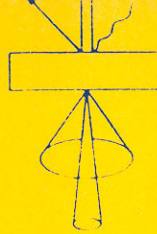


SEMT

Society of Electron Microscope
Technology



Affiliated to the Royal Microscopical Society

PREPARATIVE TECHNIQUES –

when to spit on it !

Friday, 15 May 1992 at 2.00 p.m.

IMPERIAL CANCER RESEARCH FUND
Lincoln's Inn Fields, London WC2

This meeting will take the form of a workshop on EM techniques to provide opportunities for the informal exchange of expertise.

Several of our members will provide a focus for discussions on the topics listed below, and we will be able to circulate between the various groups. Fellow experts will have much in common to talk about, and there will be a particular welcome to those seeking advice on an unfamiliar technique or wishing to discuss problems that have arisen in the course of work.

SEM stub preparation	Don Claugher & Sue Barnes
Negative staining	Anne Drewe
Support films & immunostaining	Heather Davies
Section cutting	Steve Cham
Immunogold-labelling	Catherine Sarraf & Pauline Barber
Pop-off techniques	John-Paul Cassella
Coffee will be available at 2.00 p.m.:	tea & biscuits at 3.30 p.m.

To the Secretary:

Dr Jill Lewis, Electron Microscope Unit,
St Bartholomew's Hospital Medical College,
Charterhouse Square, London EC1M 6BQ

I hope to attend the meeting at ICRF on 15 May 1992

Name Telephone

Address

I have expertise in

I am seeking general advice on

My particular problem is

.

A recommended method of picking up grid films is onto a luggage label on a slide.

Immuno-EM

If antiserum is dried onto the grid first, only the antibody will stick, so you get a much cleaner preparation and it is easier to see what is there.

? which antisera are available for my viruses ?

Negative stain - sodium silico-tungstate gives lower contrast, but is more tolerant of gunge etc.

Anti-capillary forceps are useful.

NEGATIVE STAINING

Negative staining is used for small whole structures - viruses, macromolecules or bacterial flagella. The stain does not adhere positively to the object, but forms a pool around it.

DIAGRAM

The object must be supported on a film over the grid, and I prefer formvar with carbon evaporated onto it. Faults in the film may collect stain and give rise to spurious images - streaks or blobs where stain has collected, or holes in the film.

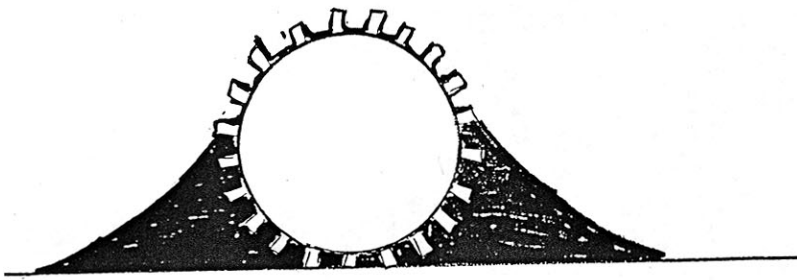
The most widely-used stain is still phosphotungstic acid (PTA), usually at pH 6.5 approx. Other stains can be used, especially ammonium molybdate or sodium silicotungstate. For routine work it is best to stick to a few stains, as the stain and pH used may affect the appearance obtained. Some people find that uranyl acetate severely damages viral structure.

If starting from a specimen with a lot of large debris, it may be useful to spin first in a bench centrifuge at about 3000 rpm for 10 minutes. Then spin the supernatant in an ultracentrifuge at 30,000 rpm for one hour; re-suspend the pellet in a couple of drops of liquid and place on parafilm. Float the grid on the drop - the length of time will depend on the amount of debris remaining and the amount of virus; I use about 3 minutes for faecal specimens, but 15 minutes for vesicle fluid. Drain the grid by touching to filter paper, then immediately put a drop of stain on and draw it off again. Since my material may contain pathogens, I leave the grid in formalin vapour in a moist chamber for 10 minutes before transferring it to the EM.

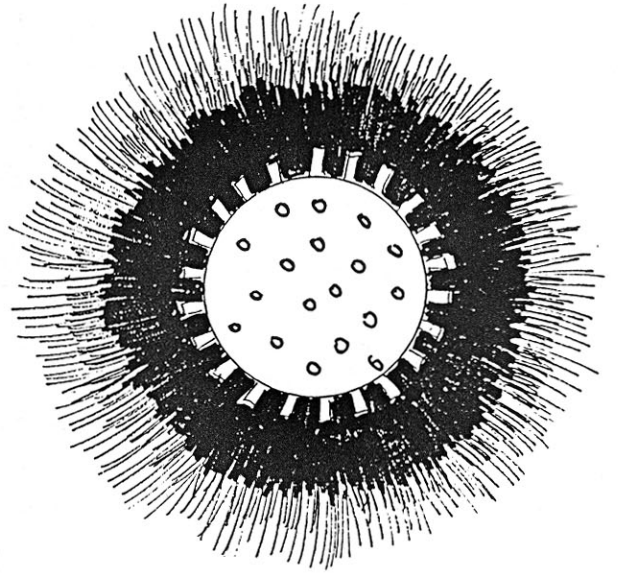
If you are looking for specific viruses etc for which you have antisera, you can obtain good clean preparations by drying a film of antiserum onto the grid film; ^{first} this will capture the specimen that you want but not much of the debris,

Anne Drewe

following workshop meeting 15-5-92



side view of virus on grid



appearance on screen