

APPENDIX E

**PRESUMPTIVE BLOOD TESTING
BLIND STUDY**

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By

Barbara J. Gundy, Ph.D.

ABSTRACT

This document reports on the results of a blind study involving the testing for the presence or absence of blood, also called presumptive blood testing, on replicated archaeological lithic artifacts. The goal of undertaking the presumptive blood testing blind study was to provide scientific research which would support or refute the usefulness of the technique as a means to streamline traditional blood residue studies, and to develop a procedure which could be recommended for general use in future archaeological research. To these ends, presumptive blood testing is a means in which to identify the presence and relative quantity of blood on an artifact prior to sending the artifact for blood residue taxa identification, thus ensuring that the costly taxa testing will not yield negative results due to the absence of blood.

The current presumptive blood residue testing blind study has proven that the use of Bluestar[®] Forensic as a reagent is a reliable means to identify blood residue on replicated lithic artifacts. With the exception of one specimen, the blind test reliably indicated the presence of blood on all of the specimens which had been exposed to blood. In addition, several variables involving exposure to non-blood organics including bone, vegetal material, and soils were tested in order to determine if false positives would result. The blind study did not yield any false positives.

The current presumptive blood residue testing blind study provides information that effectively advances the goals of archaeology in the identification of useful pre-contact period subsistence studies. It is a supplemental cost-and-time-efficient methodology that can be used to streamline and enhance traditional blood taxa studies. In addition, since the reagent does not destroy the DNA associated with the blood residue, the artifacts that react positively to the reagent can be re-used in further blood residue or genetic material studies.

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1.0 INTRODUCTION

A blind study involving the testing for the presence or absence of blood on replicated and archaeological pre-contact period lithic artifacts, also called presumptive blood testing, was undertaken as a part of the Blue Ball Transportation Improvements project, Site 7NC-B-54 (Ronald McDonald House) Phase III data recovery. The goal of undertaking the presumptive blood testing blind study was to provide scientific research which would support or refute the usefulness of the technique as a means to streamline traditional blood residue studies, and to develop a procedure which could be recommended for general use in future archaeological research.

2.0 BACKGROUND

Traditionally, blood residue studies in archaeology have been concerned with the determination of the taxa of blood residue present on pre-contact period lithic artifacts. It was hoped that by defining the taxa of animals indicated by blood on lithic artifacts, that information regarding subsistence and seasonality could be obtained. However, identification of blood residue taxa is a costly and uncertain proposition. Many times lithic artifacts are sent for blood residue taxa testing, only to yield negative results because the artifact did not contain blood, or large enough quantities of blood, necessary for the test.

Presumptive blood testing is a means to identify the presence and relative quantity of blood on an artifact prior to sending the artifact for blood residue taxa identification, thus ensuring that the costly testing will not yield negative results due to the absence of blood. Several methods of presumptive blood testing have been used in archaeological research previously; however, they were costly; impracticable on a large scale or under non-laboratory conditions; originally developed for uses other than blood detection; and none have had their effectiveness tested using blind studies (Custer *et al.* 1988; Fogel *et al.* 1994; Kavanagh and Ebright 1988; Loy 1983; Loy and Hardy 1992; Vish and Yeshion 2004; Yeshion 1991, 2001). For a complete discussion of previously completed archaeological blood residue studies conducted in Delaware, Pennsylvania, and Maryland, the reader is referred to Appendix F of this report.

3.0 RESEARCH DESIGN

The presumptive blood residue test blind study was designed to provide scientific research which would support or refute the usefulness of the technique to streamline traditional blood residue studies, and to develop a procedure which could be recommended for general use in future archaeological research. To these ends, the following research topics were proposed:

1. **Advancing Archaeology.** How can presumptive blood residue testing advance the goals of archaeology?
2. **False Positive Reactions.** Does presumptive blood residue testing work on archaeological materials reliably?
3. **Cost.** Can presumptive blood residue testing be accomplished inexpensively on large quantities of archaeological artifacts?
4. **Ease of Use.** Can presumptive blood testing be accomplished easily in an archaeological field or laboratory setting?

Using a blind study for the presumptive blood residue testing on lithic artifact reproductions, while controlling for multiple variables, should allow the research to begin answering questions associated with the proposed research topics.

3.1 Methodology

The process for the presumptive blood residue test blind study included five steps outlined below.

3.1.1 Collection of Lithic Raw Materials for Use in Replication of Artifacts

The lithic raw material chosen for the manufacture of replicated artifacts to be used in the presumptive blood residue test blind study is argillite, which was collected from the Pennsylvania/New Jersey border. Despite the lack of argillite in the archaeological record at the site, this raw material was selected because similar lithic raw materials were widely used during the pre-contact period elsewhere; because it is relatively easy to knap compared to cobble quartz or quartzite, the main lithic raw

material type recovered from the excavations at Site 7NC-B-54 (Ronald McDonald House); because it is dark charcoal to black in color, which hides blood stains better for use in the blind study; and because large packages of the raw material were easily accessible, eliminating the concern of package to package variability in the study's raw materials. William Schindler informed Skelly and Loy personnel of the argillite source.

3.1.2 Replication of Lithic Artifacts

For the purposes of the presumptive blood residue test blind study, only expedient flakes were manufactured. Large blocks of argillite were reduced to flakes of various sizes using both hammerstone and antler hammer methods. No flakes containing natural cortex were used in the research, to ensure that non-weathered, uncontaminated, fresh surfaces were available on all of the tested replicated artifacts. Following the manufacture of the replicated artifacts, each artifact was labeled with a discreet number for tracking purposes. This discreet number allowed the presumptive blood residue test results to be interpreted at the completion of the blind study.

3.1.3 Control Variables for Replicated Artifacts

Since presumptive blood residue testing was originally developed for forensic not archaeological purposes, most of the literature discussing the reliability of the technique deals with variables which would be present at a crime scene (e.g., presence of detergents and/or bleach used to wash the blood stains, exterior blood stains that have been diluted by rain water, etc.), not in an archaeological setting. Therefore, several variables more closely associated with the manufacture, use, and preservation of archaeological artifacts were considered during the presumptive blood residue test blind study. All of the replicated artifacts were manufactured using one of two methods, antler or hammerstone. This variable considers how the reagent will react to the presence of organic matter (i.e., antler) other than blood that occurs on the replicated artifacts because of manufacturing techniques, not function or use. The antler and stone manufactured replicated artifacts were then used for four different functions, including no use, butchering muskrat meat, scraping muskrat hide, and chopping/slicing cattails (Photographs E-1, E-2, and E-3). These variables consider how the reagent will react to



Photograph E-1. Knapping a lithic using a hammerstone.



Photograph E-2. Butchering a muskrat with the replicated flakes.



Photograph E-3. Scraping muskrat hide.

just a few of the uses of archaeological artifacts, including those involving organic materials other than blood. Finally, half of the replicated artifacts were buried for 30 days, while the other half remained in plastic bags on a shelf in the laboratory. This variable considers the viability of the presumptive blood residue test on buried artifacts. Establishing that the reagent can reliably denote the presence of blood on artifacts from buried contexts is very important, since most artifacts on archaeological sites are buried. Additional presumptive blood residue testing was accomplished on three soil samples taken from the general vicinity, but not the exact burial location of the artifacts. This testing was conducted to determine if elements in the soil itself could cause a false positive reaction on artifacts buried in that soil.

3.1.4 Presumptive Blood Testing

The presumptive blood residue test blind study completed as part of the Site 7NC-B-54 (Ronald McDonald House) Phase III data recovery and discussed in this document, makes use of an inexpensive and easy to use latent bloodstain reagent that

is marketed under the brand name Bluestar® Forensic. Bluestar® Forensic was chosen for this blind study because it is easily obtained, inexpensive, comes in an easy to use tablet form, has a strong and long-lasting luminescence that does not require total darkness to be visible, its luminescence is different for true blood reactions versus false positives, it does not alter DNA, and it appears to react well to old and/or diluted blood stains (Gavrilovic and Webb 2006:3).

The actual blood residue testing is a simple process which can be completed quickly. Two tablets of the Bluestar® Forensic are combined in a spray bottle with 125 ml (4 oz) of distilled water. The mixture is swirled gently for one to two minutes until the tablets are completely dissolved. The spray bottle is set on the finest mist setting available and the artifact surfaces are misted with the mixture. A side to side sweeping motion is used to spray the artifacts. The sprayed surfaces are then immediately checked for a bluish luminescence indicative of the presence of blood residue. The blood residue testing of the 48 replicated artifacts was completed in under three hours.

3.1.5 Photography

Both digital and 35mm photographs were used during the presumptive blood residue test blind study to record the process and results. However, despite the use of a tripod, 400 ASA film and a slow f stop, the low lighting conditions prohibited the 35mm photographs from capturing the luminescence. Flash photography was not used to ensure that the luminescence would not be washed out on the photographs. Only the digital photography was able to capture the luminescence sufficiently to be visible in the photographs (Photograph E-4). In some instances, the digital photographs had to be electronically brightened in order to enhance the visibility of the replicated artifact from the dark background and the luminescence.

Prior to the actual presumptive blood residue test blind study, the obverse and reverse surfaces of each replicated artifact were scanned using a flat bed scanner in order to have an image of the artifact on which could be marked the areas of luminescence during the actual testing. This would provide a permanent record of where the blood occurred on each artifact and could be used to guide any future taxonomic identification.



Photograph E-4. Luminescence resultant from positive reaction of reagent with blood.

4.0 RESULTS

A total of 48 replicated lithic artifacts (expedient flakes) were included in the presumptive blood residue test blind study. Table E-1 presents the results of that testing.

**Table E-1.
Results of the Presumptive Blood Residue Test Blind Study Replicated Artifacts**

Replica Number	Hammer/Retouch	Description of Use	Buried	Expected Presumptive Blood Residue Test Result	Actual Presumptive Blood Residue Test Result
3	antler/antler	none	yes	negative; potential false positive from organics other than blood residue or from soil components	negative
10	antler/antler	none	no	negative; potential false positive from organics other than blood residue	negative
17	antler/antler	none	no	negative; potential false positive from organics other than blood residue	negative
19	antler/antler	none	yes	negative; potential false positive from organics other than blood residue or from soil components	negative
26	antler/antler	none	yes	negative; potential false positive from organics other than blood residue or from soil components	negative
36	antler/antler	none	no	negative; potential false positive from organics other than blood residue	negative
44	antler/antler	none	no	negative; potential false positive from organics other than blood residue	negative
45	antler/antler	none	yes	negative; potential false positive from organics other than blood residue or from soil components	negative
4	stone/stone	butcher muskrat	yes	positive	positive
7	stone/stone	butcher muskrat	no	positive	positive
12	stone/stone	butcher muskrat	no	positive	positive
16	stone/stone	butcher muskrat	yes	positive	positive
27	stone/stone	butcher muskrat	yes	positive	positive
28	stone/stone	butcher muskrat	no	positive	positive
30	stone/stone	butcher muskrat	no	positive	positive
31	stone/stone	butcher muskrat	yes	positive	positive
32	stone/stone	butcher muskrat	yes	positive	positive
38	stone/stone	butcher muskrat	no	positive	negative*
43	stone/stone	butcher muskrat	no	positive	positive

**Table E-1.
Results of the Presumptive Blood Residue Test Blind Study Replicated Artifacts
(Continued)**

Replica Number	Hammer/Retouch	Description of Use	Buried	Expected Presumptive Blood Residue Test Result	Actual Presumptive Blood Residue Test Result
46	stone/stone	butcher muskrat	yes	positive	positive
2	stone/stone	none	yes	negative; potential false positive from soil components	negative
5	stone/stone	none	no	negative	negative
11	stone/stone	none	no	negative	negative
13	stone/stone	none	yes	negative; potential false positive from soil components	negative
20	stone/stone	none	yes	negative; potential false positive from soil components	negative
21	stone/stone	none	no	negative	negative
22	stone/stone	none	no	negative	negative
25	stone/stone	none	yes	negative; potential false positive from soil components	negative
39	stone/stone	none	yes	negative; potential false positive from soil components	negative
41	stone/stone	none	no	negative	negative
42	stone/stone	none	no	negative	negative
48	stone/stone	none	yes	negative; potential false positive from soil components	negative
24	stone/stone	scrape dry muskrat hide	yes	negative; potential false positive from organics other than blood residue or from soil components	negative
34	stone/stone	scrape dry muskrat hide	no	negative; potential false positive from organics other than blood residue	negative
40	stone/stone	scrape dry muskrat hide	no	negative; potential false positive from organics other than blood residue	negative
47	stone/stone	scrape dry muskrat hide	yes	negative; potential false positive from organics other than blood residue or from soil components	negative
6	stone/stone	slice cattail root	yes	negative; potential false positive from organics other than blood residue or from soil components	negative
15	stone/stone	slice cattail root	no	negative; potential false positive from organics other than blood residue	negative

**Table E-1.
Results of the Presumptive Blood Residue Test Blind Study Replicated Artifacts
(Continued)**

Replica Number	Hammer/Retouch	Description of Use	Buried	Expected Presumptive Blood Residue Test Result	Actual Presumptive Blood Residue Test Result
18	stone/stone	slice cattail root	no	negative; potential false positive from organics other than blood residue	negative
35	stone/stone	slice cattail root	yes	negative; potential false positive from organics other than blood residue or from soil components	negative
1	stone/antler	none	yes	negative; potential false positive from organics other than blood residue or from soil components	negative
8	stone/antler	none	no	negative; potential false positive from organics other than blood residue	negative
9	stone/antler	none	no	negative; potential false positive from organics other than blood residue	negative
14	stone/antler	none	yes	negative; potential false positive from organics other than blood residue or from soil components	negative
23	stone/antler	none	yes	negative; potential false positive from organics other than blood residue or from soil components	negative
29	stone/antler	none	no	negative; potential false positive from organics other than blood residue	negative
33	stone/antler	none	no	negative; potential false positive from organics other than blood residue	negative
37	stone/antler	none	yes	negative; potential false positive from organics other than blood residue or from soil components	negative

* Actual presumptive blood residue test result does not support expected presumptive blood residue test result.

As can be seen from Table E-1, the presumptive blood residue testing blind study yielded positive results, which fit the expected results in all but one case, and negative results, which dispelled ideas regarding the potential for yielding false positives for organic materials other than blood, and minerals or other compounds present in soil. Only artifacts used for butchering muskrat (i.e., exposed to blood) yielded positive results. No positive results were seen on artifacts subject to other, non-blood organic materials, such as antler tine, dry muskrat

hide or hair, or cattails, indicating that the testing method is sensitive only to blood residue. The testing did yield one negative result on an artifact used for butchering muskrat that should have yielded a positive result. It is unclear why this artifact did not react with the presumptive blood reagent; however, this unexpected result may be due to an error in recordation rather than the true absence of blood.

4.1 Applications

The current presumptive blood residue testing blind study has proven that the use of Bluestar[®] Forensic is a reliable means to identify blood residue on replicated and authentic lithic artifacts. With the exception of one specimen, the blind test reliably indicated the presence of blood on all of the specimens which had been exposed to blood. In addition, several variables involving exposure to non-blood organics, including bone, vegetal material, and soils were tested in order to determine if false positives would result. The blind study did not yield any false positives.

The current presumptive blood residue testing blind study provides information that effectively advances the goals of archaeology in the identification of pre-contact period subsistence studies. It is a supplemental methodology that can be used to streamline and enhance traditional blood taxa studies. As has been demonstrated by this research, presumptive blood residue testing is an easy, fast, inexpensive, and cost effective way to ensure that artifacts which do not retain blood residue do not get forwarded for additional, more expensive and time consuming taxa testing. The ability to eliminate artifacts that have no potential to provide positive results during blood taxa studies increases the efficiency of the research and saves resources.

In addition, the blind study has proven that presumptive blood residue testing is a cost and time efficient way to identify artifacts, such as expedient utilized flakes, which may retain blood residue but would not normally be reserved for blood taxa testing due to their perceived limited use expectancy. The inclusion of artifact types that have not been traditionally tested for blood taxa in the past may alter our cultural interpretations of lithic tool manufacture and use in the future. In addition, since the reagent does not destroy the DNA associated with the blood residue, the artifacts that react positively to the reagent can be re-used in further blood residue or genetic material studies.

The presumptive blood residue testing blind study demonstrated that the materials necessary for the testing (i.e., Bluestar[®] Forensic, distilled water, spray bottle) are readily

available, inexpensive, easily portable and storable, easy to use, and can be used on large quantities of artifacts. In addition, the ease of use of this testing is enhanced by the ability to accomplish the presumptive blood testing in varied settings, including the field, laboratory, or other non-technical settings (e.g., hotel room, weatherport structure, etc.). The key to the effectiveness of the setting in which the presumptive blood residue testing is accomplished is the lighting. While the blue luminescence (reaction with blood) is bright, it is best viewed in dim light to darkness, especially when only small amounts of blood are present on an artifact.

4.2 Recommendations

Even though presumptive blood testing, as manifested in this blind study, appears to be an efficient and reliable tool which archaeologists can use to augment their research, future studies using the technique should continue to be performed. Future presumptive blood residue testing should concentrate on refining the methods used to record the testing results so that quantifiable information can be obtained; should use other types of archaeological remains to determine if functional interpretations need to be modified; should be completed on private and/or curated collections to augment their information potential; and should attempt to eliminate other variables which might cause false positives.

5.0 REFERENCES CITED

- Custer, J.F., J. Ilgenfritz, and K.R. Doms
1988 Application of Blood Residue Analysis Techniques in the Middle Atlantic Region. *Journal of Middle Atlantic Archaeology* 4:99-104.
- Fogel, H., D. Knepper, and M. Petraglia
1994 *Archaeological Investigations at the Kettering Park Site (18PR174), Prince Georges County, Maryland*. Maryland State Highway Administration, Archaeological Report Number 78.
- Gavrilovic, N.L., and S.K. Webb
2006 *Luminol vs. Bluestar® Initial Study*. Website at http://www.bluestar-forensic.com/pdf/en/St_Louis_comparison_study.pdf. Accessed January 3, 2007.
- Kavanagh, M., and C. Ebright
1988 *Archaeological Testing of Four Prehistoric Sites in Town Creek Valley, Allegany County, Maryland*. Department of Natural Resources, Maryland Geological Survey, Division of Archaeology. File Report Number 216.
- Loy, T.H.
1983 Prehistoric Blood Residues: Detection on Tool Surfaces and Identification of Species of Origin. *Science* 220:1269-1270.
- Loy, T.H., and B.L. Hardy
1992 Blood Residue Analysis of 90,000-year-old Stone Tools from Tabun Cave, Israel. *Antiquity* 66:24-35.
- Vish, A.T., and T.E. Yeshion
2004 The Use of Luminol as a Presumptive Blood Test for Prehistoric Archaeological Artifacts. *North American Archaeology* 25(2):153-159.
- Yeshion, T.E.
1991 Bioluminescence and Chemiluminescence: Current Status. In *Proceedings of the VIth International Symposium on Bioluminescence and Chemiluminescence*, September 1990, pp. 379-384. John Wiley & Sons, Cambridge, England.
2001 Presumptive Blood Testing. *Law Enforcement Technology*, 28(1):75-79.