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Alternative Fixation Procedures for the Inactivation of Dry, Bioforensic Samples for Examination by Electron Microscopy

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Inactivation of potentially infectious microorganisms in dry bioforensic samples poses numerous challenges for microscopists. Dry bioforensic samples may contain components that will dissolve in traditional aqueous fixatives, bacterial endospores that are difficult to inactivate, hydrophobic components that do not mix with aqueous fixatives, or components that may react with aqueous fixatives. Bioforensic samples can vary in their composition and may contain: purified bacterial cultures, tissue culture cell pellets, solid surfaces, aqueous solutions, non-aqueous solutions, dry cakes or powders, water soluble samples or unknown organisms. Destructive procedures used to inactivate/sterilize many types of bioforensic samples can destroy sample morphology, change sample chemistry, selectively remove soluble ions, and disturb overall sample integrity. Harsh chemicals such as ozone or hydrogen peroxide [1] readily cause inactivation, but at the expense of losing ultrastructural preservation [2]. Numerous techniques for surface sterilization are documented for use in hospital settings. These include sterilization with gases such as ethylene oxide [1], formaldehyde vapor or solutions such as 10% bleach or quaternary ammonium compounds [3]. The disinfection method of choice for medical devices for many years has been a 2% glutaraldehyde solution for 20 minutes [4, 5]. Therefore alternative inactivation procedures were implemented for use on dry samples to preserve the cellular ultrastructure and overall sample morphology for electron microscopy (EM).

The purpose of this study was to develop and demonstrate methods for the inactivation of potentially infectious organisms in dry samples for both scanning (SEM) and transmission (TEM) electron microscopy analysis. It is currently necessary to treat specimens contaminated with bacteria for extended periods of time with chemical fixative solutions to assure inactivation. This treatment can have detrimental effects not only on the sample composition, but also on cellular ultrastructure. This study examined procedures using a non-aqueous osmium tetroxide solution or an osmium tetroxide vapor for the inactivation of bacteria while preserving sample integrity. Dry samples were fixed with either osmium tetroxide vapor from crystals, or 1% osmium tetroxide in 100% ethanol. For a control, a portion of the dry sample was treated with 4% paraformaldehyde and 1% glutaraldehyde in 0.1 M sodium cacodylate buffer (a traditional aqueous fixative solution) [6].

Samples from all three treatments (osmium vapor, osmium in ethanol, and traditional fixative) were examined via SEM and/or TEM for any visible differences in ultrastructure. Fixation in traditional aqueous fixative readily preserves cellular ultrastructure, but all soluble portions of the sample matrix dissolve. Fixation with osmium tetroxide vapor rapidly inactivates samples for SEM analysis, preserving extra-cellular and sample matrix material for imaging and documentation. Fixation in non-aqueous liquid 1% osmium tetroxide in 100% ethanol provides a rapid method for inactivation of samples that can be imaged by both SEM and TEM. The vapor and non-aqueous liquid preserved both the cellular ultrastructure and water soluble dry components of the sample. These alternative methods of dry sample inactivation will allow for rapid analysis, preservation of cellular ultrastructure as well as overall sample morphology of dry bioforensic samples.

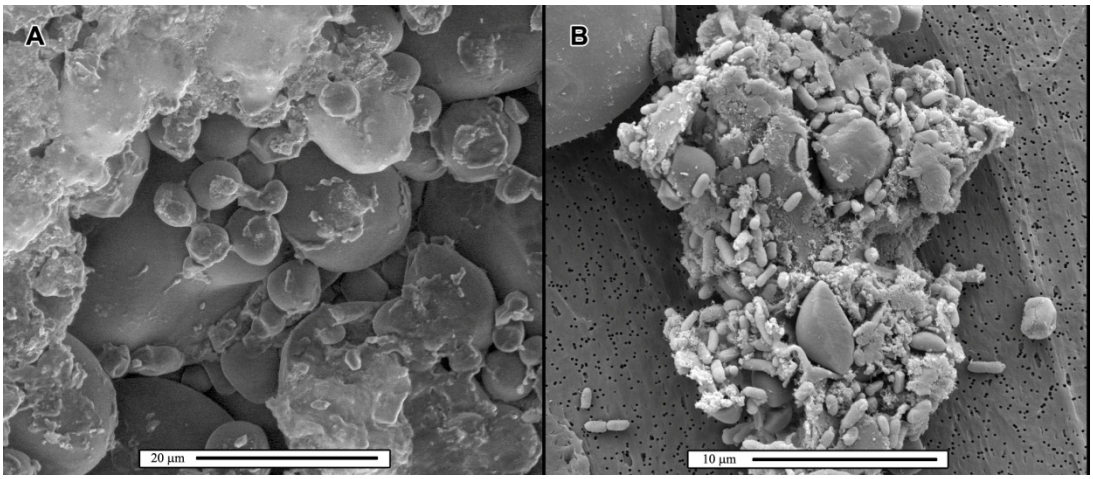

Figure 1. Fixation of casework unknown with associated water soluble matrix with osmium tetroxide vapor. **A** Sample fixed in osmium vapor and imaged uncoated in environmental mode in the SEM. **B** Sample fixed in osmium vapor and subsequently coated with gold/palladium and imaged in a high vacuum environment in the SEM.

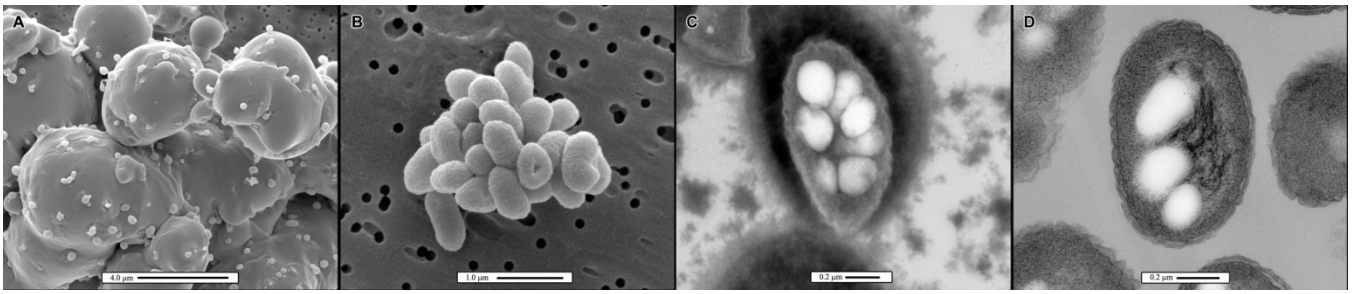

Figure 2. Fixation of *Burkholderia thailandensis* dried samples with osmium tetroxide in ethanol (**A, C**), or traditional fixative (**B, D**). **A** SEM analysis of a dry sample of *B. thailandensis* fixed in osmium/ethanol. **B** SEM analysis of traditionally fixed dry *B. thailandensis*. **C** TEM analysis of osmium/ethanol fixed dry *B. thailandensis*. **D** TEM analysis of traditionally fixed *B. thailandensis*. Note the disappearance of the water soluble portion in **B** and **D**.

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