Video Article

Principles and Practices for Safe Manipulation of Pathogenic Mycobacteria in a BSL-3 Environment Part 3

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Abstract

Mycobacterium tuberculosis, one of the most successful bacterial pathogens, is transmitted by aerosol. Despite the prevalence of tuberculosis infection, estimated to include one third of humanity, our knowledge of the biology of the pathogen is limited. In order to hasten the development of new therapeutics, a deeper understanding of how tuberculosis is able survive and thrive in the host is urgently required. The safe experimental manipulation of *M. tuberculosis* requires the use of proper techniques in a BSL-3 laboratory.

In these three protocols we demonstrate the proper techniques required to safely perform microbiological and molecular biological experiments on *M. tuberculosis* in a BSL-3 laboratory. The core principles of minimization of the generation of infectious aerosols and redundant containment and decontamination are emphasized. Specific topics include the use of personal protective equipment, setting up the biosafety cabinet, inoculation of cultures, plating bacteria, picking bacterial colonies, centrifugation, sonication, electroporation, fixation of cultures, disposal of contaminated items, and decontaminating the biosafety cabinet.

Video Link

The video component of this article can be found at http://www.jove.com/video/3050/

Protocol

1. Taking Down the Biosafety Cabinet

1. Dispose of all waste by rinsing with vesphene inside and out; dispose of waste bottle.
2. Rinse all surfaces of reusable racks with vesphene; place in save bag and seal with a rubber band.
3. Open vortex bag; wipe cord and openings; drop cord out of cabinet.
4. Create a clean area; wipe all items to be saved (pipette tip boxes, media, etc.) to clean area; change gloves.
5. Remove vortex mixer from bag; prepare outer save bag with label "save" name and date.
6. Wipe inner save bag into outer save bag; seal and wipe opening.
7. Wipe remaining items to be saved out of cabinet.
8. Seal and wipe pipette waste; put on a second sleeve to close.
9. Move all items to side with waste bag; pour vesphene pot on absorbant pads; wipe all surfaces of pads and dispose.
10. Wet wiper with vesphene, wipe all surfaces of interior of cabinet on clean side.
11. Wipe each item to be saved to clean side, dispose of all disposables.
12. Seal waste bag and wipe to clean side; wipe remainder of cabinet; change gloves.
13. Wipe and remove waste to waste barrel. Seal large waste bag, remove vesphene pot and heat blocks and rinse.
14. Wet water wiper and wipe interior of cabinet.
15. Wet ethanol wiper and wipe interior of cabinet.
16. Plug in pipette aid charger, distribute remaining items evenly, close screen and turn on UV light.
17. To exit BSL-3, spray feet and hands/arms with vesphene, remove outer gloves and sleeves.

2. Spill Recovery

1. If there is a spill inside the cabinet, cover with blue towel, soak with vesphene change gloves, close screen, and return in 1 hour.
2. If there is a spill outside the cabinet, hold breath, inform colleagues, cover with towels and soak with vesphene, leave containment lab and notify biosafety coordinator.

Discussion

The following are critical principles to which you should strictly adhere:
All viable cultures must be double contained before removal from the biosafety cabinet.
Once a viable culture is opened in the cabinet, the cabinet is "dirty" and all items must be double wiped before removal from the cabinet.
Outer "dirty" gloves are removed after the first wipe and no "dirty" item may be touched when entering with clean gloves for the second wipe, only the vesphene towel and the once wiped items.

Disclosures

No conflicts of interest declared.

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