### 10.1.5 Protein Residue Analysis of Twenty-three Artifacts and Two Soil Samples from Site 7K-F-11, the Gray Farm Site in Delaware by Robert M. Yohe II and Summer Gibbons

# Protein Residue Analysis of Twenty-three Artifacts and Two Soil Samples from Site 7K-F-11 the Gray Farm Site in Delaware

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## Introduction

The use of chemical and molecular biological techniques in the analysis of archaeological materials can provide significant new information for the interpretation of their use. The identification of organic residue from lithic and ceramics artifacts, coprolites and soils have provided archaeologists with specific data regarding prehistoric exploitation of animals and plants. Although ancient protein residues may not be preserved in their original form, linear epitopes are generally conserved which can be identified by immunological methods (Abbas et al. 1994).

Immunological methods have been used to identify plant and animal residues on flaked and groundstone lithic artifacts (Allen et al. 1995; Gerlach et al. 1996; Henrikson et al. 1998; Hyland et al. 1990; Kooyman et al. 1992; Newman 1990, 1995; Petraglia et al. 1996; Shanks et al.1999; Yohe et al. 1991) and in Chumash paint pigment (Scott et al. 1996). Plant remains on artifacts also been identified through chemical (opal phytoliths), and morphological (use-wear), studies (Hardy and Garufi 1998; Jahren et al. 1997, Sobolik 1996). Plant and animal residues on ceramic artifacts have been identified through the use of gas-liquid chromatography, high performance liquid chromatography and mass spectrometry (Bonfield and Heron 1995; Evershed et al. 1992; Evershed and Tuross, 1996; Heron et al. 1991, Patrick et al. 1985). Serological methods have been used to determine blood groups in skeletal and soft tissue remains (Heglar 1972; Lee et al. 1989) and in the detection of hemoglobin from 4500-year-old bones (Ascenzi et al. 1985). Human leukocyte antigen (HLA) and deoxyribonucleic acid (DNA) determinations made on human and animal skeletal and soft tissue remains have demonstrated genetic relationships and molecular evolutionary distances (Hänni et al. 1995; Hansen and Gurtler 1983; Lowenstein 1985, 1986; Pääbo 1985, 1986, 1989; Pääbo et al. 1989). Successful identification of residues on stone tools, dated between 35-60,000 B.P., has been made by DNA analysis (Hardy et al. 1997), while recently, residues on surgical implements from the American Civil War were identified by immunological and DNA analysis (Newman et al. 1998). A recent study demonstrated the viability of identifiable immunoglobulin G in 1.6 million-year-old fossil bones from Venta Micena, Spain, (Torres et al. 2002). Horse exploitation was identified by immunological analysis of residues retained on Clovis points dated to ca. 11,200 B.P. (Kooyman et al. 2001).

The use of forensic techniques in the investigation of archaeological materials is appropriate as both disciplines deal with residues that have undergone changes, either deliberate or natural. Criminals habitually endeavor to remove bloodstains by such means as laundering, scrubbing with bleach, etc. yet; such degraded samples are still identified by immunological methods (Lee and De Forest 1976; Milgrom and Campbell 1970; Shinomiya et al. 1978, among others). Similarly it has been shown that immunological methods can be successfully applied to ancient human cremations (Cattaneo et al. 1992). Forensic wildlife laboratories use immunological techniques in their investigation of hunting violations and illegal trade, often from contaminated evidence (Bartlett and Davidson 1992; Guglich et al. 1993; Mardini 1984; McClymont et al. 1982). Immunological methods are also used to test the purity of food products such as canned luncheon meat and sausage, products which have undergone considerable degradation (Ashoor et al. 1988; Berger et al. 1988; King 1984). Thus the age and degradation of protein does not preclude detection (Gaensslen 1983:225).

## Materials and Methods

The method of analysis used in this study of archaeological residues is cross-over immunoelectrophoresis (CIEP). Prior to the introduction of DNA fingerprinting this test was used by forensic laboratories to identify trace residues from crime scenes. Minor adaptations to the original method were made following procedures used by the Royal Canadian Mounted Police Serology Laboratory, Ottawa (1983). The solution used to remove possible residues is five percent ammonium hydroxide which is the most effective extractant for old and denatured proteins without interfering with subsequent testing (Dorrill and Whitehead 1979; Kind and Cleevely 1969). Artifacts are placed in shallow plastic dishes and 0.5 ml of five percent ammonia solution applied directly to each. Initial disaggregation is carried out by floating the dish and contents in an ultrasonic cleaning bath for five minutes. Extraction is continued by placing the dish and contents on a rotating mixer for thirty minutes. For large ground stone items, such as metates, stone bowls, etc., the ammonium hydroxide is applied directly to the worked surface, agitated periodically with a sterile orangewood stick, and allowed to sit for one half hour. The resulting solution is drawn off, placed in a numbered, sterile plastic vial and stored at -20$^{o}$C prior to testing. In the case of soil samples, one gram is placed in a vial and 0.5 ml of 1 M Tris buffer solution ($H_2NC[CH_2OH]_3$) is used instead of ammonium hydroxide. The vial is placed in a rotating mixer overnight. The resulting solution is drawn off, placed in a numbered, vial and stored at -20$^{o}$C prior to testing.

A series of paired wells is punched into an agarose gel. Approximately 2 µl. of antiserum is placed into one well and the same amount of the unknown sample extract is placed in the other. An electric current is then passed through the gel. The antiserum and unknown sample migrate through the gel and come into contact. If there is protein in the unknown which corresponds with the antiserum, an antigen-antibody reaction occurs and the protein precipitates out in a specific pattern. The precipitant is detected when the gel is pressed, dried and stained. Control positives are run simultaneously with all the unknown samples. Sterile equipment and techniques are used throughout the analysis.

## The Samples

Twenty-three artifacts and two soil samples were submitted for immunological analysis by Archaeological & Historical Consultants, Inc. from site 7K-F-11. Residue was removed from the artifacts as discussed above. The residues were tested against the suite of plant and animal antisera (Table 1). Animal antisera provided by Cappel Research and Lampire Biomedical, and plant antisera produced at the University of Calgary, provide family level identification only. The relationship of antisera to some of the possible species identified is shown in Table 2.

## Results

Three artifacts registered positive reactions with available antisera. Rabbit protein residue was found on three artifacts; a PPK (sample #3), scraper #4 and drill #8. Twenty-two samples registered no positive reaction (Table 3). The absence of identifiable proteins on an artifact may be due to poor preservation of protein, insufficient protein, or that they were not in contact with any of the organisms included in the available antisera.

**TABLE 1: ANTISERA USED IN ANALYSIS**

| Animal Antiserum | Source | Plant Antiserum | Source |
|---|---|---|---|
| Bear | " | Agave | University of Calgary |
| Bovine | " | Amaranth | " |
| Camel | Lampire Biomedical | Aster | " |
| Cat | Cappel Research | Capparid | " |
| Chicken | " | Cedar | " |
| Deer | " | Chenopod | |
| Dog | " | Pine | " |
| Elephant | Lampire Biomedical | Walnut | " |
| Horse | " | | |
| Human | Cappel Research | | |
| Rabbit | " | | |
| Rat | " | | |
| Sheep | " | | |
| Swine | " | | |
| Trout/Salmon | Lampire | | |

**TABLE 2: POSSIBLE SPECIES IDENTIFIED**

| Antiserum to: | Reacts with: |
|---|---|
| Bear | Black, grizzly, etc. |
| Bovine | Bison, cow, musk ox |
| Camel | All camelids (New & Old world) |
| Cat | Bobcat, cougar, lynx, etc. |
| Chicken | Quail, grouse, & other gallinaceous fowl |
| Deer | Deer, elk, moose |
| Dog | Coyote, dog, wolf |
| Elephantidae | Elephant, mammoth |
| Horse | Horse, donkey, kiang, etc. |
| Human | Human |
| Rabbit | Rabbit, hare, pika |
| Rat | All rat & mouse species |
| Sheep | Bighorn & other sheep |
| Swine | Pig, possibly javelina |
| Trout | Trout and salmon species |
| Agave | Agave, yucca |
| Amaranthaceae | Amaranth, pigweed, *quelite*, etc. |
| Asteraceae | Rabbitbrush, sagebrush, sunflower, thistle |
| Capparidaeceae | Beeplant, bladderpod, stinkweed, etc. |
| Cedar | Cedar, cypress, juniper |
| Chenopodiaceae | Goosefoot, greasewood, pickleweed, saltbrush, etc. |
| Pinon | Fir, hemlock, pine, spruce |
| Walnut | Walnut, hickory |

**TABLE 3: RESULTS**

| Sample | Item | Bag | Inv. # | Feature | Strat | Lvl | Comments | Results |
|---|---|---|---|---|---|---|---|---|
| 1 | Abrader | 1071 | | 371 | 1 | 7 | Pairs with sample 13. | Negative |
| 2 | PPK | 185 | 11 | | | | Rossville. Hair. | Negative |
| 3 | PPK | 1580 | 30 | | | | Untyped. Skin. | Rabbit |
| 4 | Scraper | 195 | 31 | | | | Hair | Rabbit |
| 5 | Scraper | 1423 | 6 | | | | Hair | Negative |
| 6 | Microtool | 889 | 37 | | | | Skin | Negative |
| 7 | Microtool | 1676 | | | | | Plant tissue/resin | Negative |
| 8 | Drill | 226 | 31 | | | | Poss. blood | Rabbit |
| 9 | Drill | 1583 | 41 | | | | Hair | Negative |
| 10 | Drill | 1844 | 2 | | | | Hair | Negative |
| 11 | Abrader | 364 | 38 | | | | Plant tissue | Negative |
| 12 | Abrader | 1580 | 30 | | | | Plant fiber. Recent break. | Negative |
| 13 | Abrader | 1071 | 39 | | | | Plant tissue. Pairs with sample 1. | Negative |
| 14 | Ceramic | 1849 | | 185 | | | Selden Island #1. Inked | Negative |
| 15 | Ceramic | 402 | | 10 | 2 | 2 | Townsend Vessel #9. Inked | Negative |
| 16 | Ceramic | | | TU 38 | | 3 | Marcey Creek #2. Inked | Negative |
| 17 | Ceramic | | | TU 21 | 3 | 5 | Unknown Type. Inked | Negative |
| 18 | Argillite Tool | 885 | 2 | 279 | 1 | 4 | From cache | Negative |
| 19 | Abrader | 1235 | 6 | 232 | 2 | 4 | Large dia bore | Negative |
| 20 | Abrader | 1671 | 5 | 330 | 1 | 5 | Medium dia bore | Negative |
| 21 | Abrader | 1089 | 4,5 | 225 | 1 | 13 | Small dia bore | Negative |
| 22 | Steatite | 882 | | 279 | 1 | 1 | body frag | Negative |
| 23 | Steatite | 286 | | 3 | | | Rim frag. 9/9 | Negative |
| 24 | SOIL | | | TU #32 | 1 | 1 | Ap | Negative |
| 25 | SOIL | | | 10 | 1 | 1 | Ap | Negative |

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