#### PROTEIN RESIDUE COLLECTION MANUAL

Paleo Research Institute 2675 Youngfield St. Golden, Colorado 80401 (303) 277-9848

www.paleoresearch.com

January 2000

#### COLLECTION OF FIELD SPECIMENS

# Flaked Lithics

All flaked lithic specimens should be placed directly into ziplock/whirl-pak bags with <u>minimal handling</u>. Please do not spit, lick, or rub on the artifacts as this may result in positive results for human proteins. Label the outside of the bag and if desired, place a second label inside the bag.

# **Control Samples**

Since false positives may result from bacteria, animal feces, lippoproteins, and alkaline substances in the soil, it is necessary to test soil samples with the artifacts. Collect approximately 1 gram samples from soil surrounding the artifacts and place in suitable containers (film canisters work nicely). Other control samples:

Stratified sites: Collect 1 gram samples from all cultural levels and from at

least one, but no more than three, off-site areas.

Surface sites: Collect 1 or 2 one gram samples from the site as well as 1

sample from off-site.

Control samples will be processed at our discretion and at no additional cost.

#### Groundstone and Pecked Stone

This category includes manos, mortars, pestles, etc. We prefer that groundstone and pecked stone artifacts be sent to our laboratory for extraction, especially if pollen and/or phytolith analyses are also requested. We will collect a protein residue sample from one side or area of the groundstone and pollen/starch and/or phytolith samples from a separate area of the ground surface. Starch granules represent starchy foods such as seeds and roots/tubers that often are ground in preparation for cooking.

Extracts for protein residue analysis from large metates or bedrock mortars should be collected by the archaeologist in the lab or field using the methods on page 4.

#### Ceramics

Ceramics can also be tested for protein residue, pollen, starch, and/or phytoliths. Whole pots or sherds can be sent for analysis and will be treated in a similar manner as groundstone. Protein residue analysis cannot be used on carbonized materials as the protein proteins are too severely denatured to yield results. Ceramics containing visible residue are excellent candidates for phytolith analysis using a new technique that can distinguish phytoliths of wild grasses from those of *Zea mays* and also can identify variety of maize. Ceramics should be handled in the same manner as flaked lithics.

# Soils

Soils from suspected processing areas and/or kill sites frequently retain the blood of the animal(s) processed. These soils can also be tested. Small (1 gram) amounts of the soil(s) to be tested should be placed in a ziplock/whirl-pak bag or clean film canister and sent for analysis.

# **Curated Artifacts**

Curated artifacts are also possible candidates for protein residue analysis. If the curated artifacts are stored in trays, they should be placed in separate, labeled ziplock/whirl-pak bags. If these artifacts are stored in other types of bags, such as brown paper, please send them in "as is."

# ARTIFACT SELECTION/RESEARCH QUESTIONS

For very large collections of lithics, selection of artifacts sent for protein residue analysis should fit the research design. Some examples:

Finished tools, projectile points with impact fractures, flakes with obvious use/wear.

Specific classes of artifacts. For example, were projectile points of one class used on a single type of animal -- large points for large game, small points for small animals?

If artifacts were multi-purpose tools, it is sometimes possible to identify more than one type of protein residue, unless the animals are closely related such as sheep and goats, or deer and elk.

Different types of protein residue may be detected from the hafting area and from the tip of the same tool. Identification of proteins from the hafting area may identify the hafting medium. When sending artifacts for hafting area AND tip analysis, please indicate that each area is to be tested separately. These areas are considered to be two separate samples and will be charged as two samples.

Protein residue analysis can be used in evaluating different aspects of prehistory. Examples in which it may be of benefit are:

- 1. As an objective test of tool function
- 2. As a test to determine whether animals represented in faunal remains were processed at the site.
- 3. To determine a possible range of animal resources exploited.

# Reports

Our reports are interpretive, rather than providing just a list of positive reactions. We identify possible animals represented by positive results based on known distributions of the animals and known and tested reactions of the antisera.

Artifacts sent for analysis can be tested against the following antisera:

Elephant Turkey Bear Bison Goat Agave (includes yucca) Guinea pig Bovine Trout Camel Horse American eel Cat Human Gizzard shad Catfish Pig Striped bass Rabbit Chicken Atlantic croaker Deer Rat Bay anchovy Weakfish Dog Sheep

#### POLLEN/PHYTOLITH WASHES FROM GROUNDSTONE AND VESSELS

### **Equipment and Supplies:**

- Bowl to collect liquid from wash
- · Clean trowel
- Sonicating tooth brush (or you may use a stiff-bristled brush, such as a tooth brush or a paint brush with bristles cut to a length of 3/4 to 1 inch
- · Jars with rubber gaskets to contain samples, or plastic bottles (lids must seal to prevent leakage in the mail or during transport), peanut butter jars or canning jars are good. Plastic bottles with plastic screw lids also are excellent sample containers.
- · 2 plastic squirt bottles (1 for vinegar, and 1 for distilled water)
- "Canned air", pressurized air (Dust off, Tornado, etc.)
- Liquid household bleach
- Distilled water
- Vinegar (not flavored), distilled white vinegar is best

#### Instructions:

All supplies must be "sterilized" prior to collecting the sample. Mix a bleach solution (1 part bleach to 3 parts water) and use to clean the bowl, brush, and jars. All supplies must be thoroughly rinsed with tap water to remove any bleach residue. Bleach oxidizes pollen, so any residue left on the sampling supplies will destroy the sample. Rinse copiously with tap water so there is no bleach smell left on the supplies. Then rinse lightly with distilled water to remove any particles introduced by the tap water. Fill 1 squirt bottle with vinegar and the other with distilled water.

Unwrap first piece of groundstone and remove any dirt clods adhering to the grinding surface (do not include these in the sample). Next, remove additional dirt using a clean trowel. Next, use "canned air" to blow any contaminants or loose sediment off the surface. You should have a fairly clean looking surface at this point. If not, loosen more dirt with the trowel and use the "canned air" again. If you cannot see the grinding surface of the tool, call Linda for further instructions, which might include lightly washing the surface with a gentle stream of water.

Wash non-grinding surfaces so that any liquid dripping down these surfaces does not collect additional sediment to contaminate the sample. Be certain not to wash the grinding surface during this process unless it is thoroughly covered with calcium carbonate (see next paragraph).

<u>IF</u> grinding surface is <u>thoroughly</u> covered with calcium carbonate rinse the entire artifact with water to remove all dirt, scrubbing with a brush to be certain it is clean. Otherwise, skip this step and proceed.

Hold groundstone so that liquid dripping from grinding surface will drip into bowl, but not run down the underside of the artifact. Squirt grinding surface with vinegar to wet. If surface bubbles, there is some calcium carbonate present, which must be dissolved (or the groundstone is made of limestone or other mineral soluble in acid). If the artifact is soluble in acid, only a limited quantity of vinegar should be used on the surface, as it will erode the surface. The wash should be continued with distilled water just as soon as any apparent carbonate deposits are removed. For non-soluble groundstone, use the vinegar until the bubbling stops, indicating that the carbonates have been dissolved. This will uncover the original grinding surface and allow the pollen accumulated on it to be removed. The grinding surface should be scrubbed with the brush (a sonicating tooth brush works wonders getting the surface clean) while the vinegar is being applied. This is easier with 2 people -- one to hold the rock and the other to squirt the vinegar and brush, or one to hold the rock and brush and the other to squirt the liquid. Do not brush so vigorously that you spray the vinegar or acid on the counter or on people. Remember both safety and that any pollen removed from the grinding surface is contained within the drops being sprayed around. When the surface no longer bubbles, indicating that the carbonates have been dissolved, continue washing with distilled water and brushing (in circles) with the brush. The object is to get the grinding surface clean enough to eat from. This insures that any dirt particles remaining in pores of the rock have been removed, and with them any pollen that had been ground into these pores. Brush only the grinding surface, not the nongrinding areas surrounding them. When the grinding surface is clean, rinse the brush into the collecting bowl with distilled water. Pour the sample into the jar (or jars) and seal. "Sterilize" all supplies with bleach before proceeding to the 2<sup>nd</sup> wash. Remember to rinse copiously again!

### COLLECTING PROTEIN RESIDUE SAMPLES FROM GROUNDSTONE

Extraction of protein residues is usually performed with a 0.2 M Tris hydrochloride, 0.5 M sodium chloride, and 0.5 % Triton X-100 solution (most desirable), although a 5 % ammonium hydroxide solution (less desirable) also can be used. The components for these solutions can be purchased from Fisher Scientific and from Sigma Chemical Company. When washing groundstone, use as little solution as possible (1-5 mL). For an additional \$12.00 charge, we can use a Centriprep-10 centrifugal concentrator with a 10,000 molecular weight cut-off membrane. This allows us to concentrate the proteins in a large volume of liquid down to a sample about 1-2 mL in size. If you opt to use the Centriprep device, a larger volume of solution may be used to wash the artifact (up to 50 mL).

Using a sterile (new) head on an ultrasonic toothbrush (preferred) or a new regular toothbrush, vigorously scrub the surface of the groundstone where a small amount of solution was applied. Decant the liquid into clean plastic container (do <u>not</u> use a glass container) using a pipette or syringe and repeat until a small area of the ground surface has been cleaned. Use additional solution from the pipette or syringe to "wash" the bristles of the toothbrush to get the residue out of it.

The decanted solution should be placed in a clean (preferably new) screw-capped plastic container with a tight seal. This container should be placed in a labelled ziplock/whirl-pak bag. If you have groundstone that was buried in the soil, be sure to scrape off the excess soil and include a soil control from the same area as the groundstone (preferably not what was scraped off the tool). Soils commonly contain compounds such as bacteria, lippoproteins, and animal feces that can cause false positive results for buried artifacts. A 1-2 gram soil control size is all that is required, and these soil controls are run at no extra charge.

DO THIS PROCEDURE FIRST, BEFORE WASHING THE REMAINDER OF THE SURFACE FOR POLLEN/PHYTOLITHS/STARCH.

DO <u>NOT</u> USE GLASS CONTAINERS FOR STORING PROTEIN RESIDUES, AS THE PROTEIN WILL ADHERE TO THE GLASS, MAKING RECOVERY DIFFICULT OR IMPOSSIBLE.

# GUIDELINES FOR FIELD COLLECTION OF ARTIFACTS FOR PROTEIN RESIDUE ANALYSIS

# In the Field:

- 1. Handle the artifact as little as possible.
  - Use trowel to pick up and place in plastic bag Use plastic food service gloves (Some latex gloves have been shown to contain proteins)
- 2. Do not brush off or clean dirt adhering to artifact
- 3. Place artifact in clean plastic bag
- 4. In a separate plastic bag or clean film canister, add a small amount of adjacent dirt

Dirt will be used as a negative control Dirt should be from under the artifact

Dirt should not have touched the artifact in situ