Evaluation of crystal violet impregnated filter paper strips for transport and selective isolation of β-haemolytic streptococci

Specimen collection and processing is the most crucial step in the isolation of β-haemolytic streptococci (BHS). Various methods, such as transport media (Amies and Stuart), filter paper strips and silica gel containers, have been used to improve the survival of BHS, especially *Streptococcus pyogenes*, and prevent growth of commensals. The filter paper strip technique is a standard method for the transport of *S. pyogenes* (Hollinger et al., 1960). Crystal violet (CV) is used in selective media such as Pike’s medium to inhibit the growth of commensals and increase recovery of *S. pyogenes* from clinical samples. The present study was undertaken to test the efficacy of filter paper impregnated with CV in the transport and recovery of BHS from clinical samples.

A total of 112 skin swabs collected from children with pyoderma were included in the study. The swabs were collected in duplicates. One set from all 112 patients was inoculated immediately onto plain filter paper strips. The second set was inoculated onto CV filter paper impregnated with stock solutions of different concentrations of CV (20 swabs with 1 µg ml⁻¹, 78 swabs with 16.65 µg ml⁻¹ and 14 swabs with 25 µg ml⁻¹).

Filter paper strips measuring 2 × 6 cm (Whatman no. 1) were prepared using a standard protocol (Johnson et al., 1996). The three stock solutions of CV (1 µg ml⁻¹, 16.65 µg ml⁻¹ and 25 µg ml⁻¹) were made in distilled water. These concentrations were chosen based on a pilot study, which showed that 1 µg ml⁻¹ did not inhibit bacterial growth and 25 µg ml⁻¹ was inhibitory to BHS. Two hundred microlitres from each stock was placed on the surface of the filter paper strips so that the final absorbed concentration was 0.2, 3.33 and 5 µg in each filter paper. Swabs collected in duplicates were streaked heavily back and forth on the filter paper strips and transported to the laboratory within a period of 4 h. Inoculated strips were incubated on the surface of blood agar plates for 4–5 h, removed with sterile forceps and placed on another area of the same plate for few seconds before being discarded. Plates were incubated at 37 °C under microaerophilic condition for 16–18 h, after which they were inspected for the presence of BHS colonies. Growth was graded as ‘profuse’ if more than 100 colonies of BHS were present, ‘moderate’ if the number of colonies was 21–100 and ‘scanty’ if the colony count was 1–20.

Among the 20 skin swabs that were transported on 0.2 µg CV per strip filter paper, 7 specimens grew BHS. There was no difference in the isolation of BHS on CV filter paper at this concentration when compared with plain filter paper. Of 78 skin swabs transported on 3.33 µg CV per strip filter paper, 39 swabs grew BHS. These specimens when transported on plain filter paper also grew BHS in the same numbers (39/78), but the growth was accompanied by heavy growth of staphylococci and skin flora, whereas on CV filter paper, staphylococci were inhibited and there was profuse growth of BHS (Fig. 1). Of the 14 skin swabs that were processed using 5 µg CV per strip filter paper, on the corresponding plain filter paper 8 samples grew BHS, whereas only 5 of the 8 samples grew BHS on CV filter paper. The growth was sparse in all five specimens, whereas it was moderate in all the specimens transported on plain filter paper.

CV, a bacteriostatic dye, is used in the preparation of culture media for selective isolation of BHS. We have attempted to standardize a new technique for the selective isolation and transport of BHS from skin swabs by incorporating CV at various concentrations in the filter paper. It was found that CV at a concentration of 0.2 µg per strip of filter paper was insufficient to inhibit staphylococci and 5 µg per strip was inhibitory to BHS. A concentration of 3.33 µg per strip was arbitrarily chosen since it was intermediate, and it was found to effectively inhibit staphylococci, and allow pure and heavy growth of BHS on the

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**Fig. 1.** Colony growth on blood agar. A, Area of the plate incubated with CV filter paper (numerous BHS colonies); B, area of the plate incubated with plain filter paper (BHS overgrown with staphylococci).
The use of CV filter paper, in our experience, appears to be a good method for the epidemiological study of streptococcal pyoderma. It is cost effective and ideal for a tropical setting. Further studies with varying concentrations of CV to ascertain the optimum concentration for use are in progress.

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