screen both faeces and bacterial cultures for toxin. The technique is quick to set up (one person can test 100 samples in a day without difficulty) and reduces the time needed to record results. It also produces considerable financial savings compared with glass tubes and pipettes, requires smaller volumes of cell suspension (we use 0.1 per well instead of the 1 ml required per tube), and economises on antiserum. Recently we have recycled transfer plates because they cost approximately £1 each. After use the transfer plate is soaked in chlorox, washed, and then rinsed repeatedly in distilled water, and finally dried in an incubator. We have not experienced contamination of cell lines due to this or detected carryover of toxin from previous tests. The basic principle can be varied to screen for toxin over a wide range of titres according to the volume and urgency of the work requested.

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Requests for reprints to: Dr R. H. George, Microbiology Department, The Children's Hospital, Ladywood Middleway, Ladywood, Birmingham B16 8ET, UK.

A low-speed centrifugation technique for the preparation of grids for direct virus examination by electron microscopy

H. K. NARANG and A. A. CODD *Public Health Laboratory, Institute of Pathology, General Hospital, Westgate Road, Newcastle upon Tyne NE4 6BE, UK*

The negative staining technique introduced by Horner and Brenner (1958) has been extremely useful as a rapid means of visualising virus particles from clinical specimens. However, limitations arise for three main reasons: too small a sample, too low virus concentration in the sample, and too much tissue debris relative to virus. We describe a simple technique for collecting specimens from skin lesions and preparing negatively stained grids for the electron microscope that greatly reduces the difficulties encountered in conventional techniques.

¹Type 10, 1 ml, Venture Plastics, Chessington, UK

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Method

Samples from 29 patients on wards and in casualty and outpatient departments who presented with skin lesions clinically suspicious of virus infection were examined. The lesions were uncapped with a scalpel blade and vesicle fluid, usually 2-5 μ l, was aspirated with a finely drawn Pasteur pipette. Flat-bottomed 6 mm diameter polythene tubes¹ were prepared by cutting down to a length of 15 mm. To each of two tubes 8 drops (200-250 μ l) of distilled water was added. Carbon-coated grids were lowered into the water at the side of the tubes and tilted so that the grids dropped to the bottom with the carbon/formavar film facing upward. The vesicle fluid was expelled into one tube by gentle rinsing in the distilled water and a single drop was transferred to the second tube, thereby giving a dilution of 1:9 between the tubes. When the lesions were dry the material on the tip of the scalpel blade was washed in 8 drops of distilled water which was transferred into an empty plastic tube and a grid emplaced as described. These tubes were put into 5 $\times$ 1 cm glass tubes and centrifuged horizontally in an MSE minor bench centrifuge for 30 minutes at a speed of 3700 rpm, giving a calculated gravita-

Technical methods

tional force of 2100 g. The plastic tubes were lifted out of the glass tubes, and grids were picked out with fine forceps after tilting the tubes on the side. The grids were dried on filter paper for a few minutes and then floated on a drop of phosphotungstate (PTA) pH 6.6 for half to one minute. Excess stain was blotted off with a filter paper. All grids were left under ultraviolet light for at least 15 minutes to inactivate viruses before examination in the AEI electron microscope at a standard magnification of $\times 25\,000$.

Results

Of the 29 samples examined, 27 were positive for virus particles by electron microscopy (EM): herpes simplex was found in 9, chicken pox/zoster in 7, vaccinia in 6, orf in 4, and molluscum contagiosum in 1. Both negative EM samples were negative by culture and serology for virus infection. One of these was subsequently diagnosed as Sweets' syndrome and the other as an allergic rash. In the majority of cases examination of the grids from both tubes revealed many virus particles. In some instances where there was a lot of cell debris in the vesicle fluid some grid holes in the first grid were thick and black, but many other squares could be examined as virus particles were found in almost all holes. Many of these particles were found individually, but in some cases they were also seen in small clumps of 2-6 particles.

Comments

A frequent difficulty when examining specimens from skin lesions is the paucity of material. Very

often it is not possible to extract more than a few microlitres (2-10 μ l) of vesicle fluid. In these circumstances the specimen may easily be lost in transport on the side of the container. Since it is necessary to have at least one drop of fluid, 20-25 μ l, to process a grid conventionally, eluting the fluid by adding a drop of distilled water to the specimen may impose an unacceptable dilution factor. Further, even in an adequate specimen processed by dipping, the grid usually results in a very uneven distribution, that is, more particles are seen in certain areas than in others. Both these difficulties are overcome by the present centrifugation method as in scanty specimens all the vesicle fluid can be transferred into the water in the tube overlying the grid by rinsing. On centrifugation many of the particles were uniformly distributed on the grid. It took only a few minutes to find virus particles by EM and their absence from the first few grid squares examined implied a negative sample. This contrasts with a conventionally prepared grid where it is frequently found that particles are absent from the first five or six grid squares but subsequently appear to be plentiful in other areas examined.

Reference

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Requests for reprints to: Dr H. K. Narang, Public Health Laboratory, Institute of Pathology, General Hospital, Westgate Road, Newcastle-upon-Tyne NE4 6BE, UK.

Letters to the Editor

Fetal adrenal cytomegaly

We report a case of fetal adrenal cytomegaly associated with maternal Metrolen ingestion.

Case history

A 30-year-old woman, who had two normal, healthy children, was first seen in outpatients when she was 16 weeks' pregnant by size. Because of prolonged vaginal bleeding starting at four weeks in an undiagnosed pregnancy she had been given a two-month course of

Metrolen from six to 14 weeks. At the end of this time she remained amenorrhoeic, and pregnancy had become obvious. In view of the possibility of the hormone administration causing fetal abnormality, such as a major cardiac anomaly or masculinisation of a female, a prostaglandin termination was performed at 17 weeks.

Macroscopic findings

An autolysed male fetus weighed 115 g, with a crown-rump length of 13.5 cm, a crown-heel length of 19.5 cm, and a head circumference of 15 cm. The combined kidney weight was 0.85 g. These

measurements are consistent with a 15-week gestational age. Both adrenal glands were enlarged but of a normal morphology. They had a combined weight of 0.88 g, which is over the second standard deviation above the normal for this body weight (Tanimura *et al.*, 1971). There were no other unusual features.

Microscopic findings

Cytomegalic cells were present in the inner third of the cortex of one adrenal gland. These cells were large with large nuclei, which showed a prominent chromatin network (Figure). There were no other unusual features.