PROTOCOL:

Critical Point Drying (CPD) with the E3100

KIRSCH NOTES
PROTOCOLS & TIPS FOR MICROSCOPY

Critical point drying is an established method of dehydrating biological tissue prior to examination in the Scanning Electron Microscope (SEM).

What to Do Before Starting

- Make sure all valves are closed, including the CO_2 tank.
- Place processed specimens in designated CPD holders and in final dehydrant.

NOTE #1: Dehydrants, especially acetone/propylene oxide, are volatile and specimens cannot be allowed to dry **at Step #3.1**.

Procedure

1. Turn on **COLD** water and run through the unit. Wait until it gets to 20˚C or less.
2. Unscrew the back threaded cap and remove specimen boat from the chamber.
3. Quickly load specimens into boat and insert boat into the chamber.
4. Thread the cap on until tight!
5. Open the CO_2 inlet valve on the top of the unit.
6. Open valve on the top of the CO_2 tank all the way.
7. Open the top exhaust valve slightly and leave open while observing the level of the liquid CO_2 through the front guarded viewing window.

NOTE #2: Always observe the level of the CO_2 and never let it fall below the level of the specimens.

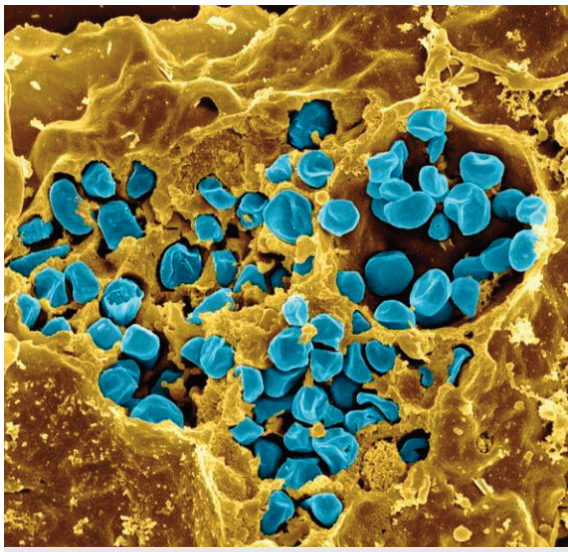
8. When the liquid fills the chamber, close the exhaust valve and wait 5 minutes.
9. Carefully open the bottom exhaust valve and let gas escape for 2 minutes or until the odor of the final dehydrant is no longer detectable. **See Note #2 above!**
10. Wait 5 minutes and then repeat Step #9 two more times, for 2 minutes each, so that the specimens have been flushed a total of three times.
11. Close ALL valves securely, including the CO_2 tank valve.
12. Start running warm water through the unit to increase the chamber temperature.
13. The temperature and pressure should rise past the critical point of 31.1˚C and 1071 psi.
14. Let the chamber reach 40˚C and 1200 psi and wait 15 minutes for biologicals, or 30 minutes minimum for MEMS.

NOTE #3: If the desired pressure, 1200 psi, is achieved before the temperature, carefully bleed off some pressure very slowly by opening the top valve. Never let the pressure exceed 1300 psi.

15. Open the top exhaust valve slightly and bleed off the pressure at a rate of about 100 psi/min.

NOTE #4: Be sure to maintain the critical temperature with warm water!

16. Once the chamber has reached atmospheric pressure, open the threaded cap, remove samples and store in the desiccators until needed.



Scanning electron micrograph of a murine macrophage infected with Francisella tularensis strain LVS. Macrophages were dry-fractured by touching the cell surface with cellophane tape after critical point drying to reveal intracellular bacteria. Bacteria (colorized in blue) are located either in the cytosol or within a membrane-bound vacuole.

Courtesy: National Institute of Allergy and Infectious Diseases

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