Preparation of viral samples within biocontainment for ultrastructural analysis: Utilization of an innovative processing capsule for negative staining

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BACKGROUND

Negative staining is a process that provides contrast to a biological specimen, allowing it to be viewed under a transmission electron microscope (TEM). The process involves briefly applying a heavy metal salt solution to a TEM grid, with the virus sample already attached, in an attempt to surround the virus without infiltrating it. This creates a dark border and maps out the particles shape. In this project we introduce a new method for negative staining TEM grids in biocontainment that utilizes mPrep/g capsules, a capsule based device for grid handling and negative staining. The mPrep/g capsule encloses two TEM grids and thereby protects the sample and minimizes direct handling, thus making damage less likely. The mPrep/g capsule attaches directly to a single or multichannel pipette where they work similar to a pipette tip, allowing for application of various liquids using aspiration. This enables simultaneous preparation of multiple samples with duplicate grids. The purpose of this study is to evaluate mPrep/g capsules as a new sample preparation method for preparing viruses in biocontainment. The mPrep/g capsule method is compared side by side to the manual droplet method, a proven method, to determine its effectiveness. This study also compares EM image quality after two different virus inactivation procedures: rapid with 1% Osmium Tetroxide (OsO4) vapor versus slower with 2% glutaraldehyde, using mPrep/g capsule method. Finally, we compare the two most common used negative stains, UA and PTA, on the EM image quality.

METHODS

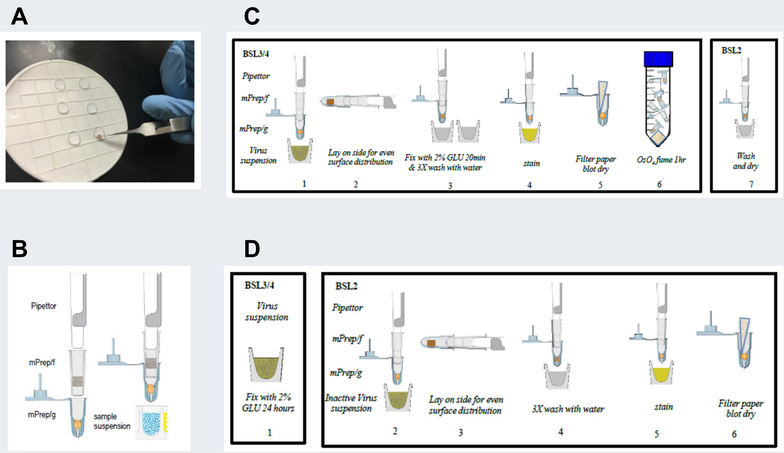

Figure 1: Negative Staining Methods overview. (A) Manual droplet method of passing EM grids along droplets of reagents and stain. (B) Setting up mPrep/g capsule method with grids and application of sample suspension. (C) Typical procedure using mPrep/g capsule method in biocontainment with short-term inactivation with 1% osmium tetroxide vapor. (D) Recommended procedure using mPrep/g capsule method in biocontainment with long-term inactivation with 2% glutaraldehyde.

RESULTS AND CONCLUSIONS

Compare and evaluate EM image quality by manual droplet method versus mPrep/g capsule method (Figure 2)

We evaluated the imaging quality generated by both manual droplet and mPrep/g capsule processing methods using Zaire ebolavirus. Figure 2 shows that the mPrep/g capsule and the manual droplet negative staining methods both have the ability to produce high quality TEM images. Both figure 2A (manual) and figure 2B (mPrep/g) have visible ebolavirus glycoproteins on the surface and clearly defined details with nucleocapside structures in the center of the virion.

Compare and evaluate EM image quality after rapid inactivation with 1% osmium tetroxide vapor versus slower inactivation with 2% glutaraldehyde only, using mPrep/g capsule method (Figure 3)

We evaluated EM image quality after two different methods of inactivation using Chikungunya virus. Inactivation is achieved using 2% glutaraldehyde for 20 min followed by a one hour exposure to 1% osmium tetroxide vapor, with the entire negative staining process done in an mPrep/g capsule method inside a BSC in biocontainment (Figure 1C). When using 2% glutaraldehyde for 24 hours to inactivate the virus, the inactivation occurs in biocontainment, but the 1% UA negative stain procedure was carried out using the mPrep/g capsule method in a BSL -2 lab outside biocontainment (Figure 1D). It is clear that figures 3C and 3D are of lower quality than figures 3A and 3B, as there are no distinguished glycoproteins visible on the Chikungunya virus and the image also appears “fuzzy” in figures 3C and 3D.

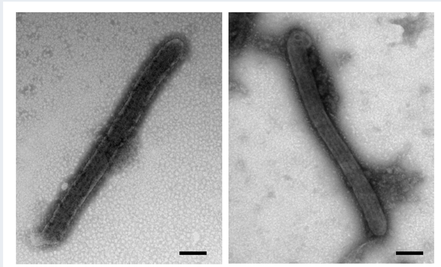

Figure 2: Comparison of ebolavirus negatively stained with 1% PTA using mPrep/g capsule method (right) and manual droplet method (left). Scale Bar 100nm.

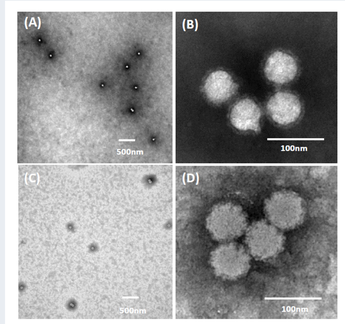

Figure 3: 1% UA Negative Staining by mPrep/g capsule method of chikungunya virus using different inactivation procedures: rapid inactivation with 1% osmium tetroxide (OsO4) vapor versus slower inactivation with 2% glutaraldehyde only. (A) 2% glutaraldehyde 24 hours inactivation, TEM low magnification. (B) 2% glutaraldehyde 24 hours inactivation, TEM high magnification. (C) 1% OsO4 vapor, 1 hour inactivation, TEM low magnification. (D) 1% OsO4 vapor, 1 hour inactivation, TEM high magnification.

Compare and evaluate EM image quality with Uranyl acetate (UA) versus phosphotungstic acid (PTA) stain (Figure 4)

The comparison between UA and PTA stain is shown in Figure 4 using virus-like-particles (VLPs) with mPrep/g capsule negative staining. Both stains display high quality results with visible glycoproteins and clearly defined borders of the Ebola nano-VLPs and Murine Leukemia VLPs (Figure 4).

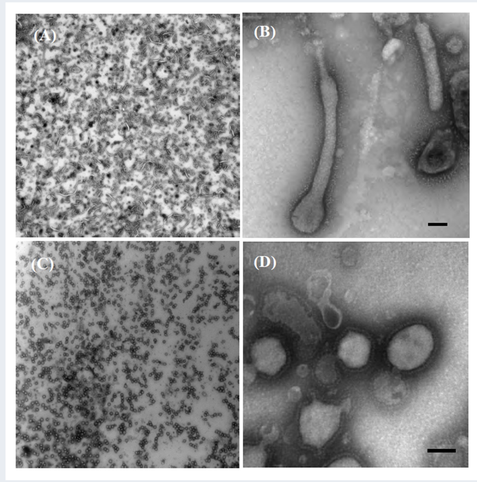

Figure 4: Comparison of Phosphotungstate (PTA) and Uranyl Acetate (UA) negatively stained Virus-Like-Particles (VLPs) using mPrep/g capsule method. (A) Low magnification overview of 1% PTA stained Ebola nano-VLPs. (B) High magnification TEM showing structural details of PTA stained Ebola nano-VLPs. (C) Low magnification overview of 1% UA stained Murine Leukemia VLPs with Ebolavirus glycoprotein on their surface. (D) High magnification TEM showing structural details of UA stained Murine LeukemiaVLPs with Ebolavirus glycoprotein on their surface. Scale Bar 100nmA

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