Pollen Sample Processing

SCD=Stir, centrifuge, and decant. Spills are greatly reduced if first vortexing sample with the residual liquid following the preceding decant, or by first adding only 1 ml of chemical, vortex and stir vigorously, then adding remaining chemical. Little subsequent stirring may be necessary using this method. Centrifuge always for 5 minutes at 2500 rpm.

Note: 5 ml refers to ca. 50% of the tube filled, 8 ml refers to 75% filled. Duplicate KOH and HF steps are rarely needed if using 0.5 ml rather than 1.0 ml of sediment.

Load tubes

1. Add 1 to 3 tablets of exotic (lycopodium) in 15 ml polyethylene tubes w/ little HCl and let it dissolve about 10 min. Note exotic batch number on data sheet.
2. Subsample sediment and add to the tubes in step 1.
3. Add 10 ml dH_2O, SCD.

Potassium hydroxide (KOH) step. Never skip this step.

4. Add 10% KOH (8 ml), heat for 10 min (@ 90°C), and centrifuge and decant.
5. Sieve samples with 200um or 250um sieves (No. 70) using dH_2O into plastic beakers. Save macrofossils if needed. Return to 50 ml tubes and repeat SCD until supernatant is fairly clear. Return to 15 ml tubes. Repeat KOH step if supernatant remains yellow, but always follow KOH step by several water rinses.

Hydrochloric acid (HCl) and Hydrofluoric acid (HF) step. Never skip this step. Use rubber gloves, face mask, and apron.

6. Add 10% HCl (5 ml). Heat samples for about 10-20 minutes in the heat block. If the reaction is too vigorous, add a few drops of 100% TBA and note on data sheet. Add more HCl to check if the reaction is complete. Centrifuge and decant supernatant.
7. Be careful with HF. Do not use glass stir rods. Add 8 ml HF, heat 40 min in heat block. Centrifuge and decant. Decant into waste HF containers.
8. Repeat HF step if necessary, or do a cold HF step overnight (e.g., for sandy samples).
9. Add 10% HCl (6 ml), stir, and heat for 3 min. Centrifuge and decant.
10. Wash w/ dH_2O, SCD.

Acetolysis step. Never skip this step. Use rubber gloves, face mask, and apron. Note: acetic anhydride reacts with H_2O.

11. Add glacial acetic acid (GAA) (6 ml), SCD.
12. Vortex sample with residual GAA.
13. Using repipet dispensers located in the fume hood, add 4 ml acetic anhydride and 0.5 ml sulfuric acid. Stir. **Heat for 3 min. Quench with GAA. Centrifuge for 3 min,** and decant.
14. Add GAA (8 ml), SCD.
15. Wash w/ dH_2O, SCD.

Sodium pyrophosphate (NaPyrP) 7um Nitex sieving step. Skip if clay content is low or samples are highly organic.

16. Turn on water bath in advance, or use hot water. Add 5% warm sodium pyrophosphate (8 ml), stir thoroughly. Set up Nitex sieves by placing mesh within threaded tubes. Sieve using warm NaPyrP. Use the dremel tool to coerce through the mesh.
17. Wash sample from nitex into a clean beaker or back into its test tube with dH_2O. **Centrifuge for 10 min** and decant.
18. Wash w/ dH_2O, stir, centrifuge, and decant.

Staining and dehydration step

19. Add 1 drop of 0.1% safranin stain, stir, and wait for 2 min. USE VERY DILUTE STAIN.
20. Add 95% EtOH (~6 ml), SCD.
21. Add 100% EtOH (~6 ml), SCD.
22. Add warm tertiary butyl alcohol (TBA) (~6 ml)
23. Wash samples in 5ml-vials w/ 1-2ml TBA (may use new, sterile glass Pasteur pipette for each sample). Cap, centrifuge and decant. Use plastic vials in centrifuge tube shields to support glass vials in the centrifuge shields.
24. Add a few drops of 2000 cs silicon oil, cover with filter paper using rubber bands, and let evaporate overnight.

Chemicals safe for stopping overnight or longer:

HF (but in fume hood, with fume hood ON)
Water
GAA
Ethanol
TBA
Sodium pyrophosphate

Chemicals not safe for pollen for extended periods:

HCl
KOH
Acetolysis (under 3 minutes, optimally).

Clean up

Test tubes: Wash in warm soapy water with a soft-bristled bottle brush. Soak in concentrated bleach overnight. Bleach oxidizes the pollen and it is re-usable. Next wash test tubes in warm soapy water. Rinse in tap water until the smell of bleach is gone. Rinse thoroughly with distilled water and let air dry.

Nitex: 7 micron sieves must be cleaned after each usage. They can be either soaked in bleach or put in an ultrasonic bath of soapy water for five minutes to remove pollen. If the bleaching method is used, the sieves must be replaced after every two or three treatments because the bleach dissolves the nitex.

Mixing reagents

Hydrochloric acid (10% HCl)

Measure 77.5 ml of distilled water. To this, slowly add 22.5 ml concentrated HCl, stirring constantly. Work in a fume hood.
Spills: For spills on skin wash immediately under faucet in large quantities of water. Wash spills on lab bench thoroughly with large quantities of water.

Potassium hydroxide (10% KOH)

To make one liter of 10% KOH, weigh out 100 gram of KOH powder or pellets. Add this slowly to approximately 800 ml distilled water, stirring constantly. When dissolved, add sufficient amount of distilled water to make one liter. Work in a fume hood.

Safranin (0.1%)

Measure 0.01 grams safranin powder and add to 10 ml dH_2O. Stir, and pour into eyedropper well.

Waste Storage and Disposal

Decant all chemicals into appropriate containers stored under the hood. Only water and KOH (diluted) should go down the drain.

Contact information for Environmental Health and Safety is posted in the Pollen Processing Room.