

Pollen Processing

John Logan, July 4, 2006

Section 1 – HF bath

Sample preparation

1. Select 12 samples, or other numbers of samples if twelve are not available, to process.
2. Remove tape or other seals from samples.
3. Fill out sample log with processor's name, date, size of sample that will be processed, sample number, provenience information, tracer information – mean count per pill, batch number.

Processor preparation

1. Put on plastic gloves
2. Put on lab coat
3. Put on plastic lab apron
4. Put on safety glasses
5. Make sure to wear long pants and shoes – no sandals or shorts

HF bath

1. Rinse cut-off bottles (numbered 1-12) w dH₂O to clean airborne pollen that may have gotten on them.
2. Fill them with 15ml of 10% HCl. The amount may vary between 10-20ml depending on the sediment aggregate.
3. Add sample – 1, 3, 5 or 15 cc to each bottle
4. Add 1 tracer to each bottle
5. Let sample set until dissolved
6. Rinse screens – 4' square window screen mesh
7. Collect batch of 12 large tubes from wash area
8. Place 12 large tubes into tube holder
9. Collect funnels, as many as possible

10. Swirl sample slurry in bottle
11. Tap on counter top
12. Rinse side of bottle with dH₂O
13. Place screen on top of funnel
14. Place funnel in mouth of large tube
15. Pour sample slurry onto screen, taking care to keep slurry over funnel
16. Fill large tube about $\frac{3}{4}$ full with screened slurry
17. Discard mud from screen into large plastic container
18. Allow mud to dry before disposing in waste basket
19. **DO NOT** put sediment down drain
20. Place centrifuge into fume hood
21. Place tube holder with tubes into fume hood
22. Use beam balance to balance tubes in pairs
23. Place tubes into centrifuge in groups of four
24. Centrifuge each group for 4 minutes
25. Decant the waste HCl/dH₂O into the waste container after centrifuging
- 26. PUT ON FACE SHIELD**
27. Pull plastic bottle (cut-off) from washing area
28. Crush ice from small refrigerator in outer lab
29. Place crushed ice into plastic bottle
30. Add some H₂O, enough to float the ice slightly
31. Put ice bath bottle under fume hood
32. Get bottle of HF from cabinet. **TAKE CARE, HF IS EXTREMELY DANGEROUS, IT CAN KILL YOU**
33. Open HF bottle

34. Hold tube with sample in ice bath
35. Add HF to about $\frac{3}{4}$ inch up tube
36. Set down HF bottle
37. Pull tube from ice bath
38. Hold in air for 2-5 seconds to insure that there is no reaction –heat, smoke. If a reaction starts place the tube back into the holder until the reaction is finished. Clean up the HF residue if needed.
39. If the sample is not reacting, add HF until the large tube is more than $\frac{1}{2}$ full
40. Fill all 12 tubes in this manner
41. Cover the tubes, in the holder, with saran wrap and seal with a rubber band
42. Label the fume hood with the sample group designation, the date and the fact that HF has been added
43. Let the samples sit for at least 48 hours. Each sample will be different, the longer that they sit in the HF bath the better. Some samples may need to sit for a week or more

Clean-up

1. Put cut-off bottles, spoons, and funnels in basin with soapy water
2. Soak for at least 5 minutes
3. Using bottle brushes clean the cut-off bottles, spoons, and funnels
4. Either towel dry them or leave them overnight to air dry
5. Put them away in the appropriate cabinet or drawer

This portion of the sample processing takes approximately **2.5** hours. There is a natural break in the processing here

Section 2 – Transfer to smaller tubes

Processor preparation

1. Put on plastic gloves
2. Put on lab coat
3. Put on plastic lab apron
4. Put on safety glasses
5. Make sure to wear long pants and shoes – no sandals or shorts

Large tubes to the small tubes

1. Find a spare large tube rack
2. Fill it with 12 empty large tubes
3. Get 12 wooden stir sticks
4. Rinse them with dH₂O
5. Place one stir stick into each of the empty tubes
6. **PUT ON FACE SHIELD**
7. Remove the saran wrap from the samples tubes in the HF bath
8. Place an empty tube with stir stick behind each large tube with sample in HF – if it is necessary to move the tubes from one rack to another, **TAKE CARE, HF IS EXTREMELY DANGEROUS, IT CAN KILL YOU**
9. Use beam balance to level the samples, add dH₂O to achieve balance
10. Stir the samples. **TAKE CARE, HF IS EXTREMELY DANGEROUS, IT CAN KILL YOU**
11. Centrifuge the samples in groups of 4 for 4 minutes
12. Decant the waste HF/dH₂O solution into the waste container
13. Fill tubes $\frac{3}{4}$ full with dH₂O
14. Use beam balance to level the samples, add dH₂O to achieve balance
15. Stir each tube

16. Centrifuge the samples in groups of 4 for 4 minutes
17. Decant the waste dH₂O solution into the waste container
18. Add $\frac{3}{4}$ inch of concentrated HCl to tube
19. Add dH₂O to tube until tube is more than $\frac{1}{2}$ full
20. Stir each tube
21. Centrifuge tubes in groups of 4 for 4 minutes
22. Decant the waste solution into the waste container. Note: If samples have a low clay content, steps 23-25 may be skipped.
23. Add dH₂O to tube until tube is approximately $\frac{2}{3}$ full
24. Stir each tube.
25. Centrifuge tubes in groups of 4 for 4 minutes
26. Pull 24 small tubes and the small tube rack
27. Make sure that they are marked 1-12 – two sets
28. Make two rows in the tube rack
29. Using the squirt bottles to rinse the large tubes, transfer the samples into the small tubes. This may involve transferring the sample into 2 or more of the small tubes. The process is dependent on the amount of the sample and whether it rinses easily from the large tube. Use as little H₂O as possible to speed-up the transfer
30. Level the small tubes with dH₂O – they don't need to be weighed
31. Centrifuge the small tubes in sets of 6 for 4 minutes
32. Decant the waste solution into the waste container
33. If there is a layer of silicates in the bottom of the tubes, add approximately $\frac{3}{4}$ inch of HCl to the tube. Stir well
34. Add dH₂O to tube until $\frac{1}{2}$ - $\frac{3}{4}$ full
35. Stir
36. Centrifuge in sets of 6 for 4 minutes

37. Decant the dH₂O/HCl waste into the waste container

38. Add dH₂O, fill the tube approximately $\frac{3}{4}$ full

39. Centrifuge in sets of 6 for 4 minutes

40. Decant the waste into the waste container

Clean-up

6. Put funnels, and large tubes in basin with soapy water

7. Soak for at least 5 minutes

8. Using bottle brushes clean the cut-off bottles, spoons, and funnels

9. Either towel dry them or leave them overnight to air dry

10. Put them away in the appropriate cabinet or drawer

*Note: This section can take from approximately **3.5-5.5** hours to complete, possibly more. The time varies with the ease of the transfer from large tubes to small tubes..*

If needed a break may be taken here. If a break is taken make sure to fill the tubes $\frac{1}{2}$ full with dH₂O and cover them with saran wrap with the batch number and "in H₂O" labeled on it.

Section 3 – Acetolysis

Processor preparation

1. Put on plastic gloves
2. Put on lab coat
3. Put on plastic lab apron
4. Put on safety glasses
5. Make sure to wear long pants and shoes – no sandals or shorts

Acetolysis

If a break was taken:

1. Level with dH₂O.
2. Centrifuge tubes in sets of 6 for 4 minutes

If no break was taken, begin with Step 3.

3. Set up Potassium Hydroxide – KOH bath.
4. Pull hot plate from cabinet
5. Place in fume hood
6. Plug in
7. Set on “Continuous”
8. Place container filled $\frac{3}{4}$ full with H₂O on hot plate
9. Place bottle with dH₂O in H₂O in container – be sure to take top off of bottle and place loosely on top of bottle. **CONTENTS WILL BOIL AND SPRAY ALL OVER OTHERWISE**
10. Place bottle with KOH in H₂O in container – be sure to take top off of bottle and place loosely on top of bottle. **CONTENTS WILL BOIL AND SPRAY ALL OVER OTHERWISE**
11. Get beaker with graduated to at least 120 ml
12. Place beaker in fume hood

13. Fill beaker with 120 ml of glacial acetic acid – $C_2H_4O_2$
14. Set pipette to 4.7 ml
15. Pipette 4.7 ml of $C_2H_4O_2$ into each tube
16. Stir
17. Centrifuge in sets of 6 for 4 minutes
18. Decant waste solution into waste beaker. **DO NOT POUR INTO WASTE CONTAINER**
19. Pour 5 ml of Acetic Anhydride – $(CH_3CO)_2O$ into each tube. Push on squeeze bottle until liquid passes the 5mL mark on bottle spout (holding upright) then pour
20. Stir
21. Set pipette to 0.55 ml
22. **PUT ON FACE SHIELD**
23. Pipette 0.55 ml of H_2SO_4 into tubes – with acetic anhydride still in them. **TILT TUBE AWAY FROM FACE.** Stir well. Be aware of the possibility of reaction with any H_2O that may have condensed in the H_2SO_4 . If the sample reacts it may be **explosive**.
24. Centrifuge in sets of 6 for 4 minutes
25. Decant waste solution into waste beaker. **DO NOT POUR INTO WASTE CONTAINER**
26. Set pipette to 4.7 ml for final $C_2H_4O_2$ wash.
27. Pipette 4.7 ml $C_2H_4O_2$ into each tube
28. Stir
29. Centrifuge in sets of 6 for 4 minutes
30. Decant waste solution into waste beaker.
31. Add Hot dH_2O – fill tubes $\frac{1}{2}$ - $\frac{3}{4}$ full. **USE HOT MITTS. BE SURE TO TIGHTEN TOP – BE AWARE THAT HOT H_2O MAY OVERFLOW FROM TUBE WHEN THIS IS DONE**
32. Stir

33. Centrifuge in sets of 6 for 4 minutes
34. Decant waste solution into waste container
35. Add KOH – fill tubes $\frac{1}{2}$ - $\frac{3}{4}$ full. **USE HOT MITTS. BE SURE TO TIGHTEN TOP – BE AWARE THAT HOT KOH MAY OVERFLOW FROM TUBE WHEN THIS IS DONE**
36. Stir
37. Centrifuge in sets of 6 for 4 minutes
38. Decant waste solution into waste container
39. Add Hot dH₂O – fill tubes $\frac{1}{2}$ - $\frac{3}{4}$ full. **USE HOT MITTS. BE SURE TO TIGHTEN TOP – BE AWARE THAT HOT H₂O MAY OVERFLOW FROM TUBE WHEN THIS IS DONE**
40. Stir
41. Centrifuge in sets of 6 for 4 minutes
42. Decant waste solution into waste container
43. Repeat this step until the solution in the tubes is clear – this may take 3 hot H₂O rinses. If the solution isn't clear after 3 hot H₂O rinses, stop.

Clean-up

1. Put pipettes and beakers in basin with soapy water
2. Soak for at least 5 minutes
3. Using bottle brushes clean the cut-off bottles, spoons, and funnels
4. Either towel dry them or leave them overnight to air dry

Put them away in the appropriate cabinet or drawer

If needed a break may be taken here. If a break is taken make sure to fill the tubes $\frac{1}{2}$ full with dH₂O and cover them with saran wrap with the batch number and “in H₂O” labeled on it.

*This section takes approximately **3.5** hours to complete.*

Section 4 – Nytex

Processor preparation

5. Put on plastic gloves
6. Put on lab coat
7. Put on plastic lab apron
8. Put on safety glasses
5. Make sure to wear long pants and shoes – no sandals or shorts

If a break was taken:

1. Centrifuge in sets of 6 for 4 minutes
2. Decant into waste container

If no break was taken start with Step 3:

3. Set up Nytex screens. Place nytex screen on mouth of cut-off bottle.
Screw on top
4. Flush sample from tube onto nytex screen
5. Add a squirt of dH₂O and detergent solution to cut-off bottle – the amount will vary, if there is more sediment, add more detergent
6. Flush the sample liberally with dH₂O – usually an amount equivalent to 1 and ½ bottles worth
7. Place funnel into tube sample was originally in
8. Carefully remove nytex screen from apparatus – taking care to avoid losing any of the sample sediment
9. Carefully place screen into funnel
10. Rinse screen into tube, rinsing all of the sample into tube
11. Centrifuge in sets of 6 for 4 minutes
12. Decant

13. Label 12 4 ml vials with batch code – for example AS, and sample number – 1, 2, 3, 4, etc.
14. Use mechanical stirring machine to loosen sample in tube
15. Pour sample from tube into vial with same number.
16. Flush remaining sample from tube. Use very little water to avoid overflowing the vial
17. Add 3-5 drops of dye to each vial
18. Centrifuge in batches of 6 in small centrifuge for 10 minutes
19. Remove liquid from sample with glass pipettes and bulb
20. Press bead valve on tube to pipette to intake liquid
21. Press bead valve on side tube to flush liquid into waste container
22. Add 3-5 drops of glycerin to each vial
23. Place in dessicator
24. Shut off valve to dessicator
25. Remove dome
26. Place caps to vials in row above vials – make sure to keep vial cap with vial that it came off
27. Place vials in dessicator
28. Replace dome
29. Move valve handle on dome to parallel to valve stem
30. Turn valve to tube 3-4 turns
31. Attempt to lift the dome. If the dessicator lifts off the ground there is a good vacuum
32. When vacuum is established, turn the valve on the dome downward until airflow is almost stopped. Recheck the vacuum by attempting to lift the dome.
33. If the vacuum is good, leave the samples for 2-3 days, less if there was very little liquid other than the glycerin, checking within 1-2 days to make

sure that the sample has not dried out. More glycerin may be added if the sample has become too dry

Clean-up

1. Put cut off bottles, nytex screens, screw caps, and small tubes in basin with soapy water
2. Soak for at least 5 minutes
3. Using bottle brushes clean the cut-off bottles, spoons, and funnels
4. Either towel dry them or leave them overnight to air dry
5. Put them away in the appropriate cabinet or drawer

*This section takes approximately **3.0** hours to complete, although it can take up to **4.5** hours if there is a great deal of sediment.*

Safranin Dye

“Stronger” dye:

1. Place one measure with silver spatula in jar
2. Fill bottle to top with dH₂O
3. Replace cap and tip bottle upside down and right side up several times to mix

“Weaker” dye

1. Place 5-8 drops of “stronger” dye into bottle
2. Fill remainder of bottle with dH₂O
3. Replace cap and tip bottle upside down and right side up several times to mix

Pollen Wash Sample Preparation

Processor preparation

1. Put on plastic gloves
2. Put on lab coat
3. Put on plastic lab apron
4. Put on safety glasses
5. Make sure to wear long pants and shoes – no sandals or shorts

Pollen Wash Preparation – One sample

1. Sample in “Ball” or “Mason” jar – add Lycopodium tablet to jar
2. After tablet dissolves, screen liquid in jar into test tubes. Screen isn't necessary if sample isn't sandy
3. Centrifuge test tubes for 4 minutes – 6 tube centrifuge
4. Decant liquid into waste container
5. Combine test tubes into one test tube
6. Centrifuge for 4 minutes
7. Decant liquid into waste container
8. Proceed to Section 1, HF bath, step 26 and continue with the processing routine