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 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 inactivation 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% ammonium tetroxide vapor. (D) Recommended procedure using mPrep/g capsule method in biocontainment with long-term inactivation with 2% glutaraldehyde.

RESULTS AND CONCLUSIONS

We evaluated EM image quality generated by both manual droplet and mPrep/g capsule processing methods using Zaire ebolavirus. Figure 2 shows that the mPrep/g capsule method (Figure 2) have visible ebolavirus glycoproteins on the surface and clearly defined details with nucleocapside structures in the center of the virion. 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 3: 1% UA Negative Staining by mPrep/g capsule method of chikungunya virus using different inactivation procedures with 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.

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