isolates tested in the presence and absence of nutrients. Additionally there was no overgrowth of any bacteria tested even in mixed culture.

Introduction

Different swab systems are used to transport a variety of specimen types to the diagnostic laboratory and these systems often differ depending upon the category of organism being investigated e.g. bacteria, viruses or fungi. The ideal swab system must absorb organisms from the infection site, maintain viability during transport and allow release of organisms from the swab to the appropriate media during cultural techniques. Liquid and gel-based swab systems have been used for many years, but have limitations as the specimen is diluted by immersion within the liquid or gel. The Sigma-swab is a new, medium free transport system and the absence of transport medium means there is no dilution of the specimen. Also, this swab system can be used for bacterial, viral and fungal culture and additionally can be used in modern molecular testing methods, e.g. PCR.

Three quantifiable parameters influence the performance of specimen transport: time, temperature and quality of transport swab. Additionally, during wound surface swabbing it is likely, that nutrients (bodily fluids and skin cells) as well as bacteria will be transferred to swab causing overgrowth during transport.

In this study, these parameters were evaluated using the standard M40-A (CLSI) method and the effect of nutrients and mixtures of bacteria, reflecting a clinical situation was also assessed.

Methods

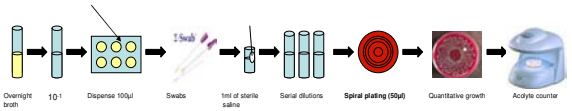

Figure 1. Method of processing the swab for evaluation of bacteria viability.

- A suspension from a freshly grown isolate of each strain (*Staphylococcus aureus* - NCTC 6571, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* NCTC 6749*)* and *Bacteroides fragilis* – NCTC 9343) was prepared in sterile saline diluted 1:10. Serial 10-fold dilutions were prepared from the suspension and plated onto nutrient agar. The plates were incubated at 37 ºC for 24h, and colony forming units counted to confirm inoculum concentration.
- Swabs were placed into the saline suspension for 10 sec allowing the fluid to absorb and then inserted back into the transport device.
- Swabs were incubated at room temperature and at 4 ºC for 0, 24h and 48h.
- After the appropriate incubation period each swab was removed and placed into 1 ml of sterile saline and mixed for 1 min.
- Serial dilutions were inoculated onto the nutrient agar using spiral plater (Don Whitley Scientific, BS5687).
- All plates were incubated at 37 ºC for 24h in appropriate aerobic and anaerobic conditions.
- After incubation, a quantitative count was performed using Acolyte counter (Don Whitley Scientific).
- All experiments were carried out in triplicate.
- All experiments were repeated using nutrient broth instead of saline to reflect for the effect of nutrients.
- All experiments were repeated using mixtures of the four organisms.

Results

Bacteria mixtures in ratio 1:1		Incubation period	
	0h	24h	48h
S.aureus	1.82 x 10^5	2.42 x 10^5	3.41 x 10^5
E.coli	2.91 x 10^5	1.80 x 10^5	7.78 x 10^5
S.aureus	2.96 x 10^5	5.14 x 10^5	4.70 x 10^5
P.aeruginosa	9.62 x 10^5	9.97 x 10^5	3.96 x 10^6
S.aureus	3.48 x 10^5	2.72 x 10^5	5.05 x 10^6
B.fragilis	5.18 x 10^6	3.22 x 10^6	2.86 x 10^6
E.coli	3.66 x 10^5	6.11 x 10^5	9.06 x 10^5
B.fragilis	3.26 x 10^6	3.54 x 10^6	3.35 x 10^6
E.coli	2.34 x 10^5	1.58 x 10^5	8.35 x 10^5
P.aeruginosa	1.96 x 10^5	8.20 x 10^5	2.88 x 10^6
P.aeruginosa	4.36 x 10^5	1.84 x 10^5	4.42 x 10^5
B.fragilis	1.13 x 10^6	2.60 x 10^6	4.03 x 10^6

Table 5. Results of the recovery of bacteria mixtures incubated at 4ºC and processed by standard procedure. **Mixtures of organisms were recovered for up to 48h without significant effect on viability.**

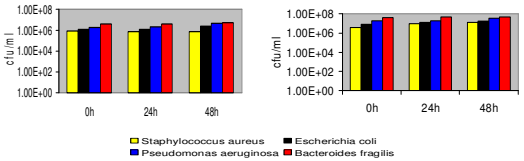

Table1. Recovery of bacteria incubated at 4ºC and processed by standard method.

Table 2. Recovery of bacteria incubated at RT and processed by standard method.

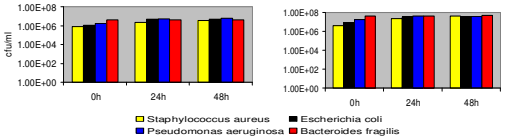

Table 3. Recovery of bacteria incubated at 4ºC and processed by modified method.

Table 4. Recovery of bacteria incubated at RT and processed by modified method.

All strains were recovered from the Sigma-swab for up to 48h of incubation at room temperature and 4ºC in the presence and absence of nutrients. There was a 1.15 log and 0.5 log increase in numbers of *S.aureus* at RT in the presence and absence of nutrients (respectively). With *E.coli* there was a similar increase seen for the same conditions (1.0 log and 0.7log increase). The numbers of *P.aeruginosa* and *B.fragilis* remained stable for 48h in all conditions.

Discussion/ Conclusion

Loss of viability during transport will have a negative effect on bacterial culture results, especially when they are present in low numbers, also, the presence of nutrients can cause overgrowth during the transport. The perfect transport device should maintain viability of bacteria and prevent overgrowth. The Medical Wire Sigma-swab met acceptance criteria at both storage temperatures for all isolates tested with excellent results of recovery.

References

1.Quality Control of Microbiological Transport Systems: Approved Standard. NCCLS document M40-A. 2003.
2. Sarina M, Lawrence D.M. Comparative Evaluation of Two New Amies Swab Transport Systems BD CultureSwab MaxV(+) (Copan) and the Fisherfinest (Starplex) Swab. ASM 105th General Convention, Atlanta 2005.

The Sigma Swabs were provided by Medical Wire & Equipment.