Alternative Staining for Electron Microscopy with Ytterbium Acetate

Sonya Layton, Swapan Bhuiyan, and Lee Hughes

Department of Biological Sciences, University of North Texas, Denton, TX

Contact Information:

Lee E. Hughes (940) 565-4137
1155 Union Circle #305220 lhughes@unt.edu
Denton, TX 76203-5017

Abstract

The current protocol for preparation of samples for electron microscopy in the SEA-PHAGES Laboratory Manual utilizes 1% uranyl acetate as the stain. While this compound provides excellent contrast for phage samples for transmission electron microscopy, uranyl acetate poses a number of safety and waste disposal issues that can make classroom use difficult. SEA-PHAGES faculty have previously shared an alternative staining method that uses phosphotungstic acid, but our campus has not had success with this protocol. However, a recent paper by Hosogi *et al.* (2015) evaluated several lanthanide salts, including ytterbium acetate, as alternatives to uranyl acetate in negative staining for electron microscopy. In the fall semester of 2015, four phage samples were prepared using both 1% uranyl acetate and 2% ytterbium acetate for comparison. The resulting images were found to be of similar quality for several of these samples. Because ytterbium acetate has fewer safety and waste disposal concerns, this stain can provide an alternative method for electron microscopy preparation that is more classroom friendly.

Introduction

One of the most powerful moments for SEA-PHAGES students is when they see the electron micrograph of their phage for the first time. At our campus, students are no longer allowed to work with the commonly used stain, uranyl acetate, due to concerns from university safety officials. As a result, we had two choices. The first was that faculty and teaching assistants could prepare the samples for the students. While this provides good results utilizing a proven method, it removes the students from the process which is not ideal. Our second choice was to find an alternate staining method. The SEA-PHAGES community had previously supplied an alternative protocol using phosphotungstic acid, but we have been unable to obtain good results using that protocol. More recently, Hosogi *et al.* (2015) report results of utilizing several lanthanide salts, including ytterbium acetate, as an alternative to uranyl acetate. This paper included EM staining of bacteriophage with good results. Ytterbium acetate does not present the same safety concerns as uranyl acetate, so is more suitable for use in a classroom setting. We tested this protocol with students at UNT during the 2015-2016 academic year.

References

Hosogi, N., Nishioka, H., & Nakakoshi, M. (2015). Evaluation of lanthanide salts as alternative stains to uranyl acetate. *Microscopy*, dfv054. http://doi.org/10.1093/jmicro/dfv054

Acknowledgements

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Direct Comparisons of Uranyl Acetate and Ytterbium Acetate Staining

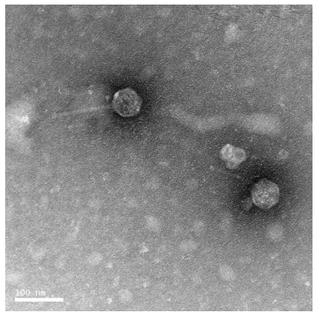

Fig. 1A. Lola – Uranyl Acetate

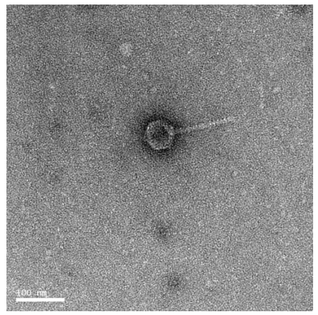

Fig. 1B. Lola – Ytterbium Acetate

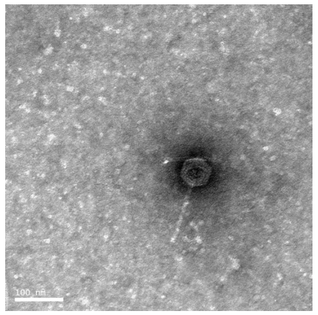

Fig. 2A. Sutton – Uranyl Acetate

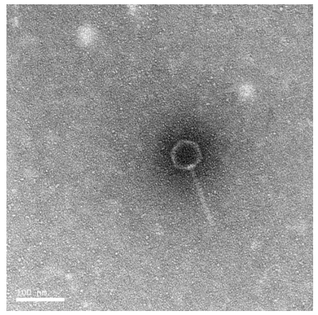

Fig. 2B. Sutton - Ytterbium Acetate

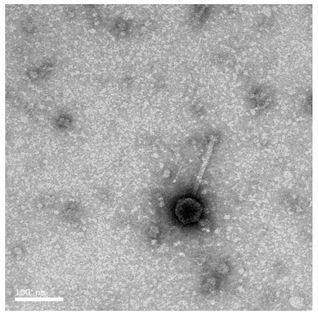

Fig. 3A. Bioscum – Uranyl Acetate

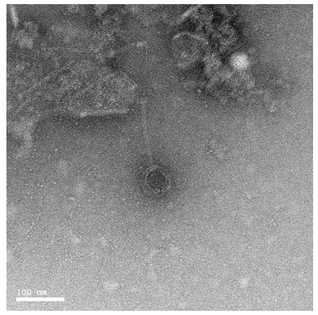

Fig. 3B. Bioscum – Ytterbium Acetate

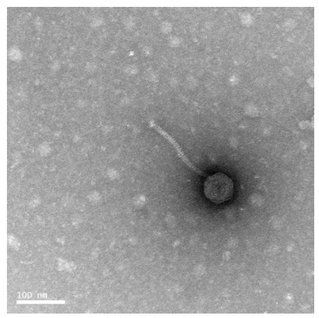

Fig. 4A. Lorelei – Uranyl Acetate

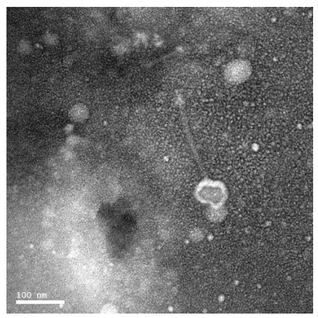

Fig. 4B. Lorelei – Ytterbium Acetate

Other Examples of Ytterbium Acetate Staining

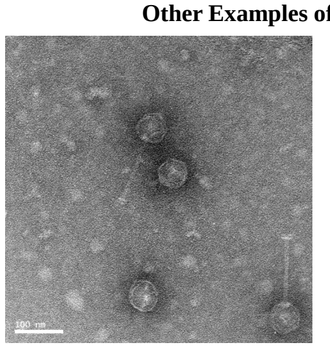

Fig. 5. LarryIsReal – Ytterbium Acetate

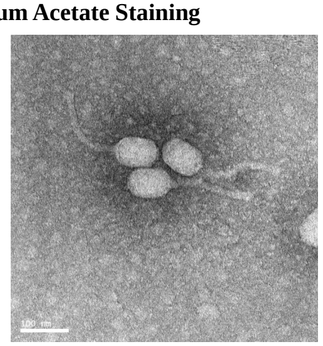

Fig. 6. BabyGotBac – Ytterbium Acetate

Methods

Ytterbium Acetate Preparation

A 2% aqueous solution of ytterbium acetate was prepared and adjusted to pH 6.5 with HCl.

Staining Procedure

A. Prepare your work area

1. Put on a fresh pair of latex or vinyl gloves (Important: we are working we toxic chemicals). Cover the designated lab counter with the plastic-faced paper to create a clean area.

2. Remove the cover paper from a 5 × 5-cm piece of parafilm, and place the parafilm into the lid of a Petri dish.

3. Using a micropipettor, transfer 15 µL of high titer lysate from the sample to the surface of the parafilm. It will make a droplet. In a separate area of the parafilm, transfer 15 µL of sterile ddH2O to make a separate droplet. In a third area of the parafilm, transfer 15 µL of 2% ytterbium acetate to make a separate droplet.

B. Collect phage on grid

1. Using the pointed tweezers, remove a fresh grid from a box of unused grids, touching only the very edge of the grid. Place the grid, shiny side DOWN, onto the first droplet ,which contains the phage HTL. The grid will float on the droplet.

2. Allow the phage to adsorb (attach to grid surface) onto the grid for a minimum of 2 minutes (time varies based on phage titer).

3. Remove the grid from the drop using tweezer. While holding carefully, use a small (2- to 3-cm) wedge of Whatman #3 filter paper or a low-lint laboratory wipe, to wick off the excess fluid.

C. Wash grid.

1. Place the grid containing phage, shiny side DOWN, onto the droplet of ddH2O. Leave for 30 seconds to wash off culture media/debris from lysate.

2. Remove the grid from the drop using tweezers. Wick off the excess fluid.

D. Stain phage sample on the grid.

1. Place the grid containing phage, shiny side DOWN, onto the droplet of 2% ytterbium acetate. Allow to stain for 2 minutes.

2. Wick off the excess stain as before using filter paper and allow the grid to air dry. Grids should be returned to the EM grid box and the box coordinates noted in your laboratory notebook.

Fig. 7A. Staining setup.

Fig. 7B. Grid on HTL.

Fig. 7C. Wicking.

Discussion

Ytterbium acetate provides a viable alternative for students to use in staining their phage samples. Variation in results was seen, with uranyl acetate generally providing the better results in a direct comparison. Yet, the ability of students to perform the work themselves is a valuable experience, and this method allows an alternative for institutions where uranyl acetate is not an option.