9.0 IMMUNOLOGICAL ANALYSES

Craig E. Skinner*, Margaret Newman**

9.1 INTRODUCTION

Seventy-two flaked and ground stone artifacts from seven archaeological sites in central and north-central Oregon were sent for immunological (blood residue) analyses to Dr. Margaret Newman, Laboratory of Archaeological Science, California State University-Bakersfield (Table 9-1, Figure 9-1).

Flaked stone tool samples were selected for immunological analysis to test for animal residues and to confirm tool use in animal procurement and processing activities. Several ground stone artifacts were selected because of staining that was suspected to be associated with animal processing or because of their close association (at 35-DS-33) with faunal materials.

The application of established immunological and chemical methods to the analysis of archaeological organic residues offers a new scientific means of evaluating prehistoric subsistence and ecology. Recent studies demonstrate that animal and plant protein residues are often preserved on artifacts for many years after their original use and that such residues can be identified to at least the family level (Heron et al. 1991; Hyland et al. 1990; Kooyman et al. 1992; Newman 1990; Newman and Julig 1989; Newman et al. 1992; Yohe et al. 1991). Information acquired from such analyses can be used in reconstructing prehistoric subsistence patterns, recreating past environments, and possibly in identifying task specific artifacts.

Although various immunological methods have been used, all are based on the antigen-antibody reaction initially observed in the classic precipitin test in the late 1800s. Following its discovery, the test quickly achieved integrity in the fields of clinical and forensic medicine and has been used extensively in medico-legal work since the beginning of this century (Gaensslen 1983). Although the successful identification of protein residues is dependent on their condition, forensic studies have demonstrated that proteins are extremely robust molecules and can withstand harsh treatment while still retaining their antigenicity and biological activity (Arquembourg 1975; Gaensslen 1983; Haber 1964; Lee and DeForest 1976; Macey 1979; Sensabaugh et al. 1971, among others). The fact that valid results from the analysis of old and severely denatured proteins are obtained in forensic medicine is of special relevance to archaeology, where "old and denatured" proteins are the norm. The sensitivity and specificity of precipitin reactions makes them an extremely effective method for the detection of trace amounts of protein (Kabat and Meyer 1967:22).

*INFOTEC Research, Inc.

**Dr. Newman is currently associated with the University of Calgary, Alberta, Canada.

Table 9-1 Summary of Immunological Analyses and Results.

Site	Flaked Stone	Ground Stone	Total	Number Positive	Percent Positive
35-DS-33	0	2	2	1	50
35-DS-263	4	0	4	2	50
35-DS-557	34	0	34	8	24
35-JE-49	8	0	8	4	50
35-JE-51B	19	0	19	3	16
35-UM-154	0	1	1	0	0
35-WS-225	3	1	4	0	0
Total	68	4	72	18	25

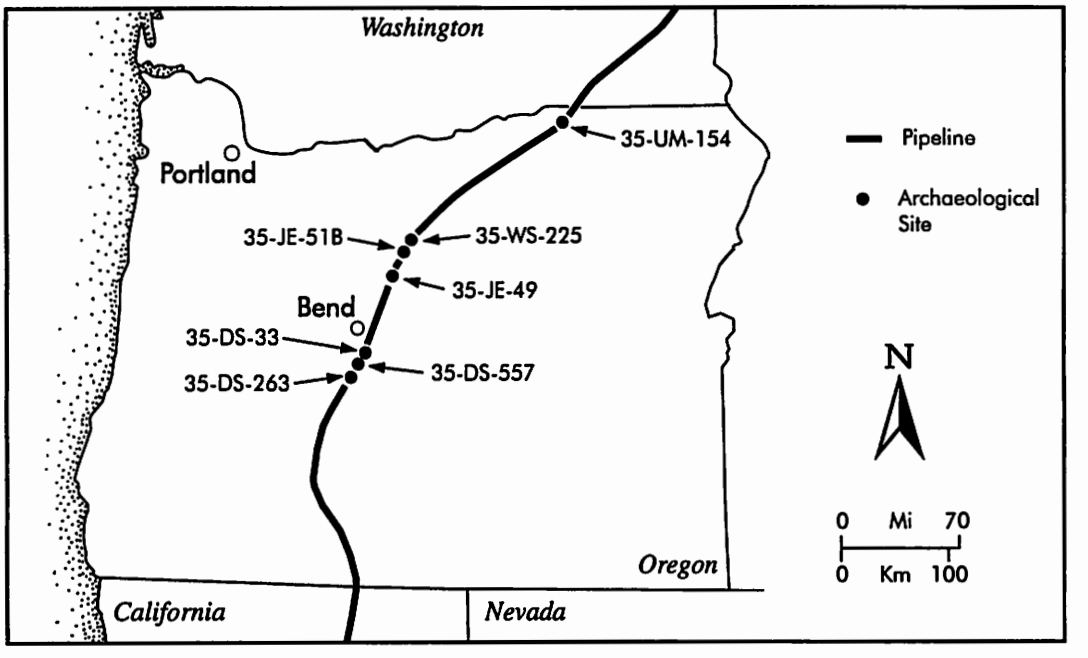

Figure 9-1 Distribution of PEP sites selected for immunological analyses.

9.2 MATERIALS AND METHODS

Crossover immunoelectrophoresis (CIEP) is a well-established method of analysis for the identification of bloodstains, body tissues, and fluids in medico-legal work (Culliford 1963; Gaensslen 1983) and is the method of analysis used by Newman. Minor adaptations to the original method were made following procedures used by the Royal Canadian Mounted Police (RCMP) Serology Laboratory in Ottawa (RCMP 1983) and the Centre of Forensic Sciences in Toronto. The test is based on the principles of the precipitin test but affords a higher degree of sensitivity and can identify 10^{-8} g of protein (Culliford 1963; Gaensslen 1983). The procedure is discussed fully in Newman and Julig (1989).

Sixty-eight flaked stone artifacts and two ground stone items recovered from seven archaeological sites in Oregon (see Table 9-1) were sent for immunological analysis of protein residues. Washes collected at the IRI laboratory from two additional large ground stone artifacts were also sent. A total of 28 soil control samples collected with the artifacts or at nearby units were also sent for testing. As contaminants in soils (such as bacteria, tannic acid, and iron chlorates) may result in nonspecific precipitation of antisera, it is important that control soil samples be included in the analysis (Gaensslen 1983).

Possible residues were removed from the artifacts using a five percent ammonium hydroxide solution. This solution has been shown to be the most effective extractant for old and denatured bloodstains and does not interfere with subsequent testing (Dorrill and Whitehead 1979; Kind and Cleevely 1969). Artifacts were placed in shallow plastic dishes and 0.5 cc of the ammonia solution was applied with a syringe and needle. Initial disaggregation of residue was carried out by floating the plastic dish and its contents in an ultrasonic cleaning bath for two to three minutes. Extraction was continued by placing the dish and contents on a rotating mixer for 30 minutes. The resulting ammonia solution was removed with a pasteur pipette, placed in a numbered plastic vial, and refrigerated prior to further testing. Approximately 1 ml of Tris buffer (pH 8.0) was added to 1 g amounts of each soil sample, mixed well, and allowed to extract for 24 hours at 4°C to prevent bacterial contamination. The resulting supernatant fluids were removed from the soils and tested against preimmune serum.

The washes collected by IRI laboratory personnel from the two large ground stone artifacts were acquired in a similar manner to those described above. A five percent solution of ammonium hydroxide solution was poured onto the suspect ground stone surfaces and was left to sit for 10 minutes. Approximately 5 ml of solution was then drawn off the surface of the artifact with a pipette, placed into a glass vial, and sealed for shipment.

The artifact and soil extracts were first tested against preimmune serum (i.e., serum from a nonimmunized animal). A positive result against preimmune serum could arise from nonspecific protein interaction not based on the immunological specificity of the antibody (i.e., nonspecific precipitation). No positive results were obtained, and testing of artifact samples was continued against the antisera shown in Table 9-2.

Except where noted, antisera are obtained from commercial sources and were developed specifically for use in forensic medicine. Where necessary, these sera are solid phase absorbed to eliminate species cross-reactivity. However, they are polyclonal; that is, they recognize epitopes shared by closely related species. For example, anti-deer will give positive results with members of the Cervidae family such as deer, moose, elk, and caribou, as well as with pronghorn (Antilocapridae family). Two additional antisera, elk and trout, were raised at the University of Calgary. The antiserum to modern elk (*Cervus canadensis*) was also raised at the University of Calgary and is species-specific. The trout antiserum is polyclonal and will recognize most members of the Salmonidae family. Immunological relationships do not necessarily bear any relationship to the Linnaean classification scheme, although they usually do (Gaensslen 1983).

Table 9-2 Antisera Used in Analysis.

Antisera	Source
Anti-bear	Organon\Teknika
Anti-bovine	forensic medicine
Anti-cat	forensic medicine
Anti-chicken	forensic medicine
Anti-deer	forensic medicine
Anti-dog	forensic medicine
Anti-guinea-pig	forensic medicine
Anti-human	forensic medicine
Anti-mouse	forensic medicine
Anti-rabbit	forensic medicine
Anti-rat	forensic medicine
Anti-sheep	forensic medicine
Anti-duck	Nordic Immunological
Anti-pigeon	Nordic Immunological
Anti-elk	University of Calgary
Anti-pronghorn	University of Calgary
Anti-trout	University of Calgary

9.3 RESULTS

The results obtained in CIEP analysis are reported in Tables 9-3 and 9-4 and are discussed below. The absence of identifiable proteins on artifacts may be due to poor preservation of protein or use on species other than those encompassed by the available antisera. It is also possible that the artifacts were not used on animal tissues.

9.3.1 35-DS-33

Positive results to rabbit antiserum were obtained from a large ground stone artifact from 35-DS-33 that was found in association with a concentration of faunal material. Although it is unusual to obtain positive reactions from stone grinding implements, it is not unknown. Ethnographic accounts of animal pulverization in California and Baja California have been recorded, and immunological studies of ground stone artifacts from southern California sites have previously yielded positive immunological results (Yohe et al. 1991). Positive results to rabbit antiserum are obtained with all members of the order Lagomorpha (rabbits, pikas, and hares). Cross-reactions with other orders do not generally occur.

9.3.2 35-DS-263

A positive reaction to cat antiserum was obtained from residues on one artifact (Specimen 1173-1). Any member of the Felidae family may be represented by this result, but cross-reactions with other orders do not generally occur. Positive results to rabbit antiserum were obtained from one artifact (Specimen 1164-1).

Table 9-3 Summary of Positive Results of Immunological Analyses of PEP Artifacts.

Site Number	Specimen Number	MAT [a]	CLA [b]	RWM [c]	Blood
35-DS-33	2172-1	GDS	HPM	BAS	Rabbit
35-DS-263	1164-1	FLS	BIF	OBS	Rabbit
35-DS-263	1173-1	FLS	BIF	OBS	Cat
35-DS-557	1477-2	FLS	PPT	OBS	Deer
35-DS-557	1850-5	FLS	UFT	OBS	Deer
35-DS-557	2049-3	FLS	UFT	OBS	Deer
35-DS-557	2068-5	FLS	BIF	OBS	Deer
35-DS-557	2117-3	FLS	BIF	OBS	Deer
35-DS-557	2117-4	FLS	BIF	OBS	Rabbit
35-DS-557	2136-3	FLS	UFT	OBS	Rabbit
35-DS-557	2236-1	FLS	BIF	OBS	Rabbit
35-JE-49	1060-6	FLS	PPT	OBS	Rabbit
35-JE-49	1109-2	FLS	PPT	OBS	Sheep
35-JE-49	1115-5	FLS	PPT	OBS	Rabbit
35-JE-49	1131-5	FLS	PPT	OBS	Guinea Pig
35-JE-51B	2013-1	FLS	BIF	CCS	Rabbit
35-JE-51B	2023-1	FLS	BIF	CCS	Rabbit
35-JE-51B	2756-1	FLS	PPT	CCS	Sheep

[a] MAT = Material, FLS = Flaked Stone, GDS = Ground Stone.
[b] CLA = Artifact Classification, BIF = Biface, HPM = Hopper Mortar, PPT = Projectile Point, UFT = Unpatterned Flaked Tool.
[c] RWM = Raw Material, BAS = Basalt, CCS = Cryptocrystalline Silica, OBS = Obsidian.

9.3.3 35-DS-557

Positive results to deer antiserum were obtained from five artifacts. A positive reaction to deer antiserum could represent any member of the Cervidae family, as well as pronghorn of the Antilocapridae family. However, as negative results to species-specific elk antiserum were obtained from these artifacts, it is suggested that deer (*Odocoileus hemionus*) is the species most likely represented by these results.

9.3.4 35-JE-49

Positive results to rabbit antiserum were obtained from two artifacts. Positive results to this antiserum are obtained with all members of the order Lagomorpha (rabbits, hares, and pikas), but cross-reactions with other orders do not generally occur.

One artifact (Specimen 1109-2) tested positive with sheep antiserum. Positive results to this antiserum are obtained with sheep and goat, but cross-reactions with other species are not known to occur.

Table 9-4 Results of Immunological Analyses of Artifacts.

Site	Specimen		Unit		Depth (cm)		RWM [a]	Class [b]		Results
35-DS-33	2172 - 1	EXU	536.00 S	528.00 E	-9.00	-21.00	BAS	GDS	HPM	Rabbit
35-DS-33	2198 - 1	EXU	538.00 S	529.00 E	3.00	-21.00	BAS	GDS	HPM	Negative
35-DS-263	1164 - 1	EXU	139.00 S	135.00 E	-81.00	-91.00	OBS	FLS	BIF	Rabbit
35-DS-263	1171 - 3	EXU	139.00 S	136.00 E	-86.00	-96.00	OBS	FLS	DEB	Negative
35-DS-263	1173 - 1	EXU	139.00 S	136.00 E	-96.00	-106.00	OBS	FLS	BIF	Cat
35-DS-263	1331 - 1	EXU	138.00 S	136.00 E	-106.00	-116.00	OBS	FLS	BIF	Negative
35-DS-557	1350 - 3	EXU	250.00 S	410.00 E	-78.00	-88.00	OBS	FLS	BIF	Negative
35-DS-557	1421 - 1	EXU	272.00 S	401.00 E	-60.00	-70.00	OBS	FLS	UFT	Negative
35-DS-557	1449 - 4	EXU	284.00 S	417.00 E	-61.00	-71.00	OBS	FLS	PPT	Negative
35-DS-557	1477 - 2	EXU	285.00 S	418.00 E	-64.00	-74.00	OBS	FLS	PPT	Deer
35-DS-557	1520 - 3	EXU	288.00 S	415.00 E	-71.00	-81.00	OBS	FLS	BIF	Negative
35-DS-557	1834 - 5	EXU	248.00 S	410.00 E	-56.00	-67.00	OBS	FLS	BIF	Negative
35-DS-557	1837 - 3	EXU	248.00 S	410.00 E	-77.00	-87.00	OBS	FLS	UFT	Negative
35-DS-557	1846 - 3	EXU	249.00 S	410.00 E	-58.00	-68.00	OBS	FLS	BIF	Negative
35-DS-557	1846 - 4	EXU	249.00 S	410.00 E	-58.00	-68.00	OBS	FLS	UFT	Negative
35-DS-557	1850 - 4	EXU	249.00 S	410.00 E	-68.00	-78.00	OBS	FLS	UFT	Negative
35-DS-557	1850 - 5	EXU	249.00 S	410.00 E	-68.00	-78.00	OBS	FLS	UFT	Deer
35-DS-557	1850 - 6	EXU	249.00 S	410.00 E	-68.00	-78.00	OBS	FLS	UFT	Negative
35-DS-557	1859 - 3	EXU	249.00 S	411.00 E	-56.00	-68.00	OBS	FLS	BIF	Negative
35-DS-557	1861 - 9	EXU	249.00 S	411.00 E	-68.00	-78.00	OBS	FLS	BIF	Negative
35-DS-557	1866 - 2	EXU	250.00 S	411.00 E	-67.00	-77.00	OBS	FLS	UFT	Negative
35-DS-557	1937 - 1	EXU	285.00 S	409.00 E	-64.00	-64.00	OBS	FLS	UFT	Negative
35-DS-557	1945 - 3	EXU	285.00 S	410.00 E	-68.00	-78.00	OBS	FLS	BIF	Negative
35-DS-557	1994 - 1	EXU	287.00 S	410.00 E	-73.00	-73.00	OBS	FLS	BIF	Negative
35-DS-557	2015 - 3	EXU	287.00 S	414.00 E	-58.00	-68.00	OBS	FLS	PFT	Negative
35-DS-557	2024 - 3	EXU	288.00 S	410.00 E	-58.00	-68.00	OBS	FLS	EMP	Negative
35-DS-557	2042 - 1	EXU	288.00 S	412.00 E	-70.00	-70.00	OBS	FLS	BIF	Negative
35-DS-557	2049 - 3	EXU	288.00 S	413.00 E	-68.00	-78.00	OBS	FLS	UFT	Deer
35-DS-557	2049 - 4	EXU	288.00 S	413.00 E	-68.00	-78.00	OBS	FLS	BIF	Negative
35-DS-557	2068 - 4	EXU	289.00 S	414.00 E	-71.00	-81.00	OBS	FLS	BIF	Negative
35-DS-557	2068 - 5	EXU	289.00 S	414.00 E	-71.00	-81.00	OBS	FLS	BIF	Deer
35-DS-557	2093 - 2	EXU	292.00 S	414.00 E	-50.00	-60.00	OBS	FLS	UFT	Negative
35-DS-557	2095 - 1	EXU	292.00 S	414.00 E	-69.00	-69.00	OBS	FLS	BIF	Negative
35-DS-557	2110 - 4	EXU	293.00 S	416.00 E	-71.00	-81.00	OBS	FLS	PFT	Negative
35-DS-557	2117 - 2	EXU	293.00 S	417.00 E	-60.00	-70.00	OBS	FLS	BIF	Negative
35-DS-557	2117 - 3	EXU	293.00 S	417.00 E	-60.00	-70.00	OBS	FLS	BIF	Deer
35-DS-557	2117 - 4	EXU	293.00 S	417.00 E	-60.00	-70.00	OBS	FLS	BIF	Rabbit
35-DS-557	2121 - 4	EXU	293.00 S	417.00 E	-80.00	-90.00	OBS	FLS	BIF	Negative
35-DS-557	2136 - 3	EXU	295.00 S	409.00 E	-67.00	-77.00	OBS	FLS	UFT	Rabbit
35-DS-557	2236 - 1	EXU	289.00 S	415.00 E	-67.00	-67.00	OBS	FLS	BIF	Rabbit

[a] RWM = Raw Material, OBS = Obsidian, BAS = Basalt.

[b] CLASS = Artifact Classification, FLS = Flaked Stone, GDS = Ground Stone, BIF = Biface, DEB = Debitage, EGS = Edge Ground Stone, EMP = Edge Modified Piece, HPM = Hopper Mortar, OTH = Other, PFT = Patterned Flaked Tool, PPT = Projectile Point, UPT = Unpatterned Flaked Tool.

Table 9-4 (continued)

Site	Specimen	Unit			Depth (cm)		RWM [a]	Class [b]		Results
35-JE-49	710 - 1	EXU	13.60 S	10.90 E	-159.00	-169.00	OBS	FLS	PPT	Negative
35-JE-49	908 - 1	EXU	18.00 S	9.00 E	-303.00	-303.00	OBS	FLS	BIF	Negative
35-JE-49	1000 - 1	EXU	22.00 S	14.50 E	-240.00	-250.00	OBS	FLS	PPT	Negative
35-JE-49	1060 - 6	EXU	26.00 S	21.00 E	-170.00	-180.00	OBS	FLS	PPT	Rabbit
35-JE-49	1109 - 2	EXU	27.00 S	21.00 E	-160.00	-170.00	OBS	FLS	PPT	Sheep
35-JE-49	1115 - 5	EXU	27.00 S	21.00 E	-180.00	-190.00	OBS	FLS	PPT	Rabbit
35-JE-49	1128 - 5	EXU	27.00 S	21.00 E	-210.00	-220.00	OBS	FLS	BIF	Negative
35-JE-49	1131 - 4	EXU	27.00 S	21.00 E	-220.00	-230.00	OBS	FLS	PPT	Guinea Pig
35-JE-51B	2013 - 1	EXU	105.00 S	79.00 E	-65.00	-65.00	CCS	FLS	BIF	Rabbit
35-JE-51B	2023 - 1	EXU	105.00 S	80.00 E	-59.00	-59.00	CCS	FLS	BIF	Rabbit
35-JE-51B	2115 - 1	EXU	118.00 S	89.00 E	-275.00	-275.00	CCS	FLS	PPT	Negative
35-JE-51B	2129 - 1	EXU	119.00 S	89.00 E	-119.00	-119.00	OBS	FLS	PPT	Negative
35-JE-51B	2135 - 5	EXU	119.00 S	89.00 E	-260.00	-270.00	CCS	FLS	BIF	Negative
35-JE-51B	2365 - 1	EXU	112.00 S	90.00 E	-184.00	-194.00	OBS	FLS	PPT	Negative
35-JE-51B	2394 - 1	EXU	113.00 S	89.00 E	-225.00	-225.00	BAS	OTH	FAR	Negative
35-JE-51B	2469 - 1	EXU	114.00 S	89.00 E	-121.00	-121.00	OBS	FLS	PPT	Negative
35-JE-51B	2600 - 1	EXU	116.00 S	89.00 E	-98.00	-98.00	OBS	FLS	PPT	Negative
35-JE-51B	2667 - 1	EXU	119.00 S	90.00 E	-105.00	-105.00	CCS	FLS	PPT	Negative
35-JE-51B	2698 - 3	EXU	120.00 S	89.00 E	-287.00	-297.00	CCS	FLS	PPT	Negative
35-JE-51B	2705 - 1	EXU	120.00 S	90.00 E	-267.00	-267.00	CCS	FLS	BIF	Negative
35-JE-51B	2756 - 1	EXU	123.00 S	83.00 E	-243.00	-243.00	CCS	FLS	PPT	Sheep
35-JE-51B	2784 - 1	EXU	124.00 S	83.00 E	-226.00	-226.00	CCS	FLS	BIF	Negative
35-JE-51B	2796 - 1	EXU	124.00 S	84.00 E	-242.00	-242.00	CCS	FLS	PPT	Negative
35-JE-51B	2803 - 1	EXU	124.00 S	84.00 E	-242.00	-249.00	CCS	FLS	PPT	Negative
35-JE-51B	2866 - 4	EXU	125.00 S	83.00 E	-210.00	-223.00	CCS	FLS	BIF	Negative
35-JE-51B	2924 - 1	EXU	125.00 S	85.00 E	-232.00	-232.00	CCS	FLS	BIF	Negative
35-JE-51B	3835 - 1	EXU	124.00 S	85.00 E	-222.00	-222.00	CCS	FLS	PPT	Negative
35-UM-154	363 - 1	EXU	88.00 S	100.00 E	-76.00	-76.00	BAS	GDS	EGS	Negative
35-WS-225	1735 - 1	EXU	112.50 S	140.00 E	-53.00	-53.00	OBS	FLS	PPT	Negative
35-WS-225	2024 - 1	EXU	108.00 S	140.00 E	-61.00	-61.00	CCS	FLS	BIF	Negative
35-WS-225	2025 - 1	EXU	108.00 S	140.00 E	-62.00	-62.00	CCS	FLS	BIF	Negative
35-WS-225	2253 - 1	EXU	115.00 S	138.00 E	-26.00	-26.00	BAS	GDS	MSL	Negative

[a] RWM = Raw Material, OBS = Obsidian, BAS = Basalt.

[b] CLASS = Artifact Classification, FLS = Flaked Stone, GDS = Ground Stone, BIF = Biface, DEB = Debitage, EGS = Edge Ground Stone, EMP = Edge Modified Piece, HPM = Hopper Mortar, OTH = Other, PFT = Patterned Flaked Tool, PPT = Projectile Point, UPT = Unpatterned Flaked Tool.

Another artifact tested positive to guinea-pig antiserum. Although other families within the order Rodentia could be represented by this result, porcupine (*Erethizontidae*), beaver (*Castor canadensis*), and squirrel (*Sciuridae*) are the most likely candidates.

9.3.5 35-JE-51B

Positive results to rabbit antiserum were obtained from residues on two flaked stone artifacts from 35-JE-51B. Another artifact tested positive against sheep antiserum.

9.3.6 35-UM-154

No positive results were obtained in the immunological testing of a single ground stone artifact from 35-UM-154.

9.3.7 35-WS-225

No positive results were obtained in the immunological testing of three flaked stone artifacts and one ground stone item from 35-WS-225.

9.4 CONCLUSIONS

Twenty-five percent of the 72 flaked and ground stone artifacts selected for immunological analyses yielded a positive reaction for the presence of blood residues. Although the overall rate of positive results (25%) is relatively low, these analyses provide direct, often species-specific, functional evidence for the animal-related use of tools at the tested sites. Through the careful selection and testing of tools, immunological analyses can provide important information about dietary choice, site use, animal processing activities, and paleoenvironmental conditions.

ADDENDUM A

Richard M. Pettigrew*

After IRI received the results of immunological analyses from Dr. Newman and drafted this chapter, a technical report by Eisele (1994) that calls into question the validity of blood protein analysis was distributed to the archaeological community. This report reviews the available techniques for detecting immunological reactions, develops one technique (gold immunoassay) said to possess great sensitivity, employs that method to examine residues from a sample of stone tools from Nevada and Oregon, and describes a test for blood residues on artifacts deliberately soaked in blood and buried for periods of up to 10 months. Eisele's discussion of the immunological antibody-antigen reaction questions the validity of the standard electrophoresis method commonly used in forensic studies. Her literature review suggests problems with many immunological methods in the detection of blood proteins. Of 159 flaked stone tools immunoassayed, only seven reacted positively; in each of these seven cases, circumstantial evidence argued that ancient proteins were not responsible for the reactions. Finally, examination of recently buried blood-stained tools showed that microbial and other degradation of immunoglobulin and albumin was rapid, especially in damp soil, and most blood protein samples were rendered biologically inviable in less than one year.

Dr. Newman was contacted during the preparation of this addendum to offer her view of Eisele's (1994) results (Margaret Newman, personal communication 1994). Her formal response to these results is in preparation. She suggested, however, that Eisele's technical procedures may be responsible for the failure to find more positive reactions on ancient tools, and pointed out that immunoglobulins are present in all body tissues, not just blood. Thus, empirical tests using blood alone do not sufficiently replicate either the use of ancient stone tools nor the mix of organic residues generated by such use.

The subject of blood residue analysis clearly has become controversial since its application to PEP studies. As a result, its validity and the reliability of interpretations based on it are in question. Proper evaluation and interpretion of PEP residue analyses no longer can be confidently determined. It is hoped that a resolution of this difficulty may be achieved soon through published airing of the different positions and by empirical studies to ascertain whether ancient blood and tissue proteins are preserved on artifacts, regularly detectable, and identifiable with sufficient confidence.

*INFOTEC Research, Inc.