AMS Pollen Preparation Procedure
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Safety
Lab coats, chemical goggles and gloves must be worn at all times. Additional safety information can be found in the Art of Pollen Preparation. Read the Art of Pollen before starting.

Introduction
In general, the AMS pollen preps are not all that different from the standard pollen preps. It is a good idea to be familiar with the standard pollen prep outlined in the Art of Pollen Preparation. AMS pollen preps require more care to prevent contamination. In the end, the condition of the pollen is not important. See the Important Notes section, below, for some important differences between AMS and standard pollen preps.

Before You Start
Bake all glassware and aluminum squares at 550˚ C for 2 hours. The only plastics that are used in the procedure are the nitex screens and screen holders. Everything else must be glass that has been baked at high temperatures to remove contaminates. The quantities listed are the minimum needed but I would recommend having extra glassware burned just in case something gets contaminated or broken. If you run out of aluminum squares, unburned high purity foil may be used instead.

8 Centrifuge tubes, glass, 40 mL
8 Stirring rods, glass
16 Vials, ½ dram, glass
8 Petri dishes, glass (optional)
16 Centrifuge tubes, glass, 15 mL
8 Beakers, glass
16+ Aluminum squares
8 Pipettes, glass

Preparing Reagents
10% Hydrochloric Acid is a 1:10 of 37% HCl (bulk) to dH_20.
10% Potassium Hydroxide is 10% by weight KOH dissolved in dH_2O.
2.5% Bleach is a 1:1 of bleach to dH_2O.

Important Notes
- Do NOT use wooden stir sticks, only burned glass stirring rods.
- Always keep the samples covered with burned aluminum foil to prevent contamination. Only remove the foil for brief moments to add chemicals and to stir.
- Do NOT use Tertiary Butanol Alcohol.
- There is no HF step because it would dissolve the glassware. If one is necessary, you must use plastic test tubes, thoroughly cleaned and rinsed with high purity water before use. Always check with the sample owner before doing this.
- ONLY use Milli Q, high purity, water.
- Make sure the spouts of the centrifuge tubes point outwards while centrifuging and boiling the samples.

Procedure

1) Fill and start the hot water bath (90+ deg. C).
2) Transfer a batch of 8 samples to 40 mL glass centrifuge tubes. Cover with baked foil squares.
3) Add ~30 mL high purity water, stir with glass stirring rod. Keep covered with baked aluminum foil squares. Centrifuge and decant.
4) Add ~30ml of 10% HCl to each sample, put in the hot water bath for 30 minutes. Stir once half way. Add water, stir, centrifuge and decant.
5) Add ~30 mL water to each sample, stir, centrifuge and decant. Repeat this water rinse until pH>6 (roughly neutral). To check the pH, the sample should be decanted onto a pH strip. Do NOT stick the pH strip in to the sample, decant the sample over the pH strip, while holding the pH strip with tongs.
6) Add ~30mL of 10% KOH to each sample, and put in hot water bath for 30 minutes. Add water, stir, centrifuge and decant. Repeat water rinses until roughly clear.
7) Pour samples through 80 µm sieve. Keep everything above 80 µm. Put the >80 micron fraction into a burned ½ dram vial or glass petri dish, label, and refrigerate.
8) Pour remaining sample through 20 micron sieves. DO NOT TOSS OR CONTAMINATE THE 20 SCREENS, they will be used again. Discard the less than 20 µm material.
9) Rinse the greater than 20µm material back into 40 mL test tubes. Add water, stir, centrifuge and decant.
10) Add ~15 mL 2.5% Bleach to each sample, and place samples in the hot bath for no more than 3 minutes. Add water, stir, centrifuge and decant.
11) Re-screen samples through 20µm mesh. Dispose of the less than 20µm fraction.
12) Transfer the greater than 20µm fraction of the samples to 12 mL test tubes. Add water, stir, centrifuge and decant.
13) Add ~6 mL 10% HCL to each sample, and put in hot water bath for 10 minutes.
14) Add water, stir, centrifuge and decant. Repeat rinses until pH>6.
15) Add ~3 mL water to each sample, and centrifuge.
16) Using a pipette, transfer the top pollen layer into ½ dram vials. Be careful not to mix the top pollen layer with all of the material at the bottom. You want as clean of a sample as possible.
17) Using the pipette remove a small amount of pollen from the ½ dram vial and make smear slides for analysis.
18) Add 1 drop of 10% HCl to each sample to prevent mold.
19) Rinse vial tops with high purity water and cap each sample.
20) Add labels to ½ dram vials.