A cost-effective and simple alternative technique for reconstitution of freeze-dried cultures of *Neisseria gonorrhoeae*

Freeze drying (lyophilization) is used to preserve micro-organisms for decades and is the preferred method for culture collections worldwide (Morgan et al., 2006). Preserved bacterial strains are critical for research, diagnostic and teaching purposes. The medium recommended by the World Health Organization (WHO) Collaborating Centre for Sexually Transmitted Diseases, Sydney, Australia, for reconstitution of freeze-dried cultures of *Neisseria gonorrhoeae* is 1 ml nutrient blood broth or rich peptone broth supplemented with 10% blood or nutrient broth. However, sheep blood or horse blood is not easily available in most laboratories, and human blood is not recommended. Normal saline (0.9%, w/v, NaCl) is generally available in all laboratories. The objective of the present study was to compare nutrient blood broth and normal saline for reconstitution of freeze-dried cultures of reference and clinical strains of *N. gonorrhoeae* and to evaluate their performance characteristics for the growth of *N. gonorrhoeae* strains.

A prospective study was undertaken between January 2011 and December 2012. Ninety-three lyophilized *N. gonorrhoeae* strains including 83 clinical isolates, ATCC 49226 and nine WHO reference strains were tested using both methods. The results were recorded in terms of colony morphology, colony size and viable gonococci.

A viable count of the content of the lyophilized ampoules by both methods was performed in duplicate after 5–6 months of storage. A spread plate technique on chocolate agar was used to determine the number of c.f.u. gonococci ml⁻¹ (Wise, 2006). Plates showing 30–300 colonies on a plate were used for viable count estimations (Breed & Dotterrer, 1916).

Statistical correlation in survival of the freeze-dried cells from the two different reconstituted suspensions was analysed using the Pearson correlation coefficient and an *r* value was generated for c.f.u. results for both methods. The statistical correlation result was considered perfect for the correlation coefficient (*r* value) of 1.00, desirable for ≥0.90 and acceptable for ≥0.80 (Armitage et al., 2002).

*N. gonorrhoeae* was successfully isolated from 89 (95.7%) lyophilized strains by both the techniques used for revival. In the other four cases, it was difficult to state with absolute certainty whether the gonococci were non-viable or whether unsatisfactory lyophilization practices were responsible for these results. Colony size

![Fig. 1. Scatter dot graph demonstrating c.f.u. ml⁻¹ in reconstituted lyophilized suspensions of *N. gonorrhoeae* strains with nutrient blood broth and normal saline.](image-url)
and colony morphology remained typical after subculture from normal saline and did not show any change in staining and biochemical characteristics. In this experiment, the $10^{-2}$ dilution was the one from both the reconstituted preparations that most closely met the criteria of 30–300 colonies per plate. The ranges of counts of surviving bacteria were $0.5 \times 10^8$–$3 \times 10^8$ c.f.u. ml$^{-1}$ in normal saline and $0.6 \times 10^8$–$3.2 \times 10^8$ c.f.u. ml$^{-1}$ in blood broth (Fig. 1). The median value for c.f.u. ml$^{-1}$ was 1.8 and 2.0 after reconstitution in normal saline and nutrient blood broth, respectively. Person’s correlation coefficient was excellent ($r=0.98$) for both methods.

To conclude, this is the first study to evaluate the usefulness of normal saline for revival of lyophilized strains of *N. gonorrhoeae*. Normal saline yielded excellent viable count results when compared with nutrient blood broth. The data obtained in the present study suggest that normal saline could be recommended as a convenient and inexpensive alternative for universal use, especially in settings with minimal microbiological resources.

Acknowledgements

The authors are thankful to the National AIDS Control Organization, India, for their financial support and to Mrs Leelamma Peter and Mr Naveen Chandra Joshi for their excellent technical assistance. We are grateful to the Indian Council of Medical Research (ICMR) for providing an SRF fellowship to V. S. and a Laboratory Technician post to M. K. This paper was presented at the STI & AIDS World Congress 2013, in Vienna, Austria.

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