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Bioforensic Microscopy Analysis of Infectious Organisms

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Bioforensic light and electron microscopy imaging is no different from traditional imaging. The challenges are getting samples, potentially containing infectious biological organisms, out of containment laboratories for processing and imaging. Biological Safety Level (BSL)-3 or BSL-4 laboratories are utilized for the processing and research on materials containing high consequence infectious bacterial and viral pathogens. Microscopes inside BSL-3 or BSL-4 containment laboratories require additional safety precautions to ensure the organism remains contained. At a minimum, vacuum systems must have HEPA filtration, and there must be ways to decontaminate microscopes for servicing. For these reasons, imaging is significantly less burdensome in BSL-1, or BSL-2 laboratories. To performing microscopy on potentially infectious biological material outside of BSL-3 or BSL-4 laboratories requires the material be proven sterile prior to imaging at the BSL-1 or BSL-2 levels. Processes for inactivation must be developed, validated, and implemented following CDC guidelines [1].

Inactivation of *B. anthracis* was validated for this study, but the procedure is similar for all bacteria and viruses. Samples are fixed with 4% paraformaldehyde with 1% glutaraldehyde; the fixative removed; and the entire sample placed into culture broth. Subsequently, the broth was plated to agar media to examine for any potential growth. For an approved validation following CDC guidelines, one set of inactivated samples must be sterility tested, while a second set of samples is spiked with viable organism (*B. anthracis* spores in this case) to demonstrate that the end-product of fixation (inactivated *B. anthracis* cells or spores) does not inhibit growth of organisms that might be present. Once validated, future samples must have at least 10% of the sample volume sterility tested to demonstrate that the material is sterile. This method has been demonstrated to inactivate vegetative cells and bacterial spores and to preserve ultrastructure [2]. This type of validation of inactivation must be repeated for other genera of bacteria and viruses. Examples of hazardous organisms that have been removed from containment laboratories, and imaged, are listed in Figure 1.

References:

[1] Guidance on the Inactivation or Removal of Select Agents and Toxins for Future Use. https://www.selectagents.gov/compliance/guidance/inactivation/index.htm.
[2] CA Brantner, et al, Microscopy and Microanalysis. 20 (2014), p. 238.
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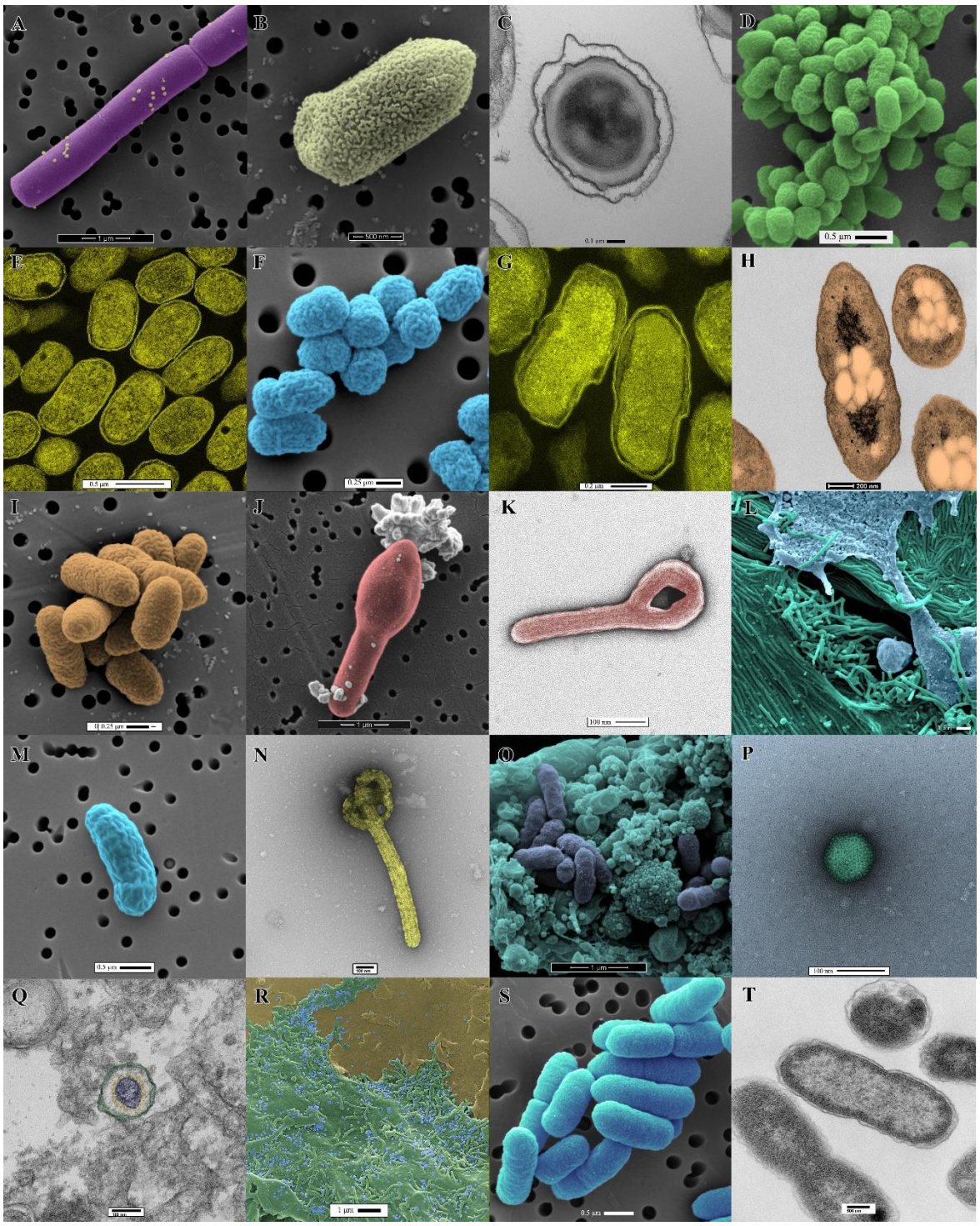

Figure 1. Scanning and Transmission Electron Microscopy images of A: *Bacillus anthracis* vegetative cells with attached bacteriophage, B-C: *Bacillus anthracis* spores, D-E: *Brucella abortus*, F: *Brucella melitensis*, G: *Brucella suis*, H-I: *Burkholderia pseudomallei*, J: *Clostridium botulinum*, K-L: Ebola

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melitensis, G: *Brucella suis*, H-I: *Burkholderia pseudomallei*, J: *Clostridium botulinum*, K-L: Ebola virus, M: *Francisella tularensis*, N: Marburg virus, O: *Rickettsia prowazekii*, P: Rift Valley Fever virus, Q-R: Severe Acute Respiratory Syndrome Corona virus-2, S-T: *Yersinia pestis*.

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