**Drying specimens using HMDS (hexamethyldisilazane), protocols for use in the Nelson Laboratory at Brigham Young University**

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We often need to dry small specimens, mostly of Diptera, for pointing from 70% ethanol. Here is an outline of procedures that have worked well in the past. If you have suggestions or modifications, please pass them on to me. This is a more detailed version of a protocol outlined by Brown (1993).

We collect most of our specimens in 70% ethanol. To dry these specimens so they don't shrink, shrivel, and distort they need to be run through a dehydration series of solutions. If simply dried from water the shear forces from the high surface tension of water distorts the specimens, crinkles the wings, and collapses the heads. We gradually remove the water by increasing the concentration of ethanol in the specimens. Then the specimens are transferred to a less polar, or non-polar solvent, such as ethyl acetate, acetone, or HMDS (hexamethyldisilazane). The resulting specimens don't have collapsed heads or other body features and this makes them much more valuable research specimens. Remember that HMDS costs about $50.00 for 250 ml and ethyl acetate less than $15.00 for 500 ml. Ethanol costs about $5.00 for 500 ml. Dasani water costs about $1.00 for 500 ml (for comparison). So be frugal yet efficient in your use of these solvents.

One of two basic protocols is followed. Your choice of protocols is based on the size of the specimens.

**Small specimens:**

If the specimens are small (up to about 5 mm) then:

Store unpinned specimens in 70% ethanol. Always keep the locality label unambiguously associated with the specimens.

Move to 85% ethanol for at least a half hour.

Move to 95% ethanol for at least a half hour.

Move to 100% ethanol for at least a half hour.

Specimens in each of these concentrations of ethanol can be stored for a long time (overnight or more) if necessary, but they are more fragile than those in 70% ethanol, so don't shake them unnecessarily.

Now work under a fume hood (Barr 2001)

Move to HMDS for 1 hour

Move to a second change of HMDS for a half hour.

Pour the specimens gently on a blotter paper in a petri dish and allow the fumes of HMDS to volatilize, this will take less than 15 minutes, usually.

Cover the dish of specimens, with appropriate locality labels, and move to a secure museum drawer for storage until time for pointing on insect pins.

Many (up to hundreds) specimens can be processed together in one vial (don't put the locality label in with the HMDS) if their legs and antennae are not tangled.

Keep track of the number of times the changes of ethanol and HMDS have been used by putting hash marks on the storage container. When about five batches of specimens have run through a given amount of solvent in a container dispose of it properly Ethanol can simply be strained and put in the waste ethanol container or used as the next more dilute alcohol concentration (for example waste 70% is strained into the big container; waste 85% is used as the next batch of 70%; waste 95% is used as the next batch of 85%, and so on). HMDS should be stored separately in a clearly labeled WASTE HMDS container.

**Large specimens:**

If the specimens are large (greater than about 5 mm) then:

Store unpinned specimens in 70% ethanol. Always keep the locality label unambiguously associated with the specimens.

Pin the specimen following standard pinning procedures, that is to say on the proper size pin, offset slightly to the right and balanced correctly both front to back and side to side. Just use regular pinning procedure but the specimen is wet.

Poke the pin into the weighted foam circle. Make sure the appendages from this specimen are not tangled with those of adjacent specimens. Quickly adjust wings and legs so they are as best as can be arranged without prop pins. Try to keep the appendages rather compact to the body, but far enough away that the sides of the main body could be visible. Choose the best specimens for pinning, meaning, in part, that the wings are entire and are best if held at an angle upright and projecting slightly away from the thorax. Make sure the legs are high enough that they won't break off when the locality label is placed on the individual pins. From time to time you might want to use a prop paper to hold up the legs during drying.

The foam pinning circle can now be moved into the appropriate bottle of solvent. Fill the bottle with solvent so that the specimen (and wings) is immersed but the pin heads are not immersed.

Move to 85% ethanol for at least a half hour. Longer if the specimens are dense.

Move to 95% ethanol for at least a half hour.

Move to 100% ethanol for at least a half hour.

Specimens in each of these concentrations of ethanol can be stored for a long time (overnight or more) if necessary, but they are more fragile than those in 70% ethanol, so don't shake them unnecessarily.

Now work under a fume hood (Barr 2001).

Move to ethyl acetate for 1 hour. Do not store specimens longer than about 5 hours in ethyl acetate. Longer times in the ethyl acetate discolors the specimens.

Lift the foam pinning circles out and let the ethyl acetate volatilize away under the fume hood until dry. The specimens usually become more colorful or at least variegated when the fine hairs of the body are all dried sufficiently. Blow on the specimen to fluff up the hairs.

Move the individual pinned specimens into a museum unit tray with an appropriate header locality label.

Move the unit tray into a covered museum drawer for storage until individual labels can be affixed to the pins.

Keep track of the number of times the changes of ethanol and ethyl acetate have been used by putting hash marks on the storage container. When about five batches of specimens have run through a given amount of solvent in a container dispose of it properly. Ethanol can simply be strained and put in the waste ethanol container or used as the next more dilute alcohol concentration (for example waste 70% is strained into the big container; waste 85% is used as the next batch of 70%; waste 95% is used as the next batch of 85%, and so on). Ethyl acetate should be stored separately in a clearly labeled WASTE ETHYL ACETATE container. This ethyl acetate is perfectly adequate for use in "dry" kill jars.

By following these procedures excellent, often better and cleaner than fresh pinned, specimens can be obtained. Make suggestions on how this protocol (or its wording) can be improved.

Literature Cited

Barr, B. 2001. A word of caution when using Hexamethyldisilazane. Fly Times 26: 11.

Brown, B.V. 1993. A further chemical alternative to critical-point-drying for preparing small (or large) flies. Fly Times. 11: 10.

Revision: 21nelson drying insect specimens 2009.docx, 29 August 2011, CRN.