APPENDIX F

BLOOD RESIDUE STUDIES SYNTHESIS

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By

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ABSTRACT

This synthesis compiles and compares blood residue studies previously presented in 33 reports on archaeological research conducted in the states of Delaware, Pennsylvania, and Maryland. It presents the methods, findings, and shortcomings of these studies, and also provides a reference collection of blood residue studies for the tri-state area. The information was used to formulate a blood residue analysis plan for Site 7NC-B-54 (Ronald McDonald House) and can provide a guide for future analysis at newly identified archaeological sites.

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1.0 INTRODUCTION

The research potential of Site 7NC-B-54 (Ronald McDonald House) rests predominately in the clarity of the site deposits. It has been possible to spatially and analytically isolate four loci of activity at this site. Each of these loci apparently reflects brief, focused use of the landform. In such a context, it is important to bring many lines of evidence to bear on interpreting the likely function of each visit. The goal of this overview of blood residue studies is to provide an informed perspective from which to design the analysis of Site 7NC-B-54.

In addition to bringing multiple lines of evidence to bear on site interpretation, the 7NC-B-54 study is stringent in requiring that all methods be explicitly defended. Our study comes after more than 30 years of intensive archaeological research in the state and surrounding region. Various researchers have taken differing approaches to methodological challenges in areas such as use-wear studies and blood residue testing. Rather than simply choose an earlier study as a model, thereby repeating the problems and limitations of the earlier study, it was appropriate to conduct a detailed, critical review of blood residue work to date. Only upon completion of this overview is it possible to select and defend the methods to be applied on the 7NC-B-54 material.

This synthesis compiles the blood residue studies presented in 33 reports from the states of Delaware, Pennsylvania, and Maryland. It presents the methods, findings, and shortcomings of these studies, and also provides a reference collection of blood residue studies for the tri-state area. The information was then used to formulate a blood residue analysis plan for Site 7NC-B-54 and can provide a guide for future analysis of new sites.

2.0 HISTORY AND CONTROVERSY OF BLOOD RESIDUE ANALYSIS IN ARCHAEOLOGY

The modern history of testing for blood on archaeological artifacts begins with Thomas Loy's 1983 article in *Science* (Loy 1983). Here, Loy used a two-prong approach that included first testing an item for the *presumptive* presence of blood, and then further analysis to ascertain taxa identification. Though methods have continued to become more sophisticated utilizing immunological techniques, the presumptive first stage methodology has remained relatively unchanged.

The second stage, that of actual taxa identification, is much more complicated and deals with an understanding of techniques recently borrowed from the immunological sciences. The original crystallization technique of Loy has given way to much more sophisticated techniques that identify unknown residue stains (antigens) by observing immunological reactions with a known antibody (antisera). Further understanding of these principals can be found in Abbas *et al.* (1991:Chapter 3), Branden and Tooze (1991:Chapter 12), and Guttman *et al.* (1989:Chapter 1).

Controversy surrounding blood residue studies started at approximately the same time as its history. Consensus has yet to be reached whether one can truly detect even the *presence* of blood, let alone identify taxa. Using these techniques on relatively fresh blood is one thing, but the large timespans at hand in archaeology have greatly complicated these matters. All the presumptive blood tests presented in reports referenced in this synthesis have a sensitivity level of approximately 1:5000 (Vish and Yeshion 2004). Many are doubtful such long timespans common in archaeology would leave enough residue intact to be detected by such a test. Others are optimistic. Loy (1983:1270) says to his knowledge "no upper limit on the presence or reactivity of blood residues has been ascertained." Fullagar *et al.* (1996:742) claim the detection of blood residues is "widely accepted" and "can be identified using relatively simple assays in series, coupled with microscopic observation." Yeshion (1991:45) claims that animal peroxidase, the reactant in luminol reactions, is extremely stable to heat and time. When Sensabaugh *et al.* (1971) found, in his experiments, that blood stored in a laboratory for eight years lost approximately 60-70 percent of its original activity, some incorrectly took this to mean blood *detection* was affected the same way (Vish and Yeshion 2004). Others feel strongly that immunologically active blood residues from their experiments did not survive (Eisele *et al.* 1994). Furthermore, manganese oxide contamination of particular presumptive tests is well documented in Custer *et al*. (1988). Recently, a new method using luminol has been introduced (Vish and Yeshion 2004), but has yet to be fully scrutinized. Clearly, there are major contradictions and uncertainties in the realm of blood detection and taxa identification.

3.0 PRESUMPTIVE BLOOD TESTS AND TAXA IDENTIFICATION

3.1 Background

Proteins are found in all plants and animals. They consist of the union of amino acids and other elements necessary to life. "Protein analysis" is a general term that encompasses chemical tests designed to detect remnant proteins. This can include searching for plant, as well as animal protein. This synthesis deals with studies that investigated blood proteins and the subsequent taxa identification that was attempted in some cases.

Blood residue studies are usually comprised of two stages. The first stage utilizes presumptive tests to determine a presence or absence of blood (proteins). Because the majority of presumptive tests can be carried out relatively cheaply, artifacts can be pre-screened before being sent for more expensive taxa analysis. Occasionally, the presumptive stage is omitted. These tests are presumptive because they do not confirm the presence of questioned matter as blood, but are reactive to other compounds. These other reactive compounds can be found in both plants and elsewhere in the environment. In a forensics case, these tests would need to be confirmed with specific, confirmatory tests. Because confirmatory tests are inherently less sensitive, these tests cannot be used in an archaeological context to confirm the findings of a presumptive test.

If an artifact is determined to have been exposed to blood residue, the second step of blood residue studies involves taxa identification. This step uses the properties of antibodies and antigens to ascertain taxa. Many of the techniques used to identify the presence of blood, as well as identify taxa, have come from established forensic methods and other scientific disciplines. Researchers have steadily tried to improve these methods as more information on the techniques, as well as draw-backs, come to light. As imagined, archaeologists are not bio or molecular chemists, so the learning curve and misunderstanding of the science can lead to major problems when these techniques are applied to archaeology. More interaction and cooperation between the disciplines will further add to the understanding of these techniques and produce more useful results.

Outlined below are the current methods employed in the presumptive stage and taxa identification stage. Further understanding of the immunological principals can be found in Abbas *et al.* (1991:Chapter 3), Branden and Tooze (1991:Chapter 12), and Guttman *et al.* (1989:Chapter 1).

3.2 Stage 1: Presumptive Blood Tests

3.2.1 Hemastix®/Chemstrip®

Loy (1983) used a presumptive blood test as the first stage in his crystal morphology studies. He employed the "Chemstrip®" *urinalysis* test as a means to detect blood on artifacts. Other studies have used a common alternative called a Hemastix®, manufactured currently by Bayer. Both of the tests are referred to as a chromogen-based presumptive test. They rely on the peroxidase-like activity of the protein hemoglobin found in erythrocytes (red blood cells) to catalyze a reagent and cause a color change on a strip housing particular reagents (Roche Diagnostics Corporation 2004). The product literature that is included instructs the user to reference a provided standard color chart to interpret results. Generally, these tests have a sensitivity level of approximately 1:5000 (Vish and Yeshion 2004). To use the Chemstrip® or Hemastix®, workers derive a solution from an artifact by either placing distilled water directly on a particular area of the artifact, or by a more sophisticated means of submerging the artifact and thus exposing residues to distilled water and submitting to sonication. Though this seems to be the accepted procedure, there are variations that have been used (Fogel *et al.* 1994; Kavanagh and Ebright 1988). Another method includes extracting proteins with a five percent ammonium hydroxide solution (i.e., Loy and Hardy 1992). Either way, the fluid is then tested with a Chemstrip® or Hemastix® by dipping the product into the solution. The important item to note here is that the already relatively low sensitivity of this method is compounded when diluting a suspected stain with distilled water or other solution.

The vast majority of blood residue studies in the eastern United States have been conducted by the University of Delaware's Center for Archaeological Research. All these studies have used the Chemstrip® presumptive blood test. Neither the University of Delaware nor Loy have ever provided a satisfactory examination of the technical aspects of the Chemstrip® method, and only through the combination of Loy (1983) and Custer *et al.* (1988) does one get a more complete understanding. Although attempts were made, no information was obtained from the University of Delaware's Center for Archaeological Research for this synthesis. Virtually all information pertaining to these two methods was received from the manufacturers or from referenced reports.

According to Loy's 1983 article, the product used was the Chemstrip® #5 from Boehringer Mannheim, now manufactured by Roche Diagnostics. Loy (1983) states the

Chemstrip® #5 will react with two other compounds; myoglobin (a protein found in muscle tissue and less commonly, blood) and chlorophyll. Chlorophyll would be considered a false positive, but myoglobin could still be useful as an indication of blood residue that could ultimately undergo taxa identification. Though Custer *et al.* (1988) found manganese oxide to cause false positives, no other false positives in the product literature such as formulin (a preservative of formaldehyde and water) or strongly oxidizing cleaning agents were mentioned in any articles. Personal communication with Roche Diagnostic's technical support gave no insight to chlorophyll or manganese oxide contamination. Personal communication from Bayer Health Care warned that using strong oxidants, such as a five percent ammonia solution used to extract residues in Loy and Hardy's 1992 study, could cause a false positive. Bayer Health Care never conducted research, nor did they have any data concerning whether Hemastix® can be used to detect dried blood from artifacts after using a five percent ammonia solution to re-hydrate dried material. Bayer Health Care went on to say it neither promotes nor recommends these procedures. This directly contradicts the statement made in Loy and Hardy (1992) when they state "The Hemastix test when used with distilled water or 5% ammonia as the solvent is not subject to false positive indication" (Loy and Hardy 1992:26). The Loy and Hardy statement is also ambiguous in that using an ammonia solution or distilled water would not prevent the possibility of a false positive from an unknown source from affecting the test. It is also apparent they were not informed that the ammonia solution they are using to re-hydrate residues could cause a false positive. It would be useful to know such background information, which could lead to understanding unforeseen relationships in complex chemical environments. With specific regard to manganese oxide causing a false positive with Chemstrips®, this may be particularly pertinent in the state of Delaware where most of the University of Delaware's Center for Archaeological Research studies have been conducted. The more acidic a soil is, the more manganese that is available in the soil (Schulte and Kelling 1999). Delaware has very acidic soils. In other locations where soils are more basic, contamination from manganese enriched fertilizers is another possibility.

It is important to note that the Chemstrip® and Hemastix® tests are *specifically* designed to be utilized in a *urine* environment in the diagnosis of particular disorders, and the manufacturer's technical support personnel went on to say (though no studies exist to back up their claim) that they could not vouch for the efficacy of the test in an environment other than urine. The consequence of that fact, as it pertains to archaeology, is unknown, but it is important to understand this product's intended use.

3.2.2 Luminol

Yeshion (1991:379) says the luminol test is a “chemiluminescent reaction based upon its oxidation in an alkaline solution in the presence of an oxidizing agent ($NaBO_3$) and a peroxidase system as found within the hemoglobin molecule of blood.” Luminol can be used as a presumptive blood test and glows a bluish white when reacting to a suspected blood stain. It is a self-contained, chemiluminescent reaction that is applied in a dark environment so that the reaction can be observed. Recently, it has been investigated as another tool, if not an alternative to Chemstrips® and Hemastix® (Vish and Yeshion 2004). Advantages over Chemstrips® include its higher sensitivity (1:1,000,000) and ease of use. No additional step of deriving a solution is needed; therefore, the maximum sensitivity can be brought to bear on the potential residue without further degrading its concentration prior to detection. Luminol is sprayed directly on the artifact, and areas of reactivity can be seen in real time. Like Chemstrips® and Hemastix®, this reaction is an oxidation reaction, which means one can use up the reactant, making any further tests negative. But luminol will not *chemically* prevent any further testing on remaining blood residue (Yeshion 2001:77). Luminol dries as a white powder and can be easily removed from an artifact.

Yeshion (personal communication 2004) reports false positives can be caused by peroxidases found in lettuce, cabbage, horseradish, garlic, tomato, and potato, none of which are native to North America. In addition, copper, nickel, and iron can cause false reactions. These can be discerned through the experience of the researcher based on the intensity and dynamics of the reaction. Future investigation of luminol should include ascertaining whether manganese oxide causes a false positive. Testing of soils for contamination prior to a study would prevent any confusion in results.

3.3 Stage 2: Taxa Identification Tests

3.3.1 Comparative Crystal Morphology

In general, comparative crystal morphology relies on the properties of hemoglobin of different species having different morphology based on the variations in amino acid sequencing. By comparing the crystallization morphology of known blood molecules to unknown molecules, taxa of the unknown molecules can be identified. This method has been abandoned for several reasons, including sample contamination (Custer *et al.* 1988; Hyland *et al.* 1990:106; Smith and Wilson 1992:238) and environmental degradation (Smith and Wilson 1992:238). For a complete understanding, see Loy (1983).

3.3.2 Isoelectric Focusing

This method employs electrophoresis (see Cross-over immuno electrophoresis section below) and compares isoelectric points of known reference samples to that of unknown blood samples, thus identifying the species of origin. According to Righetti (1983) this method has the capability of high resolution separation of different protein molecules that exceeds other electrophoresis methods. More on the technique can be found in Loy (1987), Loy and Nelson (1987), and Nelson *et al.* (1986).

3.3.3 Immunological Assays

The principals of antibodies' (antisera) reaction to foreign proteins (antigens, an unknown residue in the case of artifacts) is the basis for all immunological assays used in taxa identification. In general, antisera from a known species is brought in contact with protein from an unknown residue. If the antisera of the known species reacts with the unknown protein by forming a precipitation, then the unknown residue is determined to be of that species.

According to Newmann *et al.* (1993), soil samples should be tested for contamination of coprolites and modern feces, as these contain proteins that would skew results. This statement is partially correct, as coprolites, or fossilized feces, would certainly not contain immunologically active proteins, but unfossilized feces would contain proteins that could give erroneous results. Authors have pointed out the fact that many known

species' antibodies will react with other species' antigens (LeeDecker *et al.* 2001; Hyland *et al.* 1990). This is due to complex reasons, but simplistically, antisera from a particular animal will react with other animals that are evolutionarily closely related. Thus, for example, one may identify blood from the deer *family* (Cervidae), but this does nothing to indicate whether the blood belongs to deer, moose, or elk. It must be understood that the majority of these studies used antisera that is produced by pharmaceutical companies to be used in other biological and immunological endeavors. Archaeology adapts these products to their studies and must deal with the inadequacies inherent in these products. Some of these inadequacies include antisera that will identify blood only to the family or genus level. Very few commercially produced antisera are specific to the species level.

There are several techniques of testing proteins with anitsera that differ slightly in such aspects as sensitivity and the mechanics behind the procedures. These are outlined below.

3.3.3.1 Cross-over Immuno-electrophoresis (CIEP)

This method uses electrophoresis to separate proteins prior to testing with antisera. Electrophoresis is a method to separate individual molecules based on electrostatic properties, by running an electric current through various liquid or gel mediums. In this case, unknown proteins (unknown residue in the case of artifacts) are suspended in a gel and then separated in this fashion. Methods were developed so certain criteria could be used to separate proteins based on molecular weight or other physical properties. This ultimately lets a researcher know if a particular kind of protein is present, such as plant or animal protein. From here, these proteins are then tested with antisera from particular taxa in hopes of identifying which taxa the unknown proteins come from.

Double antibody methods are an attempt to make the taxa identification step more specific. They are all more sensitive than CIEP alone and include the following methods.

3.3.3.2 Radio Immuno Assay (RIA)

RIA uses radioactive isotopes linked to a second antibody. However, the radioactivity can make this a dangerous method to use (Hyland *et al.* 1990:106).

3.3.3.3 Enzyme-linked Immunosorbent Assay (ELISA)

In ELISA, color sensitive enzymes are linked to a second antibody. Problems include the instability of proteins in the environment (Catteneo *et al.* 1993:40). A better understanding can be found in Engvall (1980), Hyland and Anderson (1990), Hyland *et al.* (1990) and Hyland *et al.* (1994).

3.3.3.4 Gold Immuno Assay (GIA)

GIA uses colloid gold to respond to specific molecular weights of proteins. Problems include false positives because of bacterial contamination due to the extreme sensitivity of the method (Newmann *et al*. 1993:679).

3.3.3.5 Ouchterlony Double-Diffusion

Ouchterlony Double-Diffision is another method of immuno-precipitation. This method doesn't use electrophoresis to separate proteins. This assay has been criticized for its lack of sensitivity.

4.0 REVIEW OF BLOOD RESIDUE STUDIES IN THE DELAWARE REGION

A total of 32 sites from the states of Delaware, Maryland, and Pennsylvania has been evaluated for this synthesis. By far, the most numerous blood residue studies have occurred in Delaware, followed by Maryland. The sites summarized in the synthesis include extractive stations, macroband base camps, habitation sites, hunting/processing camps, small base camps or procurement staging sites, micro-band base camps, short-term transient camps, staging/processing stations, a series of single-family wintertime houses, procurement and processing stations for game and plant resources, and fishing camps. The reports are presented chronologically by state.

4.1 Delaware Studies

4.1.1 **Hockessin Valley Site (7NC-A-17)** (Custer and Hodney 1989)

The Hockessin Valley site is described as a small base camp or procurement staging site in New Castle County, Delaware. The site lies within the Piedmont Uplands physiographic province. One structure was identified and returned a date of 5,205±70 years B.P. (Custer and Hodney 1989).

The blood residue study at the Hockessin Valley site was conducted by the University of Delaware's Center for Archaeological Research using protocol outlined in Custer *et al.* (1988). This protocol utilizes the Chemstrip® presumptive test. During tests to determine any contamination, 103 of 422 soils were positive. Although the authors state the contaminated tests originated throughout the site, they claim the area of the structure was not contaminated.

Of 377 tests conducted on 264 artifacts, only seven positives were reported. Two were then dropped due to fear of contamination. As with most of the work from these authors, the over-simplified statement was made that the tools were used for either hunting or game processing. With no use-wear analysis and no taxa identification, information from these presumptive tests does little to further the understanding of the use of the artifacts.

The report included a map of excavation units that were considered to be contaminated. When looking at the distribution of the contaminated areas, there is no sense that there were any areas of the site that could have safely been tested. In addition, it seems there are areas where tests for contamination were not carried out because no excavation took place, yet these areas are next to supposedly "safe" areas where positive

blood residue results were accepted. There is no way of knowing whether these unexcavated areas could have influenced the "safe" areas where results were accepted. Also, the authors do not say what the contamination is from, only adding to the unpredictability of where and how a contaminant could affect the site. This study starts out well by investigating any potential contamination, but falls short when contamination is found and then ignored.

An alternative explanation of the contamination results is that there is a heterogeneous but low level of contamination throughout the site. At certain control points, the contamination level was sufficient to yield a positive test result, and in other areas the contamination level did not reach that threshold. Without knowing the source and mechanism of the contamination, the researchers were not justified in declaring certain areas safe for artifact testing. The subject of site genesis and how it relates to contamination and blood residue is one area that is little understood. With the probable contamination, the testing results on the artifacts from this site cannot be trusted. In addition, no taxa identification was attempted.

4.1.2 Lewden Green Site (7NC-E-9) (Custer *et al.* 1990)

The Lewden Green site is located in New Castle County, Delaware and lies within the High Coastal Plain Section of the Atlantic Coastal Plain physiographic province. This site is described as a micro-band base camp entirely within a plowzone context. Occupation periods range from Woodland I to Woodland II (Custer *et al.* 1990).

This study, conducted by the University of Delaware's Center for Archaeological Research, used the Chemstrip® method (Custer *et al.* 1988) once again as a presumptive blood test on 176 artifacts. Forty-seven positives were reported. The abstract of this report states there is a habitation area separate from several processing locations. The blood analysis contradicts this by claiming that, due to the widely dispersed positive artifacts, "no specific animal processing area is apparent" (Custer *et al.* 1990:46). Though morphology and the presence of blood on an artifact can provide some confidence in ascribed use, no use-wear analysis was done on the positive artifacts that would have underscored the stated artifact functions. The statement claiming that the negative artifacts with the same morphology as the positive artifacts had a different uses does not seem logical. Given the doubts concerning the Chemstrip® test, it would be more likely that the negative artifacts simply lost whatever blood residue may have been on them, or the amount was too small to

be detected with a Chemstrip®. An *a priori* assumption was made that the Chemstrip® test was infallible and that it was accurately identifying which artifacts had *ever* had blood on them. A use-wear analysis could have provided evidence to support or refute this position. And again, no taxa identification was attempted.

4.1.3 Paradise Lane Site (7NC-D-125) (Riley and Custer *et al.* 1994)

The Paradise Lane site is located in New Castle County, Delaware in a transitional area between the Fall Line and High Coastal Plain physiographic provinces. It is described as a staging/processing station with a Woodland I occupation (Riley and Custer *et al.* 1994).

Artifacts from this site were submitted to Chemstrip® testing using the protocol established by the University of Delaware's Center for Archaeological Research (Custer *et al.* 1988). Appropriate control tests for contamination of any kind were conducted and all tests were negative. One hundred thirty-one tests were then conducted on a total of 43 bifaces and flake tools. All of these tests were negative and no taxa identification was attempted on any of the artifacts. A closing statement is given by the authors that the results indicate that blood is not *now* present on the artifacts. Again, these results may simply indicate a lack of sensitivity on the part of Chemstrips®.

4.1.4 Brennan Site (7NC-F-61A) (Watson and Riley 1994)

The Brennan site is located in New Castle County, Delaware and lies within the Atlantic Coastal Plain physiographic province. The site served as a procurement base utilizing the nearby Delaware Chalcedony Complex. Diagnostic artifacts indicate that the site had a Woodland I occupation, approximately 3,000 B.C. to A.D. 1500 (Watson and Riley 1994).

Artifacts from this site were submitted to Chemstrip® testing using the protocol established by the University of Delaware's Center for Archaeological Research (Custer *et al.* 1988). Appropriate control tests for contamination of any kind were conducted. The authors report all the tests were "negative, or only slightly positive" (Watson and Riley 1994:26). The authors go on to establish that, based on the results of the control tests, they would only consider an artifact to be positive for blood residue if there is a "strong" positive reaction to the Chemstrip®. This demonstrates a profound misunderstanding of these tests. The Chemstrip® test can require a somewhat subjective interpretation. When used

medically, results should be compared to a color chart that comes with the test. Reactions indicated on the Chemstrip® can vary in intensity based on the concentrations at hand. A sample that is *known* to be blood may have a strong indication or a weak indication. Any contaminant that can cause a false positive with a Chemstrip® can behave the same way. The fact that the authors suggest only a "strong" positive was accepted as an indication of blood is completely unreliable. A "strong" positive indicating blood residue could likewise have been a "strong" indication for a contaminant.

According to the report, approximately 13 percent of the debitage from the site was tested with Chemstrips®. The reasoning for testing debitage is unknown, but seems to have been an attempt to perhaps screen for utilized flakes. The most useful information certainly is derived from the testing of tools or items used in everyday tasks, not items that were thrown away as debitage. Much of this work could have been done more efficiently by utilizing luminol as the presumptive test on these artifacts. Many were tested multiple times in different areas. The use of luminol could have tested the entire artifact, simultaneously indicating any areas of reactivity in real time (Vish and Yeshion 2004). All these tests were negative.

A second level of testing included a small number of tools and utilized flakes. The authors state that several of the utilized flakes were identified only after they were washed and not tested. They should have been identified in a use-wear analysis. It is unlikely the sensitivity of the Chemstrip® test would be useful in testing these artifacts after washing. However, the sensitivity of luminol may be useful in such a case by being capable of detecting minute amounts of blood (1:1,000,000), even after attempts have been made to wash the artifacts (Vish and Yeshion 2004). All tests on these tools were also negative, and no taxa identification was attempted. A closing statement by the authors claims that the results indicate that blood is not *now* present on the artifacts. Again, these results may simply indicate a lack of sensitivity on the part of Chemstrips®.

4.1.5 Site 7K-C-360 and Dover Downs Site (7K-C-365A and B)
(Riley and Watson *et al.* 1994)

Site 7K-C-360 and the Dover Downs site are located in Kent County, Delaware and lie within the Mid-Drainage Zone of the Low Coastal Plain physiographic province. These sites are described as short-term, transient camps. Artifacts from the Archaic and Woodland I periods were present at Site 7K-C-360. Locus A of the Dover Downs site

yielded material from all pre-contact periods, and Locus B was occupied in the Woodland I and Woodland II periods (Riley and Watson *et al.* 1994).

Artifacts from this site were submitted to Chemstrip® testing using the protocol established by the University of Delaware's Center for Archaeological Research (Custer *et al.* 1988). Appropriate control tests for contamination of any kind were conducted, with no contamination reported. Three hundred thirty-one individual tests were conducted on 115 tools and 634 tests were conducted on 207 pieces of debitage, for a total of 965 tests for Site 7K-C-360. At the Dover Downs site, 139 individual tests on 50 tools and 810 individual tests on 394 flakes were conducted, for a total of 949 tests. All tested artifacts at Site 7K-C-360 were negative.

At the Dover Downs site, seven artifacts returned nine "slightly" positive results. These positive tools were found among debitage that tested negative. The authors state that two of these tools made of argillite were considered to be "dubious" in their results based on the porous, weathering nature of argillite. Any blood on the surface of the tools may have disappeared with the erosion of the surface. But why doesn't this make the other positives questionable? If what is suggested about the weathering nature of the argillite is true, then the authors received a false positive on these items. It puts into question whether the other artifacts returned false positives as well.

The reasoning for testing debitage is unknown, but seems to have been an attempt to perhaps screen for utilized flakes. The most useful information certainly is derived from the testing of tools or items used in everyday tasks, not items that were thrown away as debitage. Much of this work could have been done more efficiently by utilizing luminol as the presumptive test on these artifacts. Many were tested multiple times in different areas. The use of luminol could have tested the entire artifact, simultaneously indicating any areas of reactivity in real time (Vish and Yeshion 2004).

The authors go on to interpret this information from the Dover Downs site to be an example of butchering activity, given the slightly positive tools lying among negative debitage. This is another example of a quick conclusion drawn on little evidence. No integrated use-wear was done along with this blood analysis. In light of this fact, it would be possible the artifacts were discards with blood from somewhere else, or perhaps could prove to be made by the use of a deer bone or antler as a hammer.

A closing statement by the authors is given that the negative results at Site 7K-C-360 indicate that blood is not *now* present on the artifacts. Again, these results may simply indicate a lack of sensitivity on the part of Chemstrips®.

4.1.6 **Snapp Prehistoric Site (7NC-G-101)** (Custer and Silber 1995)

The Snapp Prehistoric site is located in New Castle County, Delaware and lies in the Upper Coastal Plain physiographic province. It is described as a large base camp and has occupations spanning approximately 8,000 B.C. to A.D. 1500 (Custer and Silber 1995).

Artifacts from this site were submitted to Chemstrip® testing using the protocol established by the University of Delaware's Center for Archaeological Research (Custer *et al.* 1988). Appropriate control tests for contamination of any kind were conducted, with no contamination reported. Two hundred forty-nine tests were conducted on 110 tools. One chert flake and one biface returned positive results. Five other tests returned "semi-positive" results, including an additional test on the originally positive biface. The authors claim these tests imply that only a very faint trance of hemoglobin remained on the tools, and based on methodology, it is true that after testing the biface twice, perhaps only a small amount of blood residue remained after the first test. Based on these results, the authors tentatively suggest that butchering may have taken place at certain feature clusters of the site. The authors also state their surprise at blood on the particular biface that does not show any use as a tool (the challenges of use-wear analysis are addressed in a separate document [Espenshade 2005]). No taxa identification was attempted on any of the artifacts.

This is another example of a study that did not integrate any use-wear analysis. Though the authors claim that use-wear was conducted, no data is presented in a way that allows cross reference or comparison to artifacts submitted for blood residue analysis. With the lack of any taxa identification, many explanations are still possible for the biface that was positive for blood residue, but showed no signs of use. The artifact could have absorbed human blood in the manufacture process as a result of damaged human hands, or an individual could have had fresh blood on their hands and touched the unused biface. The biface could have pierced an animal once and shown no signs of use. The artifact could have been manufactured using a deer bone or antler. One could even imagine a biface near a kill that somehow came into contact with blood by proximity, or even endless scenarios concerning abstract ritual practices.

4.1.7 **Wrangle Hill Prehistoric Site (7NC-G-105)** (Custer *et al.* 1995)

The Wrangle Hill Prehistoric site is located in New Castle County, Delaware and lies within the High Coastal Plain Section of the Atlantic Coastal Plain physiographic province.

Occupation of this site lasted from approximately 3,000 B.C. to A.D. 1500 (Custer *et al.* 1995).

The report from this site (Custer *et al.* 1995) does not indicate how many or what kind of items were tested. All the blood residue tests were negative. No indication is given concerning the types of tests used, but again, based on similar reports from the same author, it is assumed that the Chemstrip® presumptive test was used. No taxa identification was attempted.

4.1.8 Carey Farm Site (7K-D-3) and Island Farm Site (7K-C-13)
(Custer and Watson *et al.* 1996)

These sites, located along the St. Jones River south of Dover, in Kent County, Delaware, are described as pre-contact habitation sites. The sites are within the Mid-Drainage Zone of the Low Coastal Plain physiographic province. These sites were occupied from approximately 3,000 B.C. to A.D. 1500 (Custer and Watson *et al.* 1996).

A total of 727 presumptive blood residue tests was conducted on artifacts from these sites that returned three "slightly" positive results. The report does not indicate how many or what kinds of artifacts are represented, nor does it cite what method was used to test the artifacts. The authors also do not say whether control areas were tested for false positives. Most of the studies in Delaware can be attributed to the University of Delaware's Center for Archaeological Research, whose use of the "Chemstrip®" method is better documented in other articles (Custer *et al.* 1988). It would have been more useful and consistent if these methods were outlined, regardless of the lack of results. No taxa identification was attempted.

A common theme with the Delaware studies, and any other study cited in this synthesis that used the "Chemstrip®" method, is that of sensitivity. The Chemstrip® method is a chromogen-based presumptive blood test with a sensitivity of approximately 1:5000 (Vish and Yeshion 2004). To perform the test, one must literally further dilute any minuscule amount of blood residue by using distilled water to derive a fluid from the area in question and then test it with the Chemstrip®. This fact could explain why many of the blood residue studies using Chemstrips® have reported negative presumptive tests, but then later report positives and even taxa identification in the second stage.

4.1.9 Leipsic Site (7K-C-194A) (Custer and Riley *et al.* 1996)

The Leipsic site has been described as a large pre-contact habitation site in the Leipsic River Valley in Kent County, Delaware and lies within the Low Coastal Plain Section of the Atlantic Coastal Plain physiographic province. The occupation of the site spans the period from approximately 8,000 B.C. to A.D. 1500 (Custer and Riley *et al.* 1996).

A total of 680 artifacts was tested from the Leipsic site using the Chemstrip® method outlined by Custer *et al.* (1988). The artifacts tested included tools and debitage. Soil samples from various areas of the site were tested for known false positives, but none were found. All tests were negative. No taxa identification was attempted.

The author's statement that the negative results do not indicate that blood was *never* present on the artifact, just that blood is *now* not present, is somewhat misleading. This statement was included in almost every report from the University of Delaware's Center for Archaeological Research that turned in negative results on all artifacts tested. At the time of these reports, this may have been believed to be true based on the available test used, the Chemstrip® presumptive test. But again, this test only has a sensitivity of approximately 1:5000. A more correct statement would be *the absence of positive reactions may either indicate the absence of blood residue on the artifacts, or that the Chemstrip® was not sensitive enough to detect what amount may have been present.*

4.1.10 Two Guys Site (7S-F-68) (LeeDecker *et al.* 1996)

The Two Guys site is located in Sussex County, Delaware and lies within the Atlantic Coastal Plain physiographic province. The site is described as being used on a seasonal basis from approximately 6,000-8,000 B.C. up to approximately 500 B.C.-A.D. 800 (LeeDecker *et al.* 1996).

A total of 186 artifacts was tested from the site using the Chemstrip® method (Custer *et al.* 1988). Only seven had positive results in the presumptive stage: one biface and six pieces of debitage. For the second stage of taxa identification, these seven artifacts that tested positive and 43 other artifacts that tested negative in the presumptive stage, were submitted for testing. The other 43 artifacts were included to check the efficacy of the presumptive and taxa identification stages.

Final results included identification of the following taxa: deer, rabbit, dog, guinea pig, bovine, bear, and chicken. An interesting array of inconsistencies occurred with these

tests. Of the seven artifacts that originally tested positive in the presumptive stage, only one eventually returned a positive result (and later identified taxa) in the taxa stage. A possible explanation for the other negatives in the taxa stage is that the positives in the presumptive stage were false positives. It is also possible in the presumptive stage that all residue was destroyed in the reaction, leaving nothing to detect in the taxa stage.

The second inconsistency reported was that an artifact that tested negative in the presumptive stage, tested positive in the taxa identification stage. As described earlier (see Presumptive Blood Tests and Taxa Identification section), the Chemstrip® test has an inherent dilution aspect. If a minute amount of residue is present on an artifact, diluting it with distilled water may cause the reactant (blood) to be diluted past the sensitivity of the Chemstrip®, but could still return a positive when tested with the more sensitive taxa identification tests. The authors go on to say this may have been the result of deriving a fluid from different areas for the different tests; thus, in the presumptive stage, they missed the residue, but encountered it from a different area for the taxa identification. This underscores why testing artifacts in multiple locations is problematic and should have been foreseen by the researchers. One must question what the reason is for ascertaining that blood from a point, for example, came from the tip as opposed to the margin. Researchers should have simply tested solutions derived from the entire artifact by sonication. The authors end by stating that the Chemstrip® presumptive blood test is not an effective method to test artifacts. If the researchers did have a need to delineate the areas of reactivity, the use of luminol would clearly have shown areas of reactivity in real time in relation to the artifact (Vish and Yeshion 2004).

4.1.11 Pollack Prehistoric Site (7K-C-203) (Custer *et al.* 1997)

The Pollack Prehistoric site is described as a large base camp along the Leipsic River in Kent County, Delaware and lies within the Mid-Drainage Zone of the Atlantic Coastal Plain (Custer *et al.* 1997). Major occupations occurred during the Woodland I and Woodland II time periods.

A total of 967 presumptive blood residue tests was performed, with no indication of how many artifacts this comprised. The method used is not indicated, but precedent implies the Chemstrip® method of Custer *et al.* (1988) was followed. Control tests on soils were negative for false positives. Only three artifacts returned positive results.

One flake with no signs of edge retouch or utilization from a disturbed plowzone context and a similar flake from a subsoil area were among the positives. These artifacts were interpreted to be cutting and scraping tools for processing game animals and fish (Custer *et al.* 1997), even though they showed no signs of use. The authors also interpret a positive point fragment as having been used in the killing of game. Perhaps a better explanation for blood on these tools would be that *blood arrived on the tools by unknown means*. No taxa identification was attempted.

These interpretations bring up a point to be considered in all blood residue studies. It is difficult to ascribe functions to tools based on their morphology and the presence of blood, without corroborating evidence of use or wear. Without use-wear on the two flakes from this site, it is difficult to explain the presence of blood as being from the processing of game animals or fish. It could be that someone simply had blood on their hands and touched the flakes in question, having never used them in any fashion. Alternately, low frequencies of positives on artifacts without visible use-wear may reflect the use of antler or bone hammers in the making of the flakes. Likewise, because no taxa identification was attempted, it could have been human blood deposited there as the result of cuts on the hand during knapping.

4.1.12 Lums Pond Site (7NC-F-18) (Petraglia *et al.* 1998)

This site lies next to a tributary of the former St. Georges Creek in New Castle County, Delaware and had occupation levels ranging from the Archaic through Woodland II periods. The site is within the High Coastal Plain Section of the Atlantic Coastal Plain physiographic province of Delaware (Petraglia *et al.* 1998).

The researchers at the Lums Pond site approached blood residue analysis from a skeptical angle. Not sure what to make of the then current state of blood residue analysis, they opted to have the Conservation Analytical Laboratory of the Smithsonian Institution conduct amino acid and blood residue analysis on artifacts from the site. Amino acids are constituents of proteins. This test is first carried out to ascertain if any protein residue is on the artifacts. Any positives from this analysis would qualify the artifacts to be submitted to further analysis.

Of 22 amino acid analyses, eight showed evidence of protein residues. A subset of these extracts were submitted to gel electrophoresis. Gel electrophoresis can separate proteins based on their molecular weight. This can show researchers which kind of protein

may be present in a sample (i.e., plant protein, protein from blood, or some other protein that may result from contamination). Researchers can then test the specific animal proteins of their choice with antisera of specific species. Based on the gel electrophoresis, three samples showed that the protein albumin was present. These were tested with deer antisera and one produced a reaction, suggesting the other two were of a different species. All three of these samples were again checked with horse antisera to be sure researchers were getting proper results. Since horses were extinct from North America at the time these artifacts would have been utilized, checking them with horse antisera would provide a way to ensure the tests were not compromised somehow. When tested with horse antisera, none of the samples reacted. A conclusion was reached that deer blood was truly present on only one specific artifact.

The benefit of this study lies in the logical and organized manner by which the study progressed. The only item that would have improved this study would have been the addition of a specifically tailored group of antisera, such as that used at the Puncheon Run site (see below), to test residues.

The authors conclude that immunological assays that do not use molecular weights of proteins in the analysis should not be used for taxa identification. This seems to be a valuable step in the process and can help identify particular groups of proteins as coming from plant, animal, or elsewhere. As it is, the Lums Pond site study is perhaps the most reliable analysis used in this synthesis.

4.1.13 Puncheon Run Site (7K-C-51) (LeeDecker *et al.* 2001)

The Puncheon Run site is located at the confluence of the St. Georges River and Puncheon Run in Kent County, Delaware and lies within the Atlantic Coastal Plain physiographic province. The multiple occupation areas range from 3,000 B.C. to A.D. 1500 (LeeDecker *et al.* 2001).

For a study of this size and complexity, the authors provide a very clear and useful documentation of the science and what procedures were used. The needed background and a logical critique provide an excellent blood residue narrative section, but the study results are problematic.

The researchers at the Puncheon Run site developed a creative approach to the site's blood residue analysis by developing specific antisera from *local* species to be used in conjunction with commercially bought antisera for their taxa identification study. It was

hoped that the specific, local antisera would remedy taxa results of other studies that were much too general. These specific antisera were determined by looking at environmental, traditional, and ethnographic information concerning food sources. Though this may not be possible at all sites, it could greatly improve the meaningfulness of taxa results. All positive reactions were re-tested with diluted antisera to increase specificity of the CIEP procedure and weed out false positives. It is the subsequent positives that were reported.

This brings up a controversial and very confusing point. Personal communication with one of the participating laboratories, Paleo Research, claims this dilution step was part of the procedure. However, the reports present this step as an afterthought, in response to a specific problem, concerning the over-sensitivity and lack of specificity of CIEP analysis. All artifacts that originally tested positive were resubmitted to the test using a "diluted" version of antisera. It is not apparent why a diluted concentration is simply not used from the beginning and why a secondary step is necessary. This procedure has been carried out in other blood residue studies using CIEP and usually entails diluting antisera to 1:10 or 1:20 to increase specificity. This intuitively does not make sense. What the authors seem to be saying is, based on the concentration of the antisera (i.e., if you dilute it), false positives will be eliminated and only the true immunological reaction, if present, will take place.

This dilution procedure may actually be what other authors describe as first testing the samples against pre-immune serum (see Indian Creek V site below, LeeDecker *et al.* 1991). Pre-immune serum is derived from animals that have not been inoculated and, therefore, would not cause a reaction to any of the unknown residues when their antisera is brought into contact with it. If there are any positives, this would indicate non-specific protein reactions resulting from some form of contaminant. If any positives are returned during this step, they are retested against pre-immune serum a second time with a detergent added. Adding detergent breaks the weak non-specific protein reactions, prepares the extract for testing against species antisera, and ensures no further non-specific reactions can occur. At this point, one can basically think of the extract as inert. If any positives are returned after testing against pre-immune serum once the detergent is added, these artifacts would be dropped from further testing. In light of this information, one has to wonder whether or not the word *dilute* has been used properly in this report. The method utilizing detergent is not diluting anything, it is changing the chemical properties of the extract. There is a distinct difference.

In addition to testing actual archaeological artifacts, replicated artifacts were also tested at the Puncheon Run site by processing freshly killed fish of different species. This is

another aspect of the study that makes the Puncheon Run information so useful. Many studies have been carried out on actual pre-contact artifacts, but they overlook how well researchers can use the tools of the trade on modern examples and how they can compare to actual archaeological artifacts being tested. Any current or future tests, both presumptive and taxa identification, should have a history of consistent results on fresh, replicated samples. Regardless of the outcome, this information goes far in lending confidence or causing one to have caution where certain techniques are concerned.

At the Puncheon Run site, 10 replicated artifacts were made and some were tainted with blood from known fish species, while others were not stained at all. These artifacts were then submitted for CIEP analysis along with pre-contact artifacts. The results from these replicated artifacts created an interesting and poignant scenario. If looked at as a simplified version of a study conducted on pre-contact artifacts, the results are typical, with many confusing combinations of outcomes. Of the 10 replicated artifacts, the known taxon of only one artifact was correctly identified. A second artifact returned two different positive results; one was the correct taxon. When the CIEP method was used to test replicated bifaces, some reacted to deer antisera (LeeDecker *et al.* 2001). These particular replicated artifacts had never been used to butcher, nor had they ever pierced a deer. However, deer antlers were used in the manufacture of these tools. This may be the only evidence found that claims deer antler may be responsible for a tool's reaction to deer antisera. This brings up the interesting question of whether bone and antler tools used in knapping could produce positive taxa results for the animals from which they came. Though it's a small example, if these artifacts were placed in a pre-contact context on the Lewden Green site (see above), with no use-wear analysis done, they could have been explained to have been part of a butchering and game processing area, and thus, the entire interpretation of the site would have been incorrect. Unfortunately, these results are not promising. The authors note that mold growth on the replicated artifacts may have played a role in the poor results.

The authors state they had a 40 percent "success" rate identifying the control samples, and they compare that to other studies where similar experiments were conducted. This percentage is discouraging for an experiment on known, modern blood samples and is either a reflection of the methods, or unknown contamination. Such experiments under perfect conditions would not be satisfactory unless above 90 percent success rates were reported. If this is the best we can do on known, controlled, modern samples, then the probability of archaeologists correctly identifying blood residue on pre-contact artifacts is low.

The Puncheon Run site report states that 73 pre-contact artifacts were submitted to CIEP analysis (LeeDecker *et al.* 2001: Volume II:I-7). On another page, the report states 83 artifacts in the first paragraph and 75 artifacts in the third paragraph (LeeDecker *et al.* 2001:Volume II:711). Therefore, the actual number of artifacts tested is unknown. A total of 25 positive results are reported. The authors report a 24.7 percent "success" rate and compare their results to other reports that disclose a percentage of the tested assemblage as being positive. It's unclear what value these statistics hold, let alone when comparing it to other sites.

The majority of the taxa identified included aquatic taxa. Though this site provides a very good use-wear study, it does not add much to the blood residue interpretations. Only three artifacts tested for blood residue could be found within the use-wear analysis data. These included two projectile points, one with residue identified as coming from Striped Bass, the other with residue identified as coming from Gizzard Shad. One uniface with hafting polish had residue identified as coming from American Eel. Though not extensive, these artifacts provide an example of utilizing multiple lines of available information to formulate a tool's function. In this case however, the use-wear analysis does not lend confidence to the blood residue study. It is unlikely that Striped Bass or Gizzard Shad would be spear-fished instead of netted. It is unlikely a scraper would be used in some fashion on an eel. To take the argument further, one may even question why a projectile point would be needed to spear a fish of this size when a sharpened stick and/or bone implement would work.

This discussion underscores the need to improve these analytical techniques. The Puncheon Run site is a fine example of designing a specific testing regime utilizing antisera tailored to the study. This aspect would be a recommended step in any future taxa identification study. However, the CIEP results are not reliable. As confirmation, the authors close by writing an in-depth section on subsistence and food resources available to the people at the Puncheon Run site, even though they could not correctly identify modern, known, control blood residue on artifacts they manufactured. Not having proven the worth and efficacy of the CIEP method on replicated artifacts renders their conclusions meaningless.

4.2 Pennsylvania Studies

4.2.1 **Shoop Site (36DA20)** (Hyland *et al.* 1990)

Although this Paleoindian site has had a long history, blood residue studies were not conducted until 1989 (Hyland *et al.* 1990). This study introduced a new technique of blood residue study in the form of Enzyme Immuno Sorbent/Assay (EIA).

Forty-five previously excavated artifacts from the Shoop site were submitted to blood residue analysis using Hemastix® for the presumptive stage and Enzyme Immunosorbent Assay (EIA) for the taxa identification stage. Thirteen of these artifacts returned positive results with Hemastix®. These 13 artifacts were then tested using EIA only for deer antigens. Only one artifact reacted positively to this test. The authors (Hyland *et al.* 1990) explain the lack of results for the remaining 12 artifacts as being possibly:

- dirt and other sediment incorporated during the extraction process that could have caused a mis-reading of the Hemastix®, thus a false positive;
- environmental contaminants that caused false positives;
- blood other than deer that was present on the artifacts; or
- the amount of residue that could have been reduced to the point that the EIA test could not detect it.

The authors point out a disadvantage of the EIA method as being its inability to distinguish multiple species' blood on a single artifact.

The authors close by stating that their data *clearly* indicates EIA provides an effective method of identifying taxa of blood residue. Conclusions like this, and from other reports, that state the efficacy of particular methods should be viewed skeptically. A success rate cannot be claimed if the true number of blood stained artifacts is unknown.

4.2.2 **Site 36BV292** (Knepper and Petraglia 1993)

Site 36BV292 is located in the Pittsburgh Low Plateaus Section of the Appalachian Plateaus physiographic province along a floodplain of the Connoquenessing Creek in

Beaver County, Pennsylvania. The site was occupied from the Late Archaic through Middle Woodland periods (Knepper and Petraglia 1993).

A total of 25 artifacts was analyzed from this site. The authors do not indicate whether any presumptive tests were carried out before using CIEP analysis. They state that residues were extracted using a five percent ammonium hydroxide solution, a method also used by Loy and Hardy (1992). After deriving extracts from all of the artifacts, this fluid was first tested with "pre-immune" serum to ascertain any false positives (see Puncheon Run site for more information). Two artifacts returned positive results and were subsequently dropped from further testing. Eleven artifacts ultimately returned positive results, ranging from deer, dog, chicken, guinea pig, rabbit, and mouse. Like many other studies, this analysis lacked an integrated use-wear analysis.

The authors report a percentage of the tested assemblage as being positive, and cite several other reports as doing the same. It is unclear what the value of this statistic is. If the goal is to give an expression of the ability to discern positives from *all* the artifacts found at a site, the authors are using the wrong value and are simply reporting the percentage of positives from *tested* artifacts. It would not be possible to give the true percentage of total artifacts exposed to blood at a site. If the authors wish to give a percentage reflecting the acceptability of their experiment based on preconceived distributions as they relate to morphology, then this is not recommended either. Such preconceived distributions can lead to self-fulfilling prophesies and does nothing to show that a particular testing method is working.

4.2.3 **Kettle Creek East Site (36CN199)** (Petraglia and Knepper 1994)

This site is located along Kettle Creek in Clinton County, Pennsylvania within the Deep Valleys Section of the Appalachian Plateaus physiographic province. The site contains occupations ranging from the Late Archaic through Late Woodland periods (Petraglia and Knepper 1994).

The authors do not indicate whether any presumptive tests were carried out before using CIEP analysis. They state that residues were extracted using a five percent ammonium hydroxide solution, also used by Loy and Hardy (1992). Slight confusion exists as to how many artifacts from this site were tested using CIEP. The authors state 18 artifacts were tested, but then later talk about only 13 artifacts. Regardless, three returned positive results, and all were projectile points. The authors indicate that there was no

"background contamination," but they do not explain what kind of contamination they are referring to, or whether that contamination may affect presumptive blood tests or immunological tests.

Two of the positive artifacts, identified as Susquehanna Broadspears made of rhyolite, tested positive for rabbit. The third point, made of chert and apparently non-diagnostic, tested positive for mouse. Without use-wear analysis to back up the claim that the Susquehanna Broadspear was used as a knife, the interpretation of these results is inconclusive. The authors state that the non-diagnostic chert point shows no sign of obvious use, but that it could have been used for hunting or butchering, or may retain the remnants of hafting material.

4.2.4 Site 36CO17 and Site 36CO18 (Jacoby *et al.* 1999)

These sites are located in Columbia County, Pennsylvania on a floodplain of the North Branch Susquehanna River. This area lies within the Appalachian Mountain Section of the Ridge and Valley physiographic province. Site 36CO17 contains Middle Archaic through Late Woodland occupations, while Site 36CO18 contains Middle Archaic through Woodland occupations. These sites were discovered during work to replace the Mifflinville Bridge (Jacoby *et al.* 1999).

Researchers at these sites employed the MacPhail (at the time, manufactured by Ames) and Hemastix® methods, followed by taxa identification on positive artifacts. Both the MacPhail and Hemastix® tests are chromogen-based presumptive blood tests. Although, rather unorthodox, the researchers felt that using two methods to test artifacts would add to the confidence that no false positives were interfering with the results. Perhaps this is logical, if researchers knew chemically that the two tests would cover each other's false positives. If this was the case, no indication was given in the form of an explanation pertaining to any chemical interplay. Any benefit, however, was lost when the researchers tested *separate* areas on individual artifacts with *separate* tests. If there was the possibility that separate areas on an artifact would have separate incidents of blood, then it's possible that there could be separate incidents of false positives. It would have made more sense to test the *same* areas with the two tests.

Confusion exists as to the actual numbers of artifacts tested and the results from these two sites. The authors state that a total of 70 artifacts was tested in the presumptive stage of the analysis from Site 36CO17, but make no mention of any artifacts from Site

36CO18. They state that 30 artifacts were submitted for CIEP analysis, but the authors do not break this number down by site. In the appendix of the report, they then state that 10 artifacts from Site 36CO18 returned positive results from CIEP testing. This would make a potential total of 40 artifacts submitted to CIEP testing.

No indication was given whether control samples of soils from the sites were tested to indicate any false positives in the presumptive stage. The first 37 artifacts were tested with the original, two-method scheme, but the remaining 33 artifacts were only tested with Hemastix®. The reasoning behind the sudden change in testing method was not explained. The authors' statement questioning the survivability of hemoglobin is legitimate, but their reasoning concerning the low positive results in their assemblage based on this assumption is less credible. It may simply be that the tests used were not sensitive enough to detect the amount of hemoglobin that may have been on any of the artifacts.

An indeterminate biface was the only artifact to return a positive result using Hemastix®. However, it was a false result using the MacPhail method, which questions the utility of these researchers' methods of testing the artifacts with two tests. Again, it seems there was a lack of understanding concerning the interplay between the chemistry of the two tests. This does not leave much confidence as far as the reliability of their results and the authors give neither an explanation of this, nor a discussion of how the results should be interpreted.

The second stage of taxa identification was conducted using the CIEP method. The authors ignored their results in the presumptive stage and submitted 30 artifacts for testing in stage two. Extracts were derived and initially tested with "pre-immune" antisera to ascertain any false positives (see Puncheon Run site for more information). All artifacts were negative to these tests. After testing the artifacts with specific antisera, 26 artifacts were positive. Some positive artifacts returned multiple results. Identified taxa included the species American Eel, and the families of trout, catfish, deer, turkey, and rabbit. The authors' statement that CIEP has a high sensitivity to residues, but is unable to distinguish erroneous results from true results does not sound encouraging as far as reliability of the method. To remedy this, the laboratory that conducted the study retested artifacts that were initially positive with diluted antisera to increase the specificity of the reactions. This method has also been used at other sites (LeeDecker *et al.* 2001). What the authors seem to be saying is, based on the concentration of the antisera, one could have a result for turkey while the same antisera at a different concentration could give a reaction to trout. So to

remedy this, the antisera is diluted, thereby eliminating the erroneous result based on a concentration (see the discussion of the Puncheon Run site above for further explanation).

The authors state though that there was a lack of positive results in the presumptive first stage; yet, artifacts were submitted to CIEP testing regardless of this fact. This course of action underscores the lack of confidence researchers have concerning blood residue analysis. Such low confidence in these methods results from a lack of consistent and logical results. The presumptive stage of blood residue analysis should be conducted as a means to quickly and cheaply screen artifacts before submitting them to much more expensive and sophisticated taxa identification. If these researchers are ignoring the results in the presumptive stage, then what is the point of even attempting this pre-screening of artifacts? The research is taking too large a step in trying to identify taxa, when what should first be investigated is whether researchers are receiving consistent and true results from presumptive blood tests on pre-contact artifacts. When taken as a whole, the uncertainty of many taxa identification studies usually results from inconsistent testing.

As with most sites in this synthesis, an integrated use-wear study was not conducted. Cursory descriptions of use were given, but they are not referenced to the results of the blood analysis.

4.2.5 East Bank Site (36NB16) (East *et al.* 2002)

The East Bank site is a deeply stratified, multi-component site situated in the Appalachian Mountain Section of the Ridge and Valley physiographic province. This site, located along the West Branch Susquehanna River in Northumberland County, Pennsylvania, has occupations ranging from possible Paleoindian to Late Woodland times (East *et al.* 2002).

Authors of the blood residue study for the East Bank site (Mercyhurst Archaeological Institute) provide a thorough and informative discussion, but the results are lacking (Hyland *et al.* 2000). Appropriate background concerning the methods and procedures used in the study are clearly outlined. Both presumptive tests and taxa identification were performed. Hemastix® was used for presumptive blood tests, while taxa identification used ELISA.

Though the authors talk about an ideal testing strategy of including ethnographic and environmental factors in choosing which species to test for, they note that financial limitations and availability of specific antisera played a role in this particular study, both valid points concerning other studies. White-tailed deer, eastern cottontail rabbit, and catfish

were selected for this study as the species to be investigated because these were the antisera at hand.

Seven artifacts were submitted for the analysis. Testing with Hemastix® returned only one positive result; however, all the artifacts were then submitted to the ELISA procedure. This action and the results of the ELISA procedure exemplify the conditions that have caused the lack of confidence in these procedures (see discussion of Sites 36CO17 and 36CO18, above). Testing the artifacts with Hemastix® had no bearing on the ultimate outcome of this study. Because of the lack of confidence in the results, the researchers simply ignored them and submitted all the artifacts for taxa identification.

In the ELISA phase, *all* the artifacts reacted positively to *all three* antisera: deer, rabbit, and catfish. The authors claim these were slight reactions or "background" levels. When these background levels were accounted for, only two artifacts were considered positive: one for catfish and one for white-tailed deer. Neither of these artifacts was the one that tested positive with Hemastix®. None of the antisera used to identify blood on these artifacts is specific to the particular species from which they were derived. It is doubtful an integrated use-wear study for this site would have improved the reliability of the results.

Though the reliability of the study from the East Bank site is not solid, it was this site that inspired the investigation of luminol as an alternative presumptive test to Chemstrips® and Hemastix®. Both artifacts from this study that were ultimately considered positive were subjected to the luminol test by Skelly and Loy, Inc. and ClueFinders Inc., a criminal forensics laboratory in Erie, Pennsylvania. Both artifacts were considered to show positive reactions to luminal, even after they had been subjected to submersion during sonication for the previous analysis. Though this test was very informal, and the results were certainly not conclusive, subsequent informal tests on artifacts from other sites have shown luminol to hold potential as a reliable presumptive blood test. The benefits of luminol have been introduced and outlined in Vish and Yeshion (2004). Only further testing will confirm whether it can be used with confidence.

4.3 Maryland Studies

4.3.1 Wallizer Site (18AG44), Wild Turkey Site (18AG160), Murley Branch Site (18AG60) and Whittaker Site I (18AG 14) (Kavanagh and Ebright 1988)

These four sites were all identified during an archaeological survey conducted in the Town Creek Valley, in Allegany County, Maryland. The sites lie within the Appalachian Mountain Section of the Ridge and Valley physiographic province (Kavanagh and Ebright 1988).

The Wallizer site has been described as a Late Woodland village site with earlier Archaic components. The Wild Turkey site is described as a single component lithic workshop. The Murley Branch site is described as an Early Archaic through Late Woodland base camp, and the Whittaker Site I contains a Late Archaic component (Kavanagh and Ebright 1988).

This study, along with the Kettering Park site study (see below), were among the earliest attempts by a state agency to utilize presumptive blood tests introduced by Loy (1983). Artifacts were chosen for the study based on vague judgments of utilization, thus, according to the authors, eliminating artifacts that were not utilized and therefore, not exposed to blood. This of course, is purely speculative. Just because artifacts show no signs of use does not mean they were not exposed to blood.

Ten artifacts were submitted to blood residue analysis from each site. Two positives were reported from the Wallizer site, three positives from the Murley Branch site, five positives from the Whittaker site I, and three positives from the Wild Turkey site were reported. Artifacts that were listed as “possible” and “probable” were included as positives in these totals. What the difference is between these terms is unknown.

The percentages of positives reported is claimed by the authors to be a reasonable representation based on the morphology of the assemblages tested. The authors admit only a small number of artifacts were tested from each site, which makes it difficult to draw conclusions. They are attempting, with regard to common sense, to develop a signature positive rate based on morphology. For example, one could structure an assemblage to be tested with a certain percentage of debitage and a certain percentage of projectile points. Assuming the method of detection is infallible, one would expect more blood to be on the projectile points than on the debitage, thereby establishing a “signature” blood distribution of the assemblage. This can be dangerous in the sense that it can lead to self-fulfilling prophesies. Again, this statistic can start to become meaningless when one doesn’t know the original true number of positive artifacts to begin with and if one assumes that the methods of blood detection are anything but infallible. Researchers seem to be using this approach to verify whether a method is working correctly, when this is hardly proof that one is.

Only presumptive blood tests were carried out in this study. The authors do not give an indication of the product used, but mention a “color changing, paper coated, plastic strip” (Kavanagh and Ebright 1988:VII-1). This most likely is either the Chemstrip® or Hemastix® product. Artifacts were first soaked in vials of water and tested on the first and third day of soaking. The reasoning behind this scheme was not provided. The authors do not state whether they were using distilled water. The effects of not using distilled water on these tests has not been evaluated, but anyone familiar with chemistry principles would recognize the potential of interactions with minerals and other chemicals found in ordinary tap water. For unexplained reasons, the authors needed a second stage in this procedure. The second stage of their methods included sonication of the artifacts to extract any remaining residue. This step caused sediment still attached to the artifacts to muddy the solution. It is apparent the researchers did not understand the Chemstrip®/Hemastix® test, since they claim this sediment “produces a false positive reaction, presumably due to the interfering action of fine particulates such as clay” (Kavanagh and Ebright 1988:VIII-I). This is not true; clay particles do not cause false positives in the Chemstrip®/Hemastix® test. What was most likely occurring was that the Chemstrip®/Hemastix® was being stained by the sediment, causing those who were reading it to believe there was a color change indicating blood; the sediment may also have been interfering with reading the test. The researchers apparently remedied this by allowing the extract to settle for two weeks, then testing the clear solution. Deriving a fluid from unwashed artifacts must always be accompanied with sediment in the solution. How this sediment affects the chemistry or reading of the test from this study and others, and how any researchers have dealt with the problem is a cause for concern. Loy (1983) even makes a point about preserving sediment attached to the artifact, but never explains how he dealt with muddying of the solution he was testing.

Since no studies have been done to ascertain the effects of leaving blood residue to soak in ordinary water for two weeks, the results from subsequent presumptive tests are questionable. No indication was given that any false positives were investigated and no control tests were conducted. It is unclear whether manganese oxide was known as a contaminant at this time, but it could have been a potential problem. According to the authors, pH was measured at all the sites. Only one, the Murley Branch site, had a low pH (7.9-8.1). The pH of soil can affect the amount of manganese oxide present (see Stage 1: Presumptive Blood Tests Section above).

This study is extremely questionable, particularly since this procedure of testing artifacts was in its infancy.

4.3.2 **Indian Creek V Site (18PR94)** (LeeDecker *et al.* 1991)

No background information on this site was obtained. Research at the Maryland Historical Trust Library, where archaeological reports are housed for the state of Maryland, only produced the appendix volume containing the blood residue study.

Forty-five artifacts from the Indian Creek V site were submitted to cross-over immuno-electrophoresis to attempt taxa identification of blood residues, after having been pre-screened for presence/absence of blood at the University of Delaware's Center for Archaeological Research. This most likely meant artifacts were tested with the Chemstrip® method. The technical appendix associated with this report makes no other reference to the work done at the University of Delaware as far as methods are concerned. The authors state that three soil samples were submitted as control tests. This raises the question concerning the amount of testing that is appropriate to determine contaminants. No specific study has yet been conducted concerning this point. Much is misunderstood about how contaminants can effect both presumptive tests and taxa tests and how these contaminants may relate to a site through time and site genesis. Even so, three soil samples from a large site of this size seems insufficient and does not provide much confidence in the results.

All artifacts and the soil samples were tested against pre-immune sera, which, if positive, would indicate a non-specific protein reaction resulting from some form of contaminant (see the Puncheon Run site for more information).

Thirteen artifacts returned positive results to the pre-immune serum, meaning there were positive reactions to non-specific proteins. None of the soil samples were positive. The 13 artifacts that reacted to pre-immune serum were retested using the method described in the Puncheon Run site discussion.

One artifact did return a positive when retested with pre-immune serum. But instead of dropping this from the analysis, the authors ignored their own procedure based on the positive results obtained from the presumptive stage conducted at the University of Delaware's Center for Archaeological Research. The reasoning for this is very confusing.

Final results returned 29 positive artifacts, many with multiple taxa results. Taxa identified included bovine, deer, guinea pig, dog, human, trout, fern, mouse, bear, chicken, and rabbit.

4.3.3 Dorsey VI Site (18AG168) (Wall 1993)

The Dorsey VI site (18AG168) is located in Cumberland County, Maryland. This site contains Late Archaic through Late Woodland occupations. The Middle Woodland occupation is the only component to have been functionally described and is interpreted as a series of camps established over a short time (Wall 1993).

Sixty-five artifacts were submitted to the University of Delaware's Center for Archaeological Research for presumptive blood tests. This entailed using the Chemstrip® method of detecting trace amounts of blood. Control tests of soils were performed and none were found to contain any contaminants. A total of 200 individual tests was carried out on the 65 artifacts. The authors try to ascribe a value to these presence/absence tests, and report 88 negative results, eight "slightly positive" results, and five positives. They then state that there were four "strongly positive" results and an additional three positives (Wall 1993:165). This additional statement is unclear as to meaning. In general, the chromogen-based presumptive tests used by these and other researchers should not be valued on a gradational basis, but rather, they should be simple "yes or no" answers; after all, the test is only based on "presence or absence." It is understood that these tests come with an interpretive chart, but this only serves to confuse and complicate what results are obtained, and in an archaeological context one must question the value of identifying an artifact that is "slightly" positive in contrast to one that is "strongly" positive. Only *convincingly* positive results should be accepted. It is pointless to worry about the potential of missing other artifacts that may indeed have blood on them in "slight" amounts; we will, in all of these studies, never fully detect every artifact that was exposed to blood. An assemblage of five certainly positive results is better than an assemblage of five certain positives and five "maybe" positives.

Artifacts were then submitted to cross-over immuno-electrophoresis, and taxa identification was attempted. Nineteen total artifacts were submitted, including seven positives from the presumptive stage and 12 artifacts that had not been tested for presence

or absence of blood. Nine artifacts were positive, with some providing multiple results. Included in this number were five artifacts that originally tested positive in the presumptive stage. Taxa identified included deer, elk, human, turkey, rabbit, and duck. Also of note, this report gives the first mention that human sweat can create a positive for the human taxon, using CIEP (Wall 1993:4-5).

4.3.4 **Sorrell Site (18HO190)** (Botwick *et al.* 1993)

The Sorrell site is located in Howard County, Maryland. It lies within the Piedmont physiographic province and includes Late Archaic and Woodland components. The site has been interpreted as a hunting camp (Botwick *et al.* 1993).

Five tools were tested from this site using cross-over immuno-electrophoresis, including three points, one biface, and one possibly utilized flake. Three artifacts returned positive results. The biface was positive for deer, one point tip was positive for dog, and the flake was positive for cat.

No integrated use-wear analysis was conducted in this study. The authors close by making the statement that there is evidence these animals were hunted and/or butchered. These artifacts could have been brought from somewhere else and left at the site, thus having nothing to do with activities at this particular site. This study does not explain the life history of these artifacts.

4.3.5 **Kettering Park Site (18PR174)** (Fogel *et al.* 1994)

The Kettering Park site is located at the confluence of the Western and Northern Branches of the Patuxent River in Prince George's County, Maryland. This site lies within the Western Shore Region of the Atlantic Coastal Plain physiographic province. Occupations span the Early Archaic to Late Woodland time periods (Fogel *et al.* 1994).

Like the sites from the Town Creek archaeological survey, this was one of the first attempts by a state agency to utilize a presumptive blood test introduced by Loy (1983). Artifacts were chosen for the study based on vague judgments of utilization, thus, according to the authors, eliminating artifacts that were not utilized and, therefore, not exposed to blood. This of course, is purely speculative. Just because artifacts show no signs of use does not mean they were not exposed to blood.

Of 10 artifacts chosen for the study, only one returned a positive result, two were possible, and seven were negative. The authors mention their awareness that manganese oxide could be a potential contaminant and state that none of the control tests at the site were positive. The authors state that the "probable and possible reflect weak positive reactions that were verified through repeated testing of the solution" (Fogel *et al.* 1994:98). Whether this "verified" that the reactions were weak or whether they were positives, is unclear.

The methods and procedures of this study are exactly as those from the Town Creek Valley survey (see study above by Kavanagh and Ebright 1988), with the same problems.

This study is extremely questionable, particularly since this procedure of testing artifacts was in its infancy.

4.3.6 Clifton Site (18CH358) (Barse and Beauregard 1994)

The Clifton site is located in Charles County, Maryland and contains Early Archaic through Late Woodland occupations. Site functions have been interpreted as short-term hunting or specialized extractive camps in the Archaic and Woodland components (Barse and Beauregard 1994).

Five artifacts from the Clifton site were submitted to cross-over immuno-electrophoresis to attempt taxa identification of blood residue. Soil control tests were performed using pre-immune serum to ascertain any non-specific protein reactions that would compromise the analysis (see the Puncheon Run site for more information). No contaminants were found. No artifacts returned positive results.

4.3.7 Site 18AN754 and Site 18AN756 (Gaber *et al*. n.d.)

These two sites are located in Anne Arundel County, Maryland within the Western Shore section of the Coastal Plain physiographic province. Occupations at the sites ranged from Early Archaic through Late Woodland times. The sites were interpreted as migratory camp sites (Gaber *et al.* n.d.)

Six projectile points and one soil sample were submitted to cross-over immuno-electrophoresis. This raises the question concerning the amount of testing that is appropriate to determine contaminants. No specific study has yet been conducted concerning this point. Much is misunderstood about how contaminants can affect both

presumptive tests and taxa tests and how these contaminants may relate to a site through time and space. Even so, one soil sample seems insufficient, and does not provide much confidence in the results.

All artifacts and the soil sample were tested against pre-immune sera, which, if positive, would indicate a non-specific protein reaction resulting from some form of contaminant (see Puncheon Run site for more information).

Two artifacts returned positive results after the initial test against pre-immune serum. These artifacts were retested using the method described above and were found to be negative. Thus, in this condition, the artifacts were tested against species antisera. Two artifacts were positive, including one for deer and one for both deer and elk.

5.0 DISCUSSIONS

Archaeologists must come to terms with the fact that blood residue studies and archaeology in general, are probabilistic rather than deterministic. Like many other aspects of archaeology, blood residue studies suffer from their inability to be solely scrutinized by the scientific method. It should be clearly understood by all archaeologists that archaeology is not a hard science, although it uses many sciences as a means of investigation. Most of the broad aspects of archaeological interpretation must be left to probability based on the reliability of circumstantial evidence.

The value of this synthesis lies in having determined what procedures would constitute a well planned and executed blood residue study. This is not to say such a study would be infallible. The goal here is to carry out these procedures to the highest standards, given all the tools and information at hand, and to make the most plausible interpretation without fabrication.

Many deficiencies that were outlined in this synthesis deal with the first stage of blood detection. Until a confirmatory blood test sensitive enough to detect the amounts of residue that is suspected of being on an artifact is developed, archaeologists are stuck with using presumptive blood tests if there is a need to pre-screen artifacts. One has to ask whether the presumptive step, which is basically an optional step, is necessary. The goal of this step is to lower bottom line costs by pre-screening artifacts prior to taxa identification testing. Researchers must evaluate whether a budget allows for mass processing of artifacts with no pre-screening. If money is no object, then perhaps this step is unnecessary. However, if the archaeological community decides pre-screening does hold economic or procedural benefits, then the next logical step is to assure the efficacy of these tests. If confident and consistent results in the presumptive stage cannot be produced, then doing so as a first stage in blood residue studies is not necessary. Unreliable presumptive tests only confuse and corrupt taxa results.

The University of Delaware's Center for Archaeological Research has provided Delaware with much information about that state's pre-contact period, but the blood residue studies have not been reliable. The state of Delaware has, by far, produced the largest number of blood residue studies of any state. The majority of these studies used Chemstrips® as a presumptive test. The Hockessin Valley site (Custer and Hodney 1989) demonstrates the danger with Chemstrip® contamination at archaeological sites, but doesn't describe what that contamination is. Presumably, this contamination was from manganese oxides in the soil. Based on this, the blood residue analysis should have been abandoned altogether. This point underscores the lack of understanding where contamination is concerned as it pertains to residue tests. The problem is twofold. First, possible contaminants of the presumptive blood test in question must be identified,

and second how these contaminants can effect a site both through time and space must be understood. Related to this is how blood itself behaves over such long time spans.

Another aspect that seems to have caused results from Chemstrip®/Hemastix® tests to suffer has been the difficulty in interpreting the results. The problem lies in trying to answer a yes or no question with a gradational interpretation provided by the manufacturers. Every study that used the protocol of Custer *et al.* (1988) suffered from attempting to classify results into more than two possible outcomes. Results included the terms "strong," "weak," "possible," "probable," "positive," "negative," and "slight." Researchers should have only counted results that were *clearly and undoubtably* positive, and should have refrained from using ambiguous adjectives. This would have added a sense of reliability and decisiveness to the results.

At the Brennan site (Watson and Riley 1994:26), the specious method of identifying a positive for blood residue, as opposed to a positive for contaminants, most likely made the analysis useless. This site and the East Bank site serve as examples of how researchers execute procedures and then, because they don't like the results, or because the results seem implausible, make adjustments to render the study plausible. Generally speaking, one of the unsettling aspects of blood residue studies is this persistent theme. These outcomes seem to imply either methods do not work, or researchers are incorrectly performing the tests.

Some research in Pennsylvania (Jacoby *et al.* 1999) was conducted where researchers ignored their primary results concerning presence or absence of blood and simply resubmitted negative artifacts to expensive taxa identification. This work underscored the lack of confidence and reliability of blood residue studies in general and the need to gain credible evidence that such methods can work. If researchers are this unconfident in the science, the entire method of screening artifacts with cheap presumptive blood tests becomes pointless. Much work is needed to convince archaeologists that these procedures are reliable. Many studies have reported negative results and claimed no blood residue was then present on the artifacts, when in reality it is possible some may have had residues on their surfaces that were simply too dilute to be detected with the Chemstrips® or Hemastix®.

Found most notably neglected when researching the literature was the lack of an integrated use-wear analysis with the blood residue studies. All of these sites suffered from a lack of an integrated use-wear analysis that could have lent much more support to the conclusions obtained from the presumptive stage and the final conclusions in the taxa identification stage (Custer *et al.* 1990; Custer *et al.* 1997; LeeDecker *et al.* 2001; and others). None of the reports from Maryland had integrated use-wear studies. The reliability of use-wear studies is a problem in-and-of-itself

(Smith 2000). If it is to be used in conjunction with blood residue studies, it must earn the confidence of archaeologists as well.

Chemstrips® and Hemastix® are unreliable, based on their combined lack of background testing for archaeological use, unreliable results, and less than scientific application in the state of Delaware.

Taxa identification is the most complex and specialized aspect of blood residue analysis. Perhaps the most productive endeavor would be for archaeologists to develop a very close relationship with the forensic and biological sciences, since it is from these areas that all the techniques have originated. It is not enough to simply use these sciences for archaeological means, they must be specifically adapted so that the most reliable and useful results can be obtained.

Developing useful antisera seems to be one major problem with taxa identification. All studies use antisera developed for the immunological sciences that are then adapted for use in archaeological settings. Many studies obtain antisera from multiple companies. Some antisera used in these studies is species specific (i.e., elk) and some is not. It is unclear if developing species specific antisera is simply a question of desire. Studies have, on their own, undertaken this endevor and developed specific antisera tailored to the study area at hand. Most blood residue studies are forced to use a hodgepodge of antisera that in some cases gives specific results, while in others leaves much to the imagination. An outcome of this synthesis should be to investigate whether it is possible to develop, in close relation with archaeologists, specific antisera indicative of species pertinent to pre-contact life that are specific to the species level. This should lead to an industry-wide “library” of species specific antisera, that can be readily used by all, representing native and pertinent species. If this is impossible, much of what blood residue study hopes to accomplish will never occur.

The ultimate goal of blood residue studies lies in identifying taxa. Doing so would provide far-reaching answers concerning subsistence and tool use, as well as for many other aspects of pre-contact culture. If this step cannot be accomplished, the utility of simply identifying artifacts that have come into contact with blood through presumptive tests, itself a questionable endeavor, is only barely useful. Common sense in many cases can answer whether an artifact *could* have blood on it. When the scientific methods hold no more truth than our own opinions, one must question its utility.

5.1 Integrated Approach

Blood detected on an artifact of a certain morphology, with a particular use-wear pattern, is simply circumstantial evidence that leaves interpretation open to many possibilities. Add to this unkown ritual practices, if any, and the reliability, not to mention utility, of blood residue studies can seem to exponentially decrease. This is not to suggest that blood residue studies should be abandoned. As with all other aspects of archaeological investigations, researchers must refrain from interpretations of blood residue analysis until the highest level of all lines of evidence can be combined and scrutinized. Many researchers of studies within this synthesis have attempted to make profound interpretations based on very low standards of evidence (in particular, Brennan site, Snapp site, Hockessin Valley site), partly as a result of poor testing methods and partly as a result of our limitations in understanding the workings of immunology in a pre-contact context.

The interpretation of the life history of any artifact should be derived from an integrated approach, including several lines of investigation, such as context, potential contamination, use-wear analysis, and blood residue analysis, among others. In our desire to explain what has happened in the past, many creative stories are built on scant evidence found at archaeological investigations. Blood residue studies are one of the most exaggerated lines of evidence used to interpret archaeological sites, when greater conservatism should be called into play when reporting results. Morphology, use-wear, and the results of blood residue tests should be the *minimum* amounts of information needed to make an educated *guess* or interpretation about an artifact's life history.

To present the most reliable interpretation, several lines of the highest quality of integrated evidence is needed to make interpretations based on blood residue results. If interpretations are held to the highest standards, perhaps consistency and reliability will begin to emerge. These lines of evidence include:

Use-wear Analysis -- Any claims of a particular pre-contact tool's use should be supported by clear evidence based on similar wear from replicated tools. Use-wear should include microscopic and edge angle analysis.

Artifact Morphology – This is closely tied with use-wear analysis.

Blood Residue Analysis -- While much needs to be confirmed, and accepted, a vast understanding of the chemistry and limitations of particular tests is paramount. This should all be done in cooperation with immunological scientists to fine-tune methods to the goals of

archaeology. The need for detail and reliability increase when taxa identification is attempted.

Faunal Analysis – This analysis should be included in any such study when possible. Such substantial physical evidence of species that may have been available can add meaning to blood residue results. This is largely problematic in eastern North America due to the lack of bone preservation.

Environmental Awareness – This includes site genesis, depositional and climactic awareness. Much of this is poorly understood as it pertains to the longevity of blood residue, contaminants, etc.

Logical Archaeological Associations -- Using the information gained from all the previous points, are the interpretations logical, or is fiction taking over? Logical associations will result from the gathering of as much information from all of the above lines.

Evaluating these studies was one purpose of this synthesis. It was hoped that by becoming familiar with all the known methods, that a recommended procedure could be outlined pertaining to blood residue studies. A model study would have to follow a procedure that would combine the Lums Pond and Puncheon Run site studies.

Such a model study would include a presumptive testing protocol with a proven track record on replicated artifacts, one that has taken into account possible contaminants and has accounted for such at the actual site being studied. This study would then include an amino acid analysis to identify any proteins. The amino acid step at the Lums Pond site provided clear evidence that proteins had survived, lending confidence to the next step of identifying taxa. Once this is accomplished, an immunological assay (CIEP) that separates proteins by molecular weight is recommended. Again, the Lums Pond site provided an example using this method. Separating proteins by molecular weight eliminates the testing of unknown types of proteins. Molecular weight separation allowed researchers to identify exactly which kinds of proteins were present and then specifically test them for taxa. As at the Puncheon Run site, the next step should be to utilize specifically tailored antisera. This method should have already been used to identify replicated artifacts that have been exposed to blood and used to identify the respective taxa with newly developed species-specific antisera. Integrated with this information would be all the lines of evidence described above.

5.2 Initial Proposal for Site 7NC-B-54

The introduction of luminol as an alternative presumptive blood test for archaeological artifacts (Vish and Yeshion 2004) has provided the potential to remedy many of the concerns and problems associated with the first stage of blood residue analysis. The many advantages of luminol over traditional Chemstrip®/Hemastix® testing was presented in Section 3.2.2. Testing of this method should begin immediately to ascertain its effectiveness.

In its strictest application, the scientific method would require that we create artifact replicas, use them in controlled activities, bury them for a length of time similar to the burial span of typical artifacts, and then conduct luminol testing. Not having the ability to complete experiments spanning several thousand years, it is imperative that we at least begin to delineate various factors that may create false negatives or false positives. The proposed blood residue experiment has been designed to examine various aspects of the robustness of the luminol method in archaeological contexts. The experiment will address:

- possible false positives created by antler contact during knapping;
- possible false positives created by soil contamination;
- the relative accuracy of luminol testing by an archaeologist (Al Vish of Skelly and Loy) versus a professional forensic scientist (Ted Yeshion of ClueFinders, Inc.);
- the effects of artifact burial on luminol testing;
- the effectiveness of species identification using fresh and buried artifacts.

For each artifact replication case below, there will be a set of four artifacts created and then tainted with blood. Artifact 1 will be luminol tested after 30 days of air-drying, by Skelly and Loy, Inc. Artifact 2 will be luminol tested after one week of air-drying by ClueFinders, Inc. Artifacts 3 and 4 will be buried for 30 days after their production/use and then will be luminol tested by Skelly and Loy, Inc. and ClueFinders, Inc., respectively. All items will be sent for species identification, allowing an evaluation of the accuracy of this method.

All artifacts will be produced with regionally available argillite as the raw material. A single knapper will produce all the items. The items will include medium to large flakes, generally with at

least one edge retouched (by stone, antler hammer, or pressure-flaking with an antler tine or copper rod). The inclusion of the copper rod as a retouch tool will be used as a control against informed guessing by the analysts (e.g., this fine retouch must have been done with an antler tine, so we'll test extra carefully for deer blood).

Cases 1-3. Stone-hammered, unutilized items. These 12 items will be the control population. These items will not have been exposed to blood or tissue in their manufacture or use. Gloves will be used by the knapper throughout the replication process to guard against hand-cut contamination.

Cases 4-5. Antler-hammered, unutilized items. These eight items will be produced with a deer antler hammer, but will not be exposed to any other blood or tissue. This case grouping will address the possibility that soft hammer percussion with an antler may leave sufficient biological residue to create a positive result from luminol testing (see Puncheon Run site). The species testing will establish if sufficient organic matter remains to yield an accurate species of origin.

Cases 6-7. Pressure-flaked by antler, unutilized items. These eight items will have edge retouch created through pressure flaking with an antler tine. This case should indicate if pressure-flaking with an antler leaves sufficient biological residue to trigger a positive result from luminol testing (see Puncheon Run site). The species testing will establish if sufficient organic matter remains to yield an accurate species of origin.

Case 8. Stone-hammered, used to butcher catfish. All of the cases of artifacts used in butchering single species will allow evaluation of the effects of 30-day burial, and the effectiveness of species identification testing.

Case 9. Stone-hammered, used to butcher groundhog. All of the cases of artifacts used in butchering single species will allow evaluation of the effects of 30-day burial, and the effectiveness of species identification testing.

Case 10. Stone-hammered, used to butcher deer. All of the cases of artifacts used in butchering single species will allow evaluation of the effects of 30-day burial, and the effectiveness of species identification testing. Case 10 will also provide valuable contrasts with Cases 4-5 (antler-hammered, unutilized), Cases 6-7 (pressure-flaked by antler, unutilized), and Case 11 (used to scrape deer hide).

Case 11. Stone-hammered, used to scrape deer hide. Many Delaware blood residue studies make the leap from positive results for presumptive blood tests to the taking and butchering of game. This case will determine if the post-butchering processing of low-blood material (i.e., deer hide) results in sufficient organic matter to trigger a positive Luminol result and to allow species identification.

Case 12. Stone-hammered, used to chop cattail roots. This case is used essentially as another control sample, but these items will be used in a typical, pre-contact activity lacking contact with flesh or blood. It will help evaluate how the presence of plant-derived organic matter may create false positives or spurious species identifications.

A total of 48 artifacts will be produced, luminol tested, and examined for species identification. Each item will be secretly randomly numbered to allow proper tracking without identifying the case, and introducing bias. The knapper, labeler, and user of the tools will be Chris Espenshade of Skelly and Loy, Inc. The luminol testers will be Al Vish (Skelly and Loy, Inc.) and Ted Yeshion (ClueFinders, Inc.). The species identification will be undertaken by a laboratory to be determined.

The results of the Step 1 experiment will be statistically evaluated. The robustness of luminol as a presumptive blood test on archaeological stone tools will be addressed in light of the results. Likewise, the accuracy of species identification will be modeled. It is proposed that no testing of Site 7NC-B-54 artifacts be undertaken until all interested parties are convinced of the value of such testing. The Step 1 experiment should be considered mandatory before conducting any additional presumptive testing of Delaware artifacts.

Based on the results of the Step 1 experiment, Skelly and Loy will recommend a course of action for blood residue study as it pertains to the Site 7NC-B-54 assemblage. We will strive to avoid the “check-list” mentality under which blood residue studies have been conducted, without considering baseline, scientific principals.

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