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REFRIGERATION DOES NOT IMPAIR THE RECOVERY OF NEISSERIA GONORRHOEAE FROM CHARCOAL TRANSPORT MEDIUM

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Background

Culture recovery of *Neisseria gonorrhoeae* (NG) remains important for the diagnosis of gonorrhoea, especially in females. 'Bed side' inoculation of media is optimal, but can prove impractical, or impossible, in some healthcare settings[1]. Despite emergent molecular diagnostics, the need to recover NG for susceptibility testing and epidemiological typing ensures that transport media remain indispensable in the management of gonorrhoea. This is underscored by the development of an NCCLS quality control standard for microbiological transport systems (M-40).

Although many evaluations of media and 'transport systems' have been made, it remains unclear whether refrigeration of transport swabs improves, or is detrimental to, the recovery of NG. To address this we measured the survival of 30 distinguishable strains of NG at room temperature (RT) and at 4°C. We also evaluated the effects of two suspending media for the preparation of the inocula in these experiments.

Methods

Thirty clinical isolates of NG, distinguishable by auxotyping, epidemiologically, or both were studied. For each strain two inocula were prepared. Initially a 2 mL suspension in phosphate-buffered saline (PBS, Sigma-Aldrich, Poole, UK) was prepared to a McFarland No. 1 standard; 0.5 mL of this was added to 0.5 mL of PBS (PBS inoculum), and 0.5 mL to 0.5 mL of nutrient broth (Oxoid, Basingstoke, UK; PBS:broth inoculum). Swabs were inoculated with 100mL of suspensions. 'Transwab®' rayon-tipped swabs with plastic shafts and charcoal-containing Amies medium (Medical Wire & Equipment Co, Corsham, UK) were used throughout. For each strain, eight swabs were inoculated, to allow comparison of all combinations of: the PBS or PBS:broth inocula; storage at RT or 4°C; for 24 or 48h.

At times 24h and 48h, NG were recovered from swabs by vortexing the tips in 1mL PBS for 30s. Triplicate counts were performed on the time 0h inocula and washings, using a spiral plater (Don Whitley, Shipley, UK), on chocolate agar (Oxoid, Basingstoke, UK). For each of the 30 strains, the median of the three counts was taken for each of the eight possible inoculum / medium / time interval combinations.

Results

Figures 1 & 2. The median log counts at time 0h for the PBS and PBS:broth inocula were 6.68 and 6.48, respectively (P=NS). At 48h the recovery from swabs held at 4°C was significantly better than for those held at RT, but only for the inoculum prepared in PBS rather than PBS:broth.

Discussion

Cooling specimens – including the use of dry ice[2] - to preserve gonococci during transport has been considered for many years. Although Stuart *et al* recommended refrigerating Stuart's transport medium in 1954[3], they provided no evidence to support this, and Stuart did not suggest refrigeration in his original paper[4]. Amies gave no recommendation about holding temperatures for his modification of Stuart's medium[5]. Stuart's and Amies' media may even differ in this respect: in a study of urethral pus from 58 men inoculated on to (rather than in to) three semi-solid transport media, Taylor & Phillips found that storage at 4°C improved recovery of gonococci from Stuart's, but not Amies', medium[6].

Our study includes more strains of GC than other in vitro evaluations to date. Sng *et al*, in a semiquantitative study, looked at the survival of 5 strains of NG in Amies at four temperatures (4, 18, 26 & 32°C) and found better survival at lower temperatures up to 48h[7]. Arbique *et al*, studying six isolates found that recovery was improved by refrigeration, though optimum temperature varied with transport system[8]. Perry *et al*[9], using three isolates, considered that 4°C prolonged survival. In

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other studies of NG ATCC 43069, in various transport systems, recovery has usually been improved by storage at 4°C[10-12], though not always[13].

Figure 1. Phosphate-buffered saline Inoculum

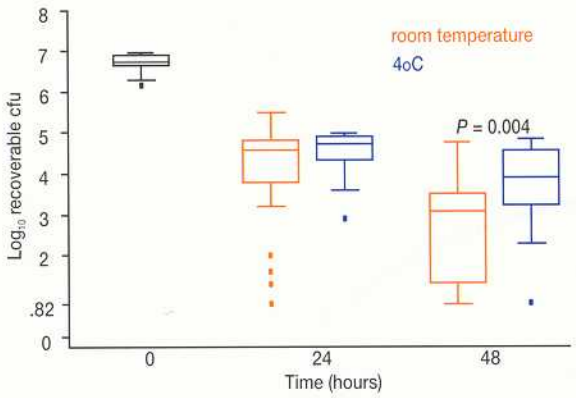
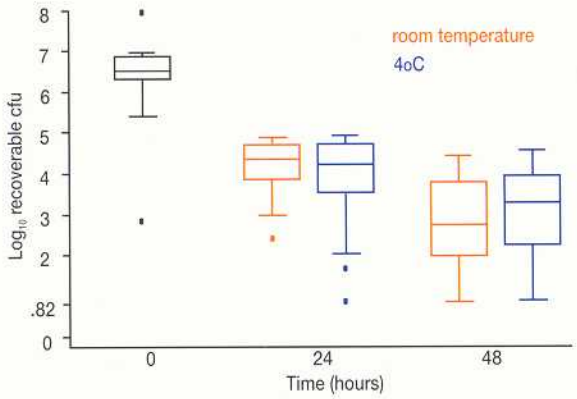


Figure 2. 50:50 phosphate-buffered saline : broth mix inoculum



Conclusions

Our results add to a growing body of evidence that refrigeration does not reduce the yield of NG and may be beneficial. This is in keeping with observations that NG autolysis is minimal at lower temperatures[14-16]. Further studies – using fresh clinical isolates - are needed to determine the degree to which survival of NG in different commercially available systems is affected by temperature. We found that the type of inoculum used - PBS or PBS:broth - affected our results: standardising the menstruum used for the inocula in quality control testing (as proposed by the approved NCCLS M-40) is essential.

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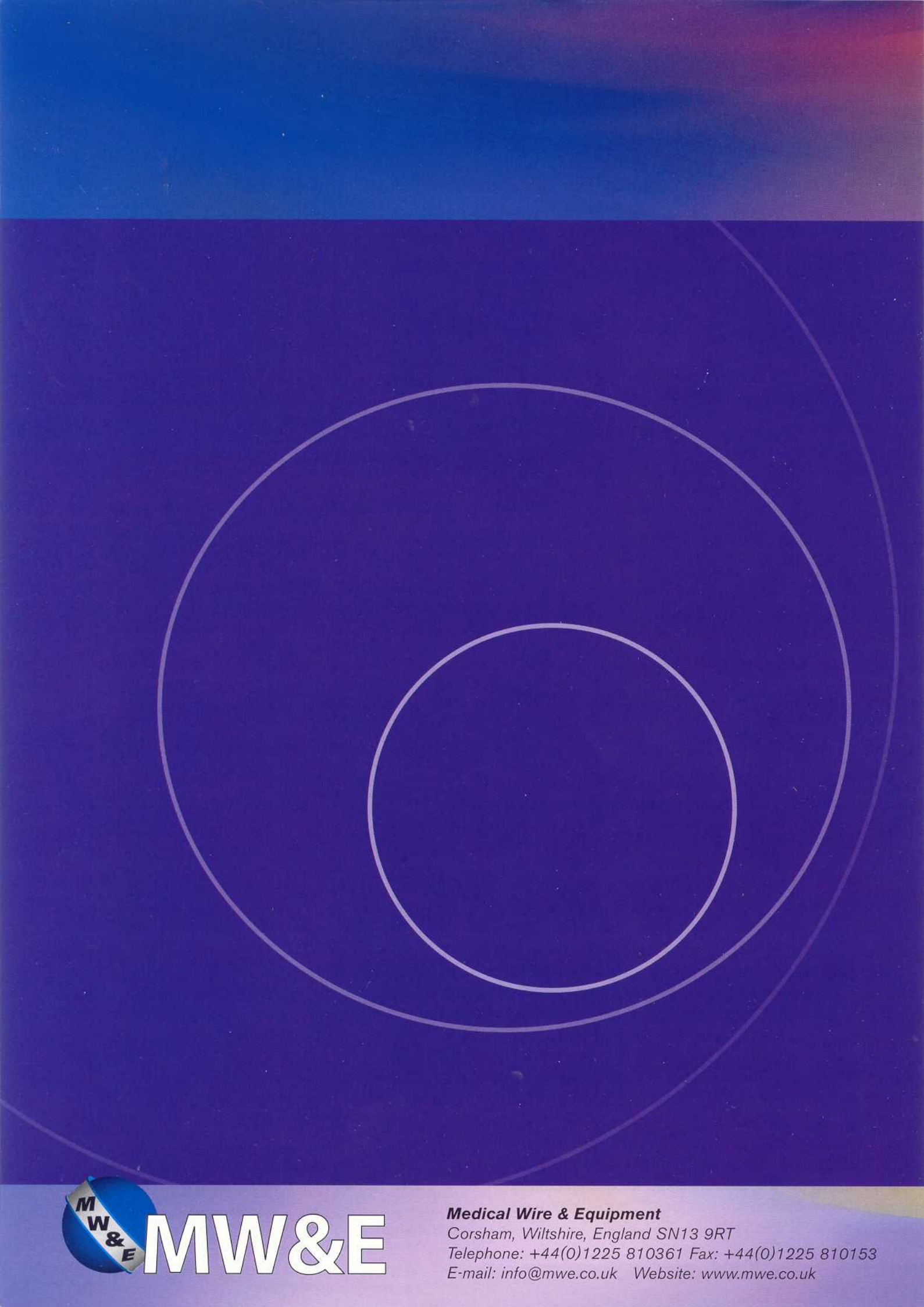

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