JOURNAL OF BACTERIOLOGY, Feb., 1967, p. 768-769
Vol. 93, No. 2

Printed in U.S.A.

Evaluation of Polyvinyl Alcohols as Semipermanent Mountants for Fluorescent-antibody Studies

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Received for publication 24 October 1966

Smears of bacteria stained with fluorescein-labeled antibodies (FA) are usually mounted with a cover slip by use of buffered glycerol as the mountant. Fluorescence of such preparations diminishes in intensity during prolonged storage, necessitating the use of freshly prepared smears for demonstration, reference, or teaching purposes. The permanent type of mountants commonly used in histopathology laboratories have not proven satisfactory for FA microscopy. The use of a polyvinyl alcohol (PVA), Elvanol 51-05 with glycerol, as a semipermanent mounting medium for FA smears was suggested by J. Rodriquez and F. Deinhardt (Virology **12**:316, 1960), and was used by C. E. D. Taylor et al. (J. Clin. Pathol. **17**:225, 1964) to mount smears of *Shigella sonnei*.

We have used two PVA preparations, Elvanol 51-05 obtained from Delkote, Inc., Wilmington, Del., and Gelvatol 3-60, supplied by Shawnigan Resins, Springfield, Mass., in combination with glycerol for mounting smears of *Escherichia coli* and *S. sonnei* from both pure culture and fecal specimens.

Several combinations of the PVA preparations and glycerol were tried with various *p*H levels and concentrations. The following were the most satisfactory: 10 and 15% Elvanol 51-05 with 30% glycerol (*p*H 7.4) and 5% Gelvatol 3-60 with 30% glycerol (*p*H 7.4). When the *p*H level dropped below 7, the fluorescence became yellow to orange and the organisms appeared smaller in size. A *p*H level above 7.4 resulted in considerable softening of the PVA. We used only one lot of the Elvanol and Gelvatol. Other lots may vary in properties so that an increased or decreased amount may be necessary to achieve the desired consistency and adhesive properties.

The following procedure for preparing the mountants was modified slightly from that described by Rodriquez and Deinhardt.

(i) Add 10 or 15 g of Elvanol 51-05 or 5 g of Gelvatol 3-60 to 70 ml of 0.85% saline buffered to *p*H 7.4 with 0.01 M Na_2HPO_4-KH_2PO_4.

(ii) Heat to 70 to 80 C until PVA is dissolved. Cool to room temperature.

(iii) Add 30 ml of glycerol to PVA-buffer solution and mix thoroughly. Check the *p*H and adjust to 7.4 if necessary with the phosphate buffer.

(iv) Add 1 ml of 1% Merthiolate to obtain a final concentration of 1:10,000. Mix well.

(v) Store in an air-tight bottle.

We evaluated the usefulness of the PVA mountants by preparing smears of *E. coli* O111:B4 and *S. sonnei* from suspensions of pure cultures and from seeded fecal specimens. After staining with the homologous antibodies, three sets of smears of both *E. coli* and *S. sonnei* were mounted with cover slips by use of the three mountants. After the mountants hardened (4 to 6 hr), one set of smears was left at room temperature, one set was stored at 4 C, and the other was stored at −20 C. Representative slides were removed at various intervals and examined for fluorescence intensity.

Smears from pure cultures of *E. coli* mounted with Gelvatol and the two concentrations of Elvanol exhibited 4+ fluorescence after 18 months of storage at all three temperatures. The preparations stored at −20 C appeared to be somewhat more brilliant than the ones stored at either room temperature or at 4 C.

The smears from pure cultures of *S. sonnei* retained their initial fluorescence for 5 months at all storage temperatures with all three mountants. After 5 months of storage, the fluorescence decreased in the smears held at room temperature. The smears stored at 4 or −20 C exhibited the maximal fluorescence for the length of the storage (18 months).

All fecal smears containing either *E coli* or *S. sonnei* were unsatisfactory after 3 months at all temperatures. The fecal material became more fluorescent, whereas the fluorescence of the bacteria decreased. No difference was noted between the fluorescence of smears mounted with the 5% Gelvatol and the 10 and 15% Elvanol.

The PVA mountants should prove useful to those concerned with teaching FA techniques and to those who wish to retain stained prepara-

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tions for reference or control purposes. The PVA hardens enough so that the cover slips are firmly attached; yet the smears do not dry out during storage. The cover slips can be dislodged with careless handling, so care should be exercised when wiping immersion oil off the surface.

FA preparations which must be examined immediately should be mounted as usual with buffered glycerol, since several hours are required for the PVA mountant to harden. If smears are examined before the PVA sets, organisms appear fuzzy and have haloes of refracted light around them so that critical focusing is impossible. Care must be exercised in using only the amount of PVA necessary to seal the cover slip to the smear; otherwise, optical difficulties may occur owing to the thickness of the mountant. Cover slips can be removed from smears mounted with buffered glycerol, and the smears can be rinsed and remounted with the PVA if so desired.