INSTRUCTIONS FOR EMS 850 CRITICAL POINT DRYER

SPECIMEN PREPARATION BEFORE DRYING:

1. Dehydrate in acetone or ethanol series (20, 30, 40, 50, 60, 70% [overnight if necessary in this percentage], 80, 90, 100, 100, 100 at 10-15 minutes for each change. If fixing in FAA, begin the dehydration series with 50% ethanol.
2. 50:50 acetone (or ethanol): amyl acetate - 15 minutes.
3. Three (3) changes of amyl acetate - 15 minutes each.
4. Critical Point Dry: You may decide to critical point dry directly after the acetone or alcohol series.
5. NOTE: Rather than use the critical point drying technique, one could impregnate the tissues with HMDS (hexamethyldisilizane) after 100% ethanol. Two changes of HMDS at 5 minute to 1 hour each followed by air drying IN THE HOOD!

OPERATING CRITICAL POINT DRYER (CPD)

1. Make sure that valves #2, #3, #4, and #5 on the CPD and the valves on both liquid CO₂ tanks are CLOSED.
2. Make sure chamber lid is loose by removing nuts. Chamber pressure should be reading zero (0).
3. Turn on the power with the switch located on the back right of the CPD.
4. Precool the chamber:
   a. OPEN valve on CO₂ Tank A.
   b. OPEN cool valve (#2) and establish a steady flow of CO₂. If when you open the cool valve (#2), there is spitting and sputtering, the cool valve (#2) should be opened just a little more.
   c. Allow the chamber to cool until the gauge on the right of the panel reads 5°C. You may have to tap the temperature gauge occasionally as it tends to stick.
   d. Once the temperature reads 5°C or lower, gently CLOSE the cool valve (#2), and the temperature will coast down to about 2°C or less.
   e. CLOSE the valve on CO₂ Tank A.
5. Transfer your specimens to the chamber on top of the apparatus:
   a. Remove the top plate.
   b. Insert your specimens. Use one of the holders if necessary.
   c. Tighten the 3 nuts in a rotational manner. Finger tight is plenty.
6. Fill the chamber with liquid CO₂.
   a. Make sure all CPD valves are gently CLOSED.
   b. OPEN valve on CO₂ Tank B.
   c. OPEN the inlet valve (#3) slowly while observing the fluid level in the small viewing window below the chamber.
   d. FILL the chamber until the fluid is at the top of the window, even with the top inlet hole.
   e. CLOSE inlet valve (#3).
7. Allow the specimens to soak for 3 minutes (perhaps longer for large samples). While the tissue is soaking, turn on the toggle switch for the magnetic stirrer to enhance solvent exchange.

8. Drain/Fill Cycle:
   a. Turn the magnetic stirrer OFF.
   b. Slowly OPEN the exhaust valve (#4) while observing the fluid level in the viewing window. The fluid level will drop. Drain until the fluid level reaches the bottom of the viewing window but not out-of-sight.
   c. REFILL the chamber by OPENING inlet valve (#3). Fluid level should now be at the top.
   d. Repeat this drain/fill step 3 times and end with the chamber FILLED (fluid level is at the top of the window).

9. NOTE: IF THE CHAMBER TEMPERATURE CLIMBS MUCH ABOVE 5°C DO THE FOLLOWING:
   a. Make sure all valves are CLOSED including both CO₂ tank valves.
   b. OPEN valve on CO₂ Tank A. CO₂ Tank B valve should be closed!
   c. OPEN cool valve (#2) and cool chamber to below 5°C (essentially repeating step 3 above).
   d. CLOSE cool valve (#2).
   e. CLOSE valve on CO₂ Tank A.
   f. OPEN valve on CO₂ Tank B.

10. Repeat steps 7 and 8 (Soak and Drain/Fill Cycle) however during the final draining (purging), check to see that the solvent exchange has been achieved:
    a. Hold a small piece of filter paper in the stream of exhausting gas. If a small damp patch appears on the filter paper, solvent is still present in your sample and you must repeat steps 7 and 8 again. If no spot appears proceed to the next step.

11. Make sure the fluid level is again at the very top of the viewing window. CLOSE ALL VALVES INCLUDING THOSE ON BOTH CO₂ TANKS.

12. Switch on the heater and allow chamber conditions to reach approximately 1150 psi and 33°C. In certain cases, the chamber pressure may not reach the 1150 psi. However, be sure to go to 33°C.

13. Turn OFF the heater. SLOWLY depressurize the chamber using the bleed valve (#5). For less delicate specimens depressurize the chamber at a rate of 100 psi/min. For more delicate specimens, depressurize the chamber at a rate of approx. 50 psi/min which equals 100 psi/2 min.

14. Once chamber pressure reads zero, OPEN chamber and remove specimens. The specimens are very dry at this point and will take up moisture so mount them on a stub and sputter coat as soon as possible or leave un-coated specimens in a desiccating cabinet.

15. Turn the power OFF (switch at back right of CPD).

16. Finish by leaving the valves (#2, #3, #4, and #5) on the CPD OPEN. Make sure that both CO₂ Tank valves are CLOSED!

17. Record your usage information in the log book.

v.3