

GENERALIZED PROCEEDURE FOR PREPARING MITES FOR MICROSCOPIC EXAMINATION

First, and Foremost, respect the person being asked to identify the specimen, be it yourself or others, by preparing a proper slide, with the expectation of excellence.

Assemble the tools

Invest in a few pairs of excellent jeweler's forceps, extra fine (best purchased from Jeweler's supply houses)

At least 2 pin vices (available from BioQuip)

Assemble a number of small to very small Pyrex dishes, be prepared to make them

Mug warmer

Ultra Sonic cleaner

Hot plate

Drying oven or slide warmer

Slides

Cover slips

The Chemicals

85% Lactic Acid (Potassium Hydroxide as a back-up)

Household Ammonia or DeCon 90 (later apparently available only in the UK)

Distilled water

70% ETOH

90% ETOH

95% ETOH

100% ETOH

Xylene and/or HistoClear (Xylene requires a hood)

Mounting medium (Permount or Canda Balsam)
Ringing compound

Problems

The concept of diffractive index

Bubbles

Incomplete desiccation

Specimens that resist clearing

The Question of Hoyer's

Specimens go through 4 main steps

1. Cleaning and clearing
2. Desiccation
3. Embedding
4. Finishing

Each step is detailed and exacting.

STEP 1 – Cleaning and clearing

Select a number of specimens to start with. Some will always be damaged, suffer bubble problems or be the wrong stage or sex.

Clean and degrease selected specimens in Ammonia bath in Ultra Sonic cleaner.

Place specimens in straight Lactic Acid for several days, until clear. May be speeded up by gentle heating, but caution required. Use KOH (cold) only for large or difficult to clear specimens. Specimens may be gently “squeezed” to help process along.

Once clear, wash specimens through an Ammonia bath, followed by several of clean water, each in the Ultra Sonic cleaner.

STEP 2 – Desiccation

Remove all water from the specimen by the following process

Transfer to 70% ETOH, and change once.

20 minutes each successively in 2 90% ETOH baths, 2 95% ETOH baths, 2 100% ETOH baths and 2 HistoClear baths. **DO NOT USE ULTRA SONIC CLEANER TO SPEED THIS UP.**

STEP 3 – Embedding

Choose the best specimens from the pool, those most clear and with no bubbles

Center a drop of non-water based mounting medium (usually Permunt) on clean slide. **DO NOT INTRODUCE BUBBLES**

Remove specimen from bath, blot gently, and place on center of medium drop – **DO NOT** truly dry specimen

Press specimen into medium, and let equalize

At this point, the remaining solvent from the last bath will dissolve the medium around the specimen, and draw it into the specimen through diffusion. This will take some time.

After specimen has equalized, place cover slip on slide – This is an art unto itself

Remove bubbles on a hot plate, do what can be done to improve positioning of specimen

STEP 4 – Finish the specimen

Add labels to slide

Place slide in a dust-free environment on a warming tray or slide oven to dry

When dry, ring specimen with ringing compound.