

## Some Hints On Clinical TEM Negative Staining For Viruses

Sara E. Miller, Duke University Medical School

I would *not*, repeat *\*not\**, recommend fixing any virus sample before attaching it to the grid. If you are concerned with pathogenicity, you can fix it after allowing it to adhere to the grid, then wash with water and stain, or you can UV irradiate both sides of the grid after staining. For speed in reporting clinical results (we do almost 1000/year), we look at negative stains of potentially infectious material without fixing by keeping a separate specimen holder for "dirty" grids and another for nonpathogenic material such as sections. The grids are then UV irradiated before storage.

Anything fixed by aldehydes, especially glutaraldehyde, becomes *less* sticky after fixation. The most important reason that viruses in a clinical sample should not be fixed before gridding is that you decrease the numbers that stick to the support film. If you are close to the threshold of detection, you may decrease the numbers below that level.

Many of the described methods for fixing viruses before gridding include ultracentrifugation to concentrate them. This may be fine for samples such as stool from gastroenteritis patients where there are likely to be lots of virus, but for cerebrospinal fluid and other liquid samples likely to have few viruses, you don't want to take the chance of losing any. If I were looking for an unknown virus in any sample, not knowing beforehand whether it were positive or negative, I would

not want to lower my chances of finding something.

Further, adding fix to a dirty sample like stool can glue contaminants together. You can form large clumps that trap viruses and then are pelleted out in a low speed spin, effectively decreasing the number that can be seen on your grid. Also, junk can be glued to viruses, coating them so that they're unrecognizable.

It has been reported that some viruses have altered morphology after fixation. If you are looking for a novel virus, you should look at fixed and unfixed virus.

### Miscellaneous Facts:

We use uranyl acetate which fixes and preserves viral structure; it does *not* kill all viruses, but we use it because phosphotungstic acid (PTA) is known to destroy some viruses, particularly reo- and rotaviruses. While you can observe them immediately after PTA staining, if you want to store them to show the professor or medical student tomorrow or next week, you have to fix them and then wash with water before staining - adding more steps. Uranyl acetate is radioactive. PTA stains the spikes on viruses better (e.g., paramyxoviruses).

### Note carefully:

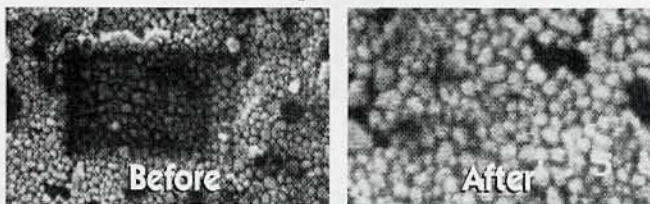
Finally, looking in stool for a virus that may be in brain may or may not yield positive results. If your question is "Does the patient who has viral encephalitis also shed virus in stool," then your search is valid. If your question is "Does the patient who has encephalitis have viral encephalitis," then you can't be sure a negative result from stool examination is valid. Not all viruses that cause viral encephalitis are shed in stool. Some never are; some are shed only for a short window of time. Some viruses that cause encephalitis could not be identified in stool, even if they were shed (e.g., alphaviruses, flaviviruses, bunyaviruses). Stool is just too dirty: there are too many membranes and vesicles that resemble viruses, and these viruses don't have a morphologically recognizable nucleocapsid. Finally some viruses cause a post infectious encephalomyelitis which appears to have an autoimmune component, and little or no virus is seen - even in brain. The only viruses you're really likely to see in feces of encephalitis patients are Picornaviruses (e.g., enteroviruses, Coxsackie viruses). ■

### A Reference:

Hayat & Miller, 1990. *Negative Staining*. McGraw-Hill. Especially the chapter on safety.

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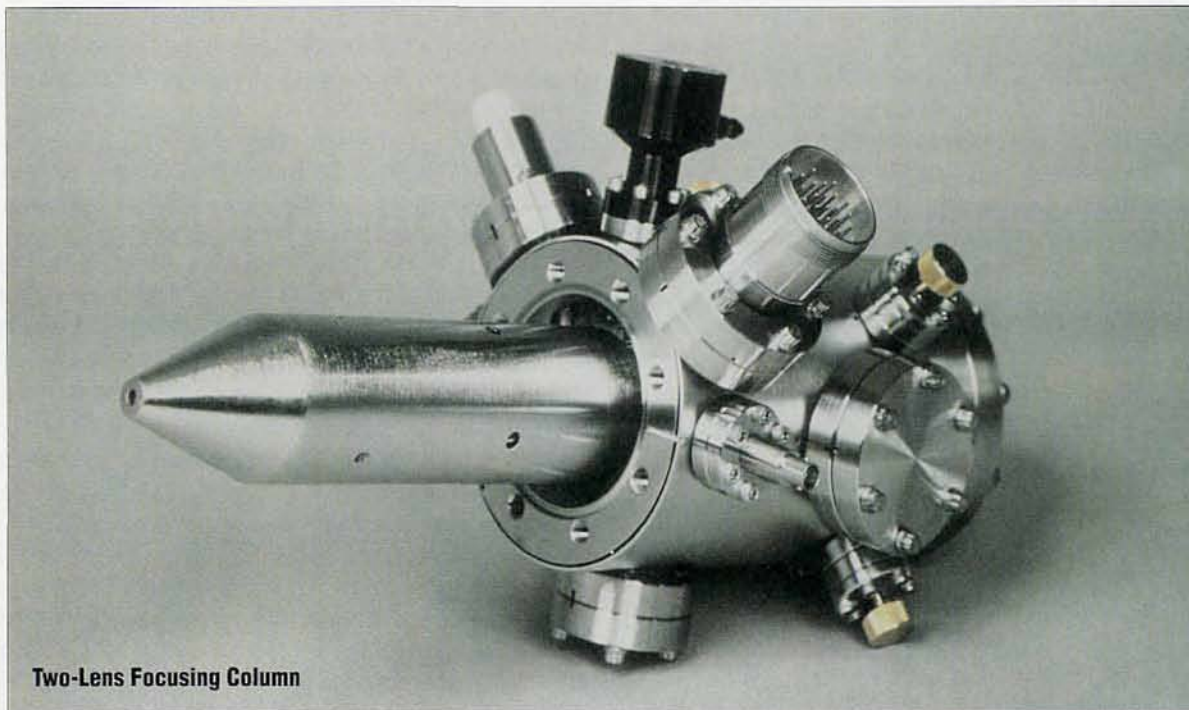
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