



Comparison of Five Swab Systems for the Transport of Pathogenic Bacteria: Effects of Charcoal in the Swab or Gel Medium

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REVISED ABSTRACT

Background: The construction of swab transport devices profoundly affects the survival of bacteria. Plain swabs with charcoal-containing gel are popular, but Stuart's original work suggested that charcoal in the swab, rather than the gel medium, was superior. In this study we compared the recovery of mainly fastidious bacteria after storage in systems with charcoal in the swab tip and in the gel medium. In addition, swab systems from 2 manufacturers were compared. **Methods:** Strains tested included 5 *N. gonorrhoeae*, 5 *S. pneumoniae*, 5 *H. influenzae*, 2 *F. nucleatum*, 1 *P. melaninogenica*, 1 *P. anaerobius*, 1 *S. pyogenes* and 1 *S. aureus*. Bibby-Sterilin systems (manufactured by Copan Italia, Brescia, Italy) were all Amies medium with rayon swabs and plastic sticks: without charcoal (108C); with charcoal in the medium (114C); and plain medium with charcoal-coated swab (193C). Medical Wire systems (Corsham, UK) were Transwab charcoal medium (MW171); and Transwab plain medium (MW170). Swabs were inoculated with 10⁶cfu organism and then held at 4°C for 0, 6, 24 and 48h in triplicate. Swab tips were then cut off and vortexed in 1mL sterile saline for 15s. Organisms in suspensions were counted by the Miles and Misra method. Mean colony counts were expressed as percent recovery compared with time 0. **Results:** *N. gonorrhoeae* showed most rapid loss of viability in all systems, but the 193C gave the highest recovery over 48h, and the MW170 the lowest. For *S. pneumoniae*, results were less consistent, but 193C and 108C systems gave the lowest counts at 48h. Individual system performance was not consistent with different strains of *H. influenzae*. Recovery of anaerobes was good with all systems, although some differences were seen. Recovery of *S. pyogenes* and *S. aureus* was good for all systems, although multiplication occurred with *S. aureus* in the MW171 system. **Conclusion:** The system with charcoal in the swab tip (193C) gave the best recovery of *N. gonorrhoeae*, but performed least well with pneumococci. There were differences between systems from different manufacturers. The MW171 system, with charcoal in the gel medium, performed generally well but allowed multiplication of *S. aureus*.

INTRODUCTION

It has been known for over 50 years that the construction of swab transport devices profoundly affects the survival of bacteria. Reduced recovery is particularly significant if transport times exceed 6 hours, and such delays are common in the UK and other healthcare systems today.

Neisseria gonorrhoeae has generally been shown to pose the most stringent test of preservation, although prevention of overgrowth of more robust species has been considered an additional advantage (Stuart, 1959).

Plain swabs with charcoal-containing gel are popular, but Stuart's original work suggested that charcoal in the swab, rather than the gel medium, was superior and allowed reading of the methylene blue reduction indicator (Stuart et al, 1954). Such grey swabs, however, have been considered aesthetically unpleasant for patients. Amies (1967) modified Stuart's formulation with charcoal in the medium and demonstrated good survival, and systems of this type have become accepted as a de facto international standard.

Few recent comparative studies have re-evaluated charcoal coating of swabs (Blomgren et al, 2001), or been well-designed or have included a wide range of isolates. In this study we compared the recovery of fastidious and robust bacteria (both reference strains and recent clinical isolates) after storage in plain transport systems and in those with

charcoal in the swab tip and in the gel medium. In addition, swab systems from 2 manufacturers were compared.

While most swabs are transported at ambient temperature there are several publications suggesting that this is not optimal and that refrigerated storage is preferable for some fastidious organisms (eg Perry, 2001; Schieven and Farrell, 2001). In view of these data, swabs were stored at 4-8°C in most experiments in this study.

METHODS

Strains tested were:

Neisseria gonorrhoeae ATCC 49226 and 4 clinical isolates.
Streptococcus pneumoniae ATCC 49619 and 4 clinical isolates.
Haemophilus influenzae ATCC 49247 and 4 clinical isolates.
Streptococcus pyogenes ATCC 12344.
Staphylococcus aureus ATCC 25923.
Fusobacterium nucleatum ATCC 10953 and NCTC 11326.
Peptostreptococcus anaerobius ATCC 27337.
Prevotella melaninogenica NCTC 9336.

Swab systems were:

1. Bibby-Sterilin plain swab with plain transport medium (108C)
2. Bibby-Sterilin plain swab with charcoal in transport medium (114C)
3. Bibby-Sterilin charcoal-coated swab with plain transport medium (193C)
4. Medical Wire plain swab with plain transport medium (MW170)
5. Medical Wire plain swab with charcoal in transport medium (MW171)

Bibby-Sterilin systems were manufactured by Copan Italia, Brescia, Italy. Medical Wire systems were manufactured by Medical Wire, Corsham, UK.

Survival of organisms on swabs:

Organisms were suspended in quarter strength Ringer's solution and photometrically adjusted to a concentration of 10⁷cfu/mL. Swabs were inoculated with 10⁶cfu organism and then held at 4°C for 0, 6, 24 and 48h in triplicate. Swab tips were then cut off and vortexed in 1mL quarter strength Ringer's solution for 15s. Organisms in suspensions were counted by the Miles and Misra method with six replicates.

RESULTS

In previous comparative studies of the performance of swab transport systems, mean colony counts have usually been expressed as percent recovery compared with counts at time 0. This has the major disadvantage that relatively small differences in counts around the inoculum density may appear as significant differences in recovery after a few hours storage when they would have little practical consequence. In addition, differences in counts of several log₁₀ dilutions after more prolonged storage, when organisms are surviving in lower numbers, are hidden. Survival at 24-48h is likely to be of practical importance in many healthcare systems. To overcome this anomaly, we have expressed results as log₁₀ percent recovery compared with time 0. This gives a clearer indication of differences in recovery rates and differences around the 3 log₁₀ reduction limit proposed by NCCLS (NCCLS, 2002) are obvious. The effect of this is illustrated in Figure 1 for a strain of *N. gonorrhoeae*.

Figures 2 – 16 show log₁₀ percent recovery compared with time 0 for most of the remaining tested strains.

RESULTS

For *S. pneumoniae*, 2 isolates (1 reference, 1 clinical) survived well in all systems (see Figure 2 for example), but the 193C and 108C systems performed less well with the other 3 clinical isolates (for example, Figure 3).

Recovery of *S. pyogenes* (Figure 4) and *S. aureus* (Figure 5) was good from all systems, although some multiplication occurred with *S. aureus* in the MW171 system at 48h.

N. gonorrhoeae showed most rapid loss of viability in all systems, but the Bibby-Sterilin 193C gave the highest recovery over 48h, and the MW170 the lowest (Figures 1, 6-8 and 16a).

Individual system performance was not consistent with different isolates of *H. influenzae*. For example, the reference strain showed good survival in all systems (Figure 9), but other isolates survived less well on the 108C and 193C (Figure 10) or on the 108C and MW170 (Figure 11).

One *F. nucleatum* gave lower recoveries from the 193C and 108C systems (Figure 12), whereas the other multiplied in all systems except 193C and 108C (Figure 13). Good survival or multiplication was seen in all systems with the *P. meloninogenica* (Figure 14), and most loss of viability of *P. anaerobius* was seen in the MW170 and MW171 systems (Figure 15).

The improved survival of some organisms at 4°C in comparison with 20°C was confirmed with a *N. gonorrhoeae* strain where recovery at 48h was around ten times higher from systems stored at 4°C (Figure 16)

CHARTED RESULTS

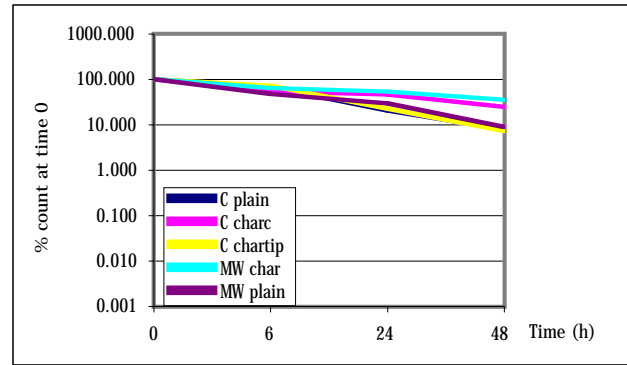


Figure 2: Survival of *Str pneumoniae* ATCC 49619 in different swab systems.

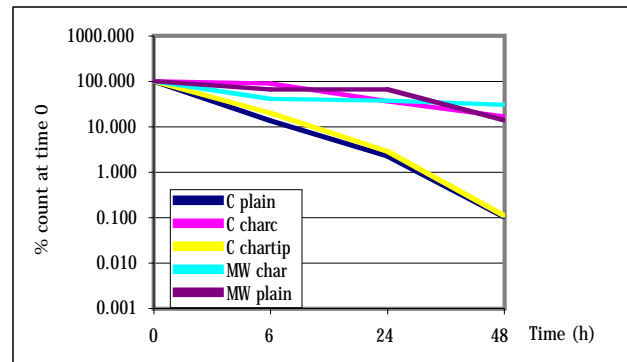


Figure 3: Survival of *Str pneumoniae* clinical isolate 10081 in different swab systems.

CHARTED RESULTS

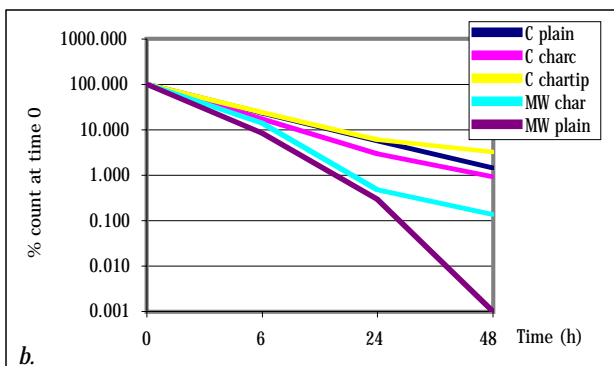
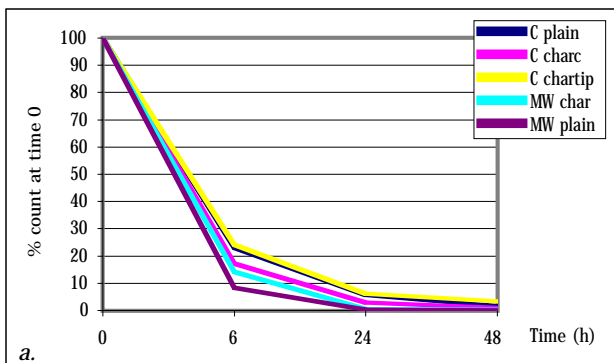


Figure 1: Survival of *N gonorrhoeae* clinical isolate 10085 in different swab systems. Results expressed as (a) Percent count at time 0, (b) Log percent count at time 0.

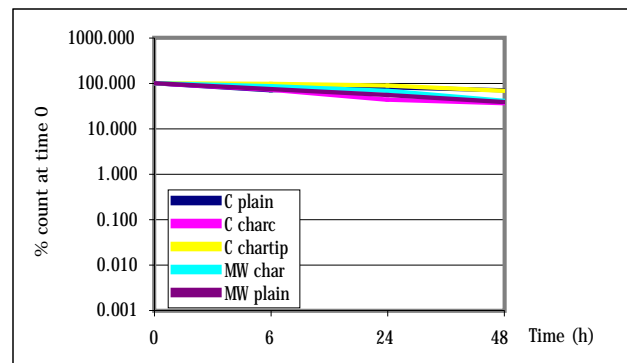


Figure 4: Survival of *S pyogenes* ATCC 12344 in different swab systems.

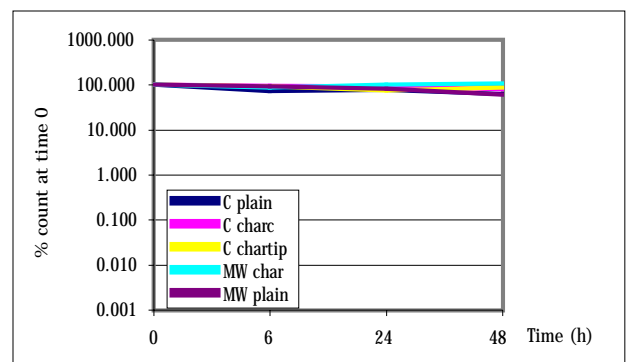


Figure 5: Survival of *S aureus* ATCC 25923 in different swab systems.

CHARTED RESULTS (continued)

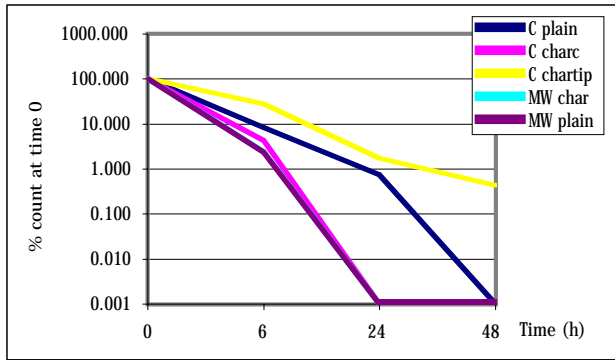


Figure 6: Survival of *N gonorrhoeae* ATCC 49226 in different swab systems.

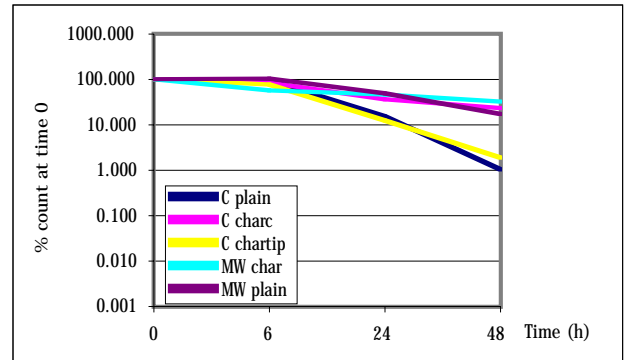


Figure 10: Survival of *H influenzae* clinical isolate 10077 in different swab systems.

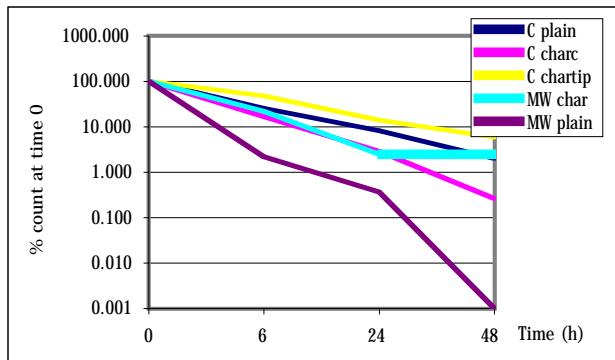


Figure 7: Survival of *N gonorrhoeae* clinical isolate 10084 in different swab systems.

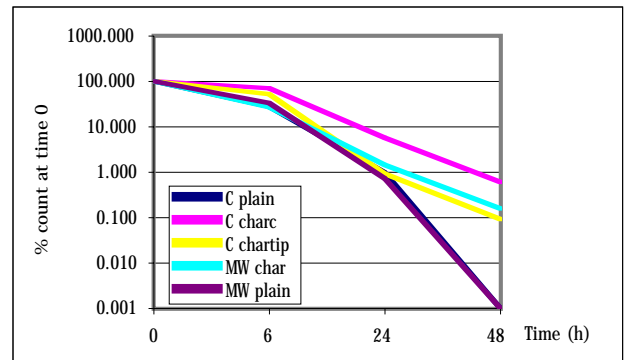


Figure 11: Survival of *H influenzae* clinical isolate 10079 in different swab systems.

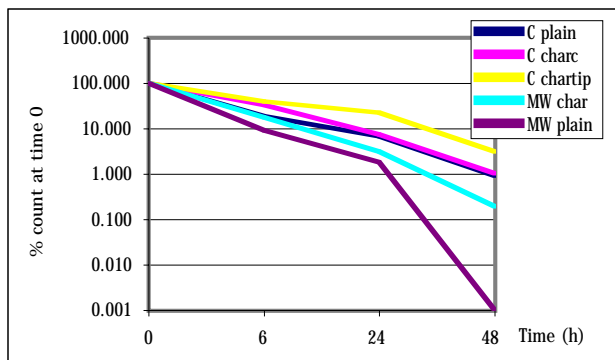


Figure 8: Survival of *N gonorrhoeae* clinical isolate 10086 in different swab systems.

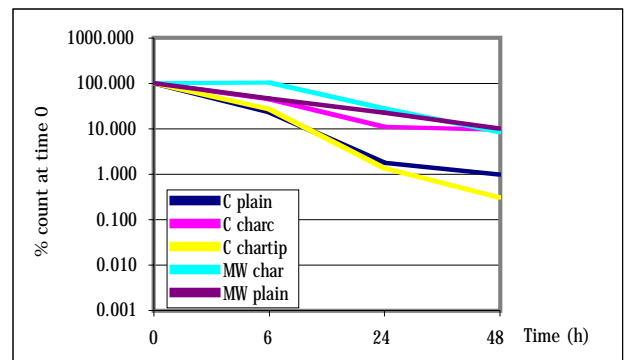


Figure 12: Survival of *Fusobacterium nucleatum* NCTC 11326 in different swab systems.

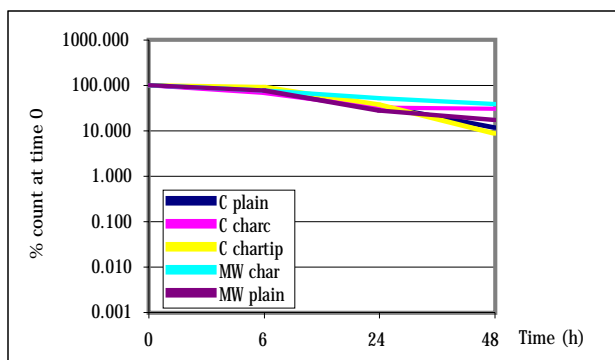


Figure 9: Survival of *H influenzae* ATCC 49247 in different swab systems.

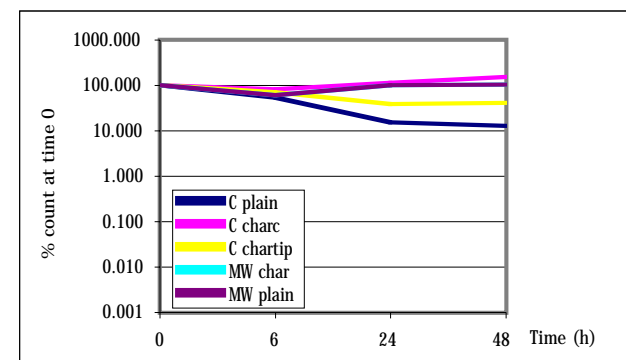


Figure 13: Survival of *Fusobacterium nucleatum* ATCC 10953 in different swab systems.

CHARTED RESULTS (continued)

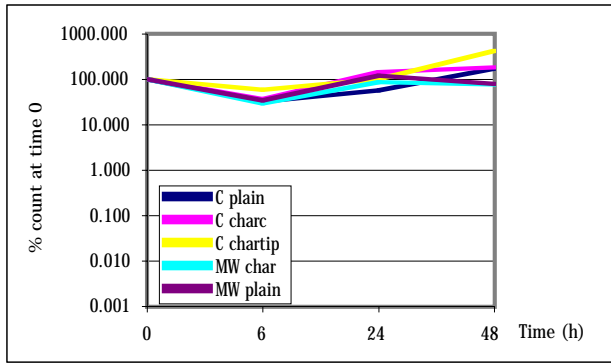


Figure 14: Survival of *Prevotella melaninogenica* NCTC 9336 in different swab systems.

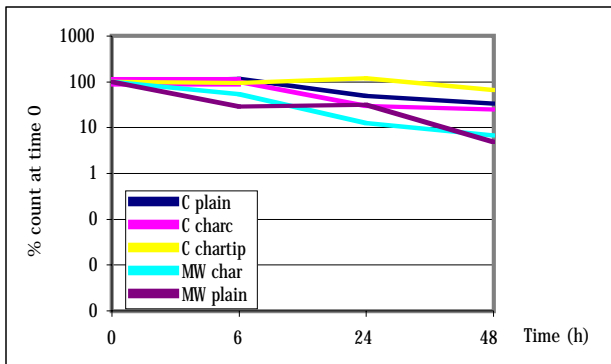


Figure 15: Survival of *Peptostreptococcus anaerobius* ATCC 27337 in different swab systems.

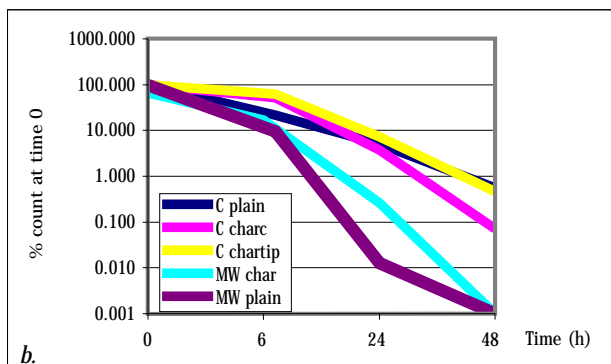
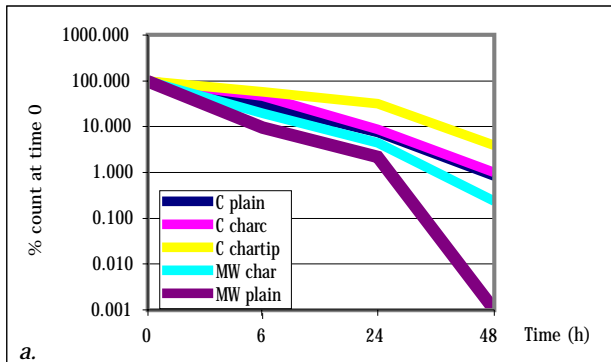


Figure 16: Survival of *N. gonorrhoeae* clinical isolate 10086 in different swab systems at (a) 4-8°C and (b) 20°C.

CONCLUSION

The system with charcoal in the swab tip (193C) gave the best recovery of *N. gonorrhoeae*, but performed least well with pneumococci.

There were differences between systems from different manufacturers among individual isolates of other bacteria, but systems containing charcoal generally gave higher recoveries than those without.

The MW171 system, with charcoal in the gel medium, performed generally well but allowed multiplication of *S. aureus*.

Survival of anaerobes was generally good, with some isolates multiplying in some transport systems.

The question of the effects of transport and storage temperature on organism survival needs to be resolved and further studies are addressing this issue.

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