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Inactivation of Bacterial Endospores in an Artificial Tissue for Electron Microscopy Analysis

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Removal of tissue samples that contain, or potentially contain, pathogenic organisms from containment laboratories for electron microscopy analysis poses unique challenges. While the extraction of nucleic acids or proteins for molecular biology or immunology analysis is fairly straightforward, these methods of extraction destroy ultrastructure, and are not suitable for samples intended for microscopy. Fixation of tissue samples containing pathogenic organisms traditionally requires several days to inactivate the organisms. Furthermore, sterility testing for pathogenic organisms can take from 2-21 days, depending on the organism. This lengthy fixation with sterility testing is not feasible for the rapid turnaround required for forensic samples. Previously, data demonstrated that treatment of bacterial endospores with 4% paraformaldehyde/1% glutaraldehyde for 240 minutes inactivates most bacterial endospores. [1]

The objective of this study was to deduce the time required to inactivate bacterial endospores in an artificial tissue matrix. *Bacillus subtilis* endospores were used for this experiment due to their known resistance to inactivation [1]. Endospores were embedded in an artificial tissue (4% agarose/20% beef liver) prepared as either 1 cm^3 or 0.5 cm^3 cubes. These sizes were used to mimic the maximum size of tissue pieces typically collected for light and electron microscopy. The cubes were treated with universal EM fixative (4% paraformaldehyde/1% glutaraldehyde) for the following times 1, 2, 4, 8, 24, 48, and 72 hours, and 4, 5, 6, and 7 days (data from days 4-7 is not shown on the graph). Following fixation, the cubes were washed with buffer three times, ground, and plated to enumerate viable spores. Inactivation of the endospores varied for the different sized cubes. The spores in the 0.25 cm^3 pieces were completely inactivated in four hours, while the spores in the 1 cm^3 pieces were completely inactivated in eight hours. The results of these studies will reduce the total time required to fix and inactivate tissue samples to enable their removal from biocontainment. Future expansion of this work will include the timed inactivation of several species of endospores, including *Bacillus anthracis*.

References

[1] CA Brantner, et al, Inactivation and ultrastructure analysis of *Bacillus* spp. and *Clostridium perfringens* spores. *Microscopy and Microanalysis.* 20 (2014), 238-244.

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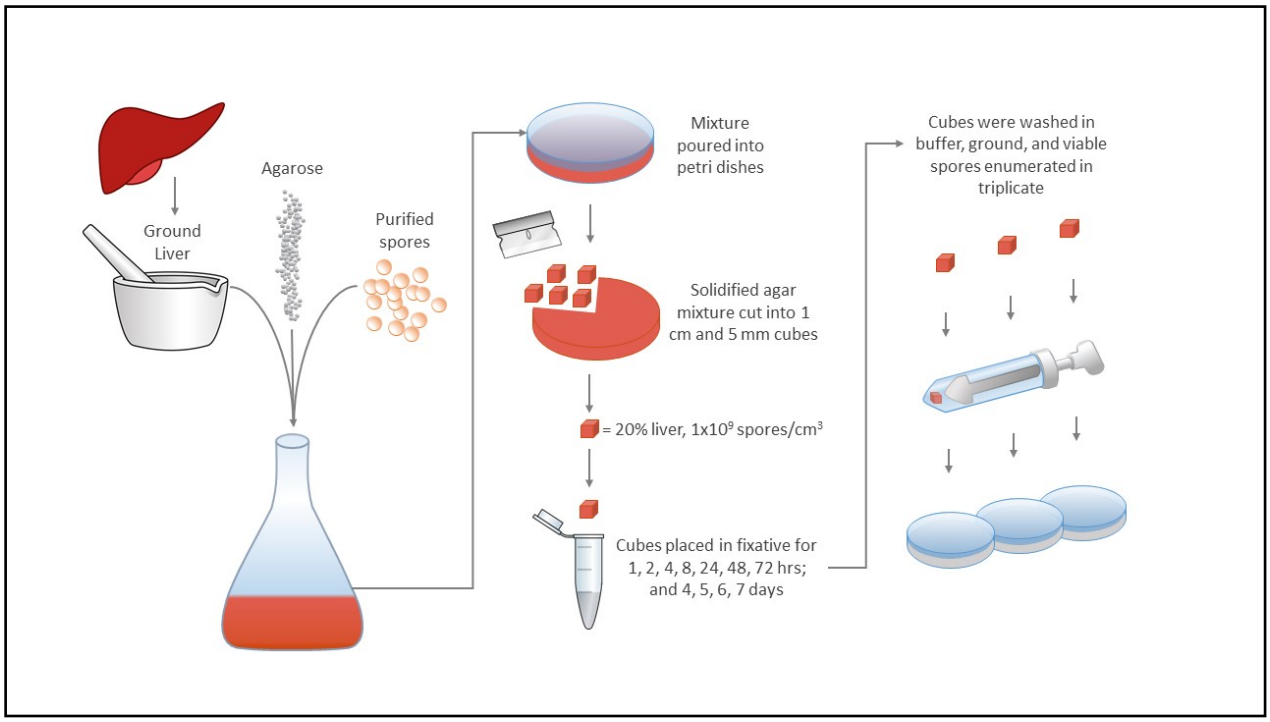

Figure 1. Procedure for the production of artificial tissue (4% agarose/20% beef liver) containing *Bacillus subtilis* endospores. The artificial tissue was placed into fixative for various times, washed with buffer, ground and the spores enumerated for viability.

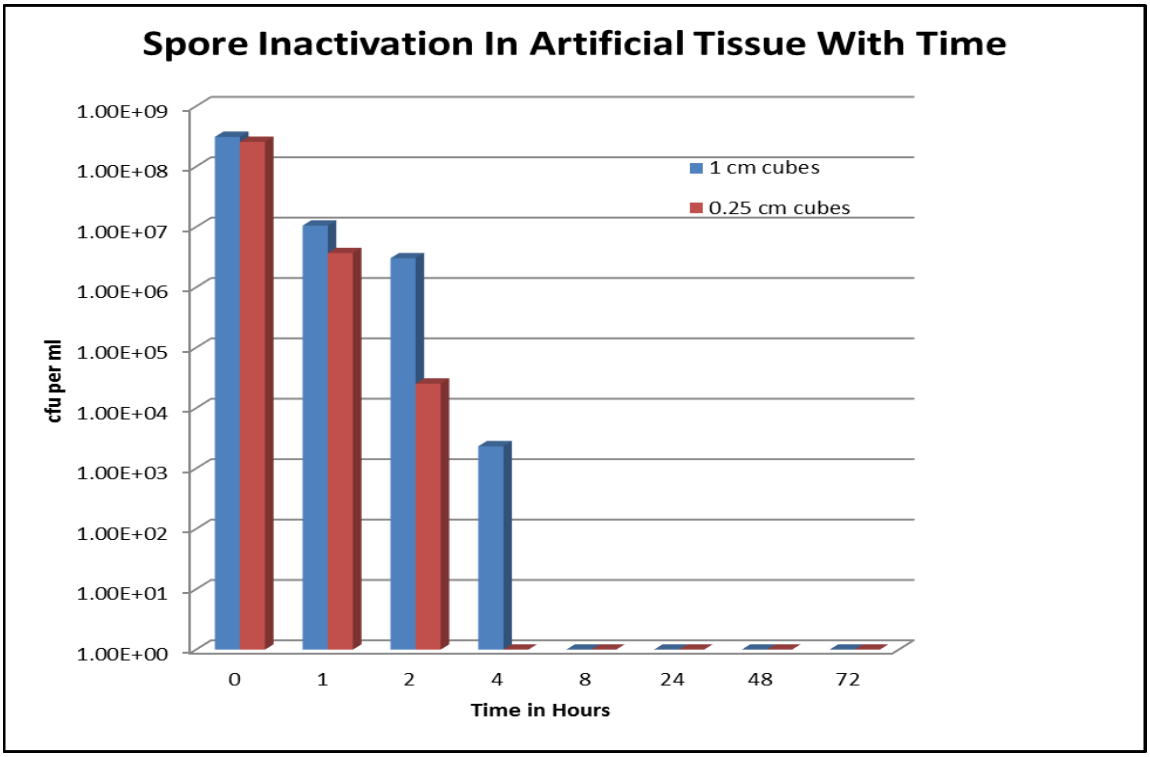

Figure 2. Decrease in spore viability after timed treatment in universal EM fixative solution. Spores in artificial tissue were fixed for times ranging from one hour up to seven days (days 4-7 not shown on graph). The time for inactivation varied for the different sized pieces of artificial tissue with the spores in 0.25 cm^3 cubes being inactivated in four hours and the spores in 1 cm^3 cubes inactivated in eight hours.

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