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Comparison of Cryo-fixation Methods used for Enhanced Ultrastructural Preservation and Immunogold Labeling of Biological Specimens.

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In biomedical research, immuno electron microscopy is a vital tool for high-resolution molecular localization. To obtain adequate immunolabeling, the antigens of interest need to be preserved for antibodies to recognize, and the ultrastructural integrity needs to be maintained to allow interpretation of the labeling. Various cryo-techniques have been described and proven to be advantageous as sample preparation methods for immunolabeling compared to the conventional chemical fixation and resin embedding. Among these techniques, cryo-fixation by high pressure freezing (HPF) is preferred as it allows for specimens of up to 300 μm in thickness to be frozen without detectable ice crystal formation and subsequent damage (Studer et al., 1989, Dahl & Staehelin, 1989, Vanhecke et al, 2008).

Recently, Leunissen and Yi (2007 and 2009) developed an alternative cryo-fixation method termed Self-Pressurized Rapid Freezing (SPRF). SPRF provides comparable ultrastructual preservation to that of HPF. The simplicity of the SPRF method means it has the potential for becoming a widely used sample preparation method for ultrastructural investigation of biological materials.

In this abstract, we evaluate the potential of SPRF as a cryo-fixation technique for immuno electron microscopy. Bacterial spores, mammalian cell suspensions, and multi-cellular organisms will be preserved by plunge freezing, SPRF and HPF methods. Subsequently, the SPRF and HPF samples will be cryo-substituted and embedded in either Lowicryl or LR White resins for detailed ultrastructural analysis and immunogold labeling. The initial results in *Bacillus subtilis* cryo-fixed by SPRF (without prior chemical fixation) and cryosubstitued in a medium which is generally compatible with immunogold labeling, has shown an adequate ultrastructure quality (Figure 1). The bilaminar structure of the membranes surrounding the forespore (arrowhead) and the development of the engulfing mother cell membrane (arrow) are well preserved. Immunogold labeling of the GFP-tagged SpoIIIAG protein and of the FLAG-tagged SpoIIIAH protein will be performed on both SPRF and HPF samples to compare the quality of labeling.

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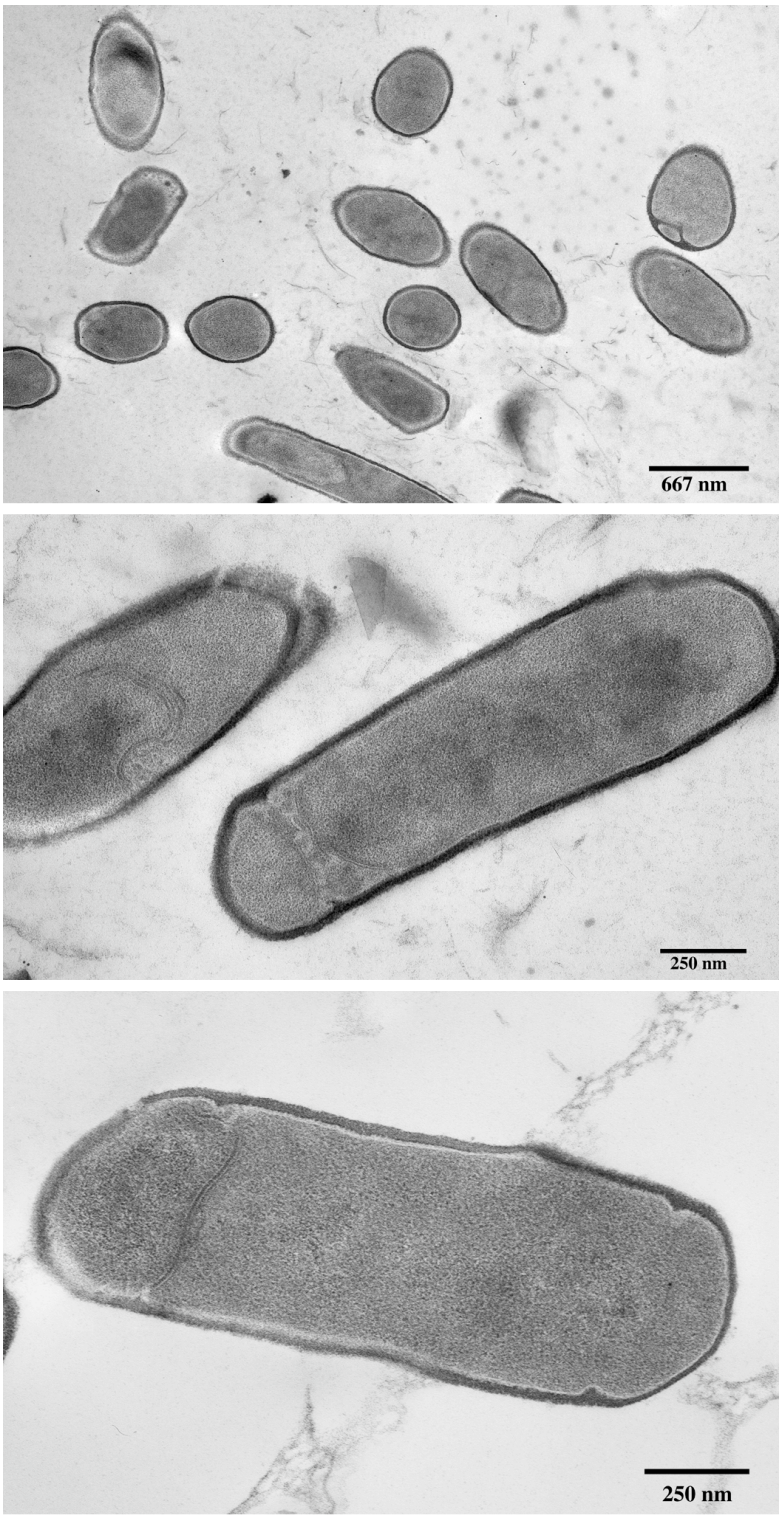

Figure 1: Bacterial cells and forming spores cryo-preserved in liquid nitrogen with the Self-Pressurized Rapid Freezing method, cryo-substituted in acetone and embedded in LR White resin. Substitution media used contained 2% uranyl acetate

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