

# Methods



## Pollen Extraction

1. Dilute 10 grams of honey with 100 mL of deionized water.
2. Add a known number of *Lycopodium clavatum* (club moss) spores to serve as a control and calculate concentration values (pollen/gram).
3. Transfer to a test tube for centrifugation to concentrate pollen and dispose of the additional water.
4. Dehydrate using 200 proof ethanol.
5. Acetolyze using a nine to one acetic anhydride to sulfuric acid solution. Acetolysis eliminates the cytoplasm in the pollen grains and allows for the structures of the pollen to be more visible.
6. Wash with 200 proof ethanol and stain to enhance the pollen structures.
7. Seal in a sample tube in glycerin USP until viewed under the microscope.



## Pollen Counting and Analysis

1. Use a light microscope at 400x magnification.
2. Count >200 pollen grains and tally the abundance of pollen types included in our “Identifiable Pollen” list (on the second page of this document), or listed during sample submission.
3. Any pollen grains encountered that are not included in the list are categorized as “Unknown”. Unknown grains are included in both relative abundance and total pollen concentration calculations.
4. Calculate concentration (pollen grains/gram) using the following formula:  
$$(\text{pollen counted} * \text{Lycopodium added}) / (\text{Lycopodium counted} * 10 \text{ grams})$$
5. Sometimes pollen grains are unidentifiable due to degradation or obscurement from other pollen. We include the unidentifiable grains in pollen concentration estimates and relative abundance calculations.

# Identifiable Pollen

*Acalypha* (copperleaf)  
*Acer* (maples)  
*Acacia* (acacia)  
*Aesculus* (horse chestnut)  
*Alnus* (alder)  
*Alternanthera* (joyweed)  
*Ambrosia* (ragweed)  
Amaranthaceae (goosefoot family)  
Anacardaceae (sumac family)  
Apiaceae (carrot family)  
*Artemisia* (sagebrush)  
*Asclepias* (milkweed)  
Asteraceae (composite family)  
*Betula* (birch)  
*Borago* (starflower)  
Boraginaceae (borage family) Brassicaceae  
(mustard family)  
*Carya* (hickory)  
Caryophyllaceae (carnation family)  
*Castanea* (chestnut)  
*Celtis* (hackberry)  
*Centaurea* (knapweed)  
*Cercis* (redbud)  
*Cornus* (dogwood)  
*Corylus* (hazel)  
Cupressaceae (cypress family)  
Cyperaceae (sedge family)  
*Diospyros* (persimmons)  
*Eragrostis* (umbrella plant)  
Ericaceae (heath family)  
Euphorbiaceae (spurge family)  
Fabaceae (bean family)  
*Fagopyrum* (buckwheat)  
*Fagus* (beech)  
*Fraxinus* (ash)  
*Hedera* (English ivy)  
*Ilex* (holly)  
*Juglans* (walnut)  
Lamiaceae (mint family)  
*Ligustrum* (privet)  
Liliaceae (lily family)  
*Lagerstroemia* (crepe myrtle)  
*Liquidambar* (sweet gum)  
*Liriodendron* (tulip poplar)  
*Lonicera* (honeysuckle)  
*Lythrum* (loosestrife)  
*Magnolia* (magnolia)  
Malpighiaceae (Barbados cherry)  
Malvaceae (mallow family)  
Melastomataceae (melastome family)  
*Melilotus* (sweet clover)  
*Mimosa* (sensitive plant)  
Myrtaceae (eucalyptus family)  
*Nyssa* (tupelo)  
Oleaceae (olive family)  
Onagraceae (evening primrose)  
*Ostrya/Carpinus* (Hop-hornbeam)  
*Oxydendrum* (sourwood)  
*Parthenocissus* (Virginia creeper)  
*Pedicularis* (lousewort)  
Pinaceae (pine family)  
*Pinus* (pine)  
*Plantago* (plantain)  
*Plantanus* (sycamore)  
*Podocarpus* (plum pine)  
*Polygonum* (knotweed)  
Poaceae (grass family)  
*Populus* (cottonwood)  
*Prosopis* (mesquite)  
*Quercus* (oak)  
Ranunculaceae (buttercup family)  
Rhamnaceae (buckthorn family)  
Rosaceae (rose family)  
Rubiaceae (bedstraw family)  
Rutaceae (citrus family)  
*Rhus* (sumac)  
*Sambucus* (elderberry)  
Sapindaceae (soapberry family)  
Scrophulariaceae (figworts)  
*Salix* (willow)  
*Tilia* (basswood)  
*Taraxacum* (dandelion)  
*Toxicodendron* (poison ivy)  
*Triadica* (Chinese tallow)  
*Trifolium* (clover)  
*Triticum* (wheat)  
*Typha* (cattail)  
*Ulmus* (elm)  
Verbenaceae (verbena)  
*Viburnum* (viburnum)  
*Vicia* (vetch)  
*Vitis* (grape)  
*Zea mays* (corn)