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THE USE OF LUMINOL AS A PRESUMPTIVE BLOOD TEST FOR PREHISTORIC ARCHAEOLOGICAL ARTIFACTS

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ABSTRACT

Much controversy exists surrounding the reliability of the science of blood residue analysis and taxa identification in archaeology. Validating simple presumptive blood tests before proceeding to taxa identification should be an immediate priority. This paper serves to introduce luminol as an additional presumptive tool in blood residue studies.

INTRODUCTION

Blood residue analysis is comprised of two general levels of investigation. The first includes determining whether any blood is present on an artifact. If time, money, and science allow, the next step is to attempt taxa identification of blood identified on the artifact. This paper deals with the first level of analysis and serves to introduce luminol as an alternative presumptive blood test for consideration. The first tests using luminol on prehistoric artifacts returned positive results from two artifacts that previously tested positive for deer and catfish antisera from the East Bank site (36NB16) (East *et al.* 2002). Subsequent testing on other artifacts has indicated that luminol holds potential as a new tool in blood residue studies. An examination of luminol as an additional option may help to secure the consistency and reliability of blood residue analysis of archaeological artifacts.

BACKGROUND

The modern history of testing for blood on archaeological artifacts begins with Loy's 1983 article in *Science* (Loy 1983). Loy used a two-prong approach that included first testing an item for the presumptive presence of blood using the "chemstrip" method.

Custer *et al.* (1988:100) gives a description of the chemstrip procedure. To test artifacts with chemstrips, an extracted solution must be derived directly from the artifacts. It is this solution that is then tested. This is a somewhat complicated procedure in that artifacts must first be protected in a way that does not disturb soil in contact with the artifact. The preservation of soil would increase the concentration of

blood residue since it would absorb any that was on the surface of the artifact. From here, distilled water is applied to areas that are suspected of having blood residue or areas that were observed under magnification to have questionable residue. The water is allowed to sit while the residue is absorbed. The fluid is removed and tested with the chemstrip. If initial presumptive blood tests gave positive chemical indications for the presence of blood, further analysis to ascertain taxa identification would be conducted.

Many contradictions and controversies exist in the literature surrounding presumptive blood tests, specifically with regard to the lack of reported sensitivity levels and specificity. Many blood residue studies report conflicting or vague taxa results. The efficacy of the presumptive first step comes into question when such results are reported (see LeeDecker *et al.* 1996 for example). Although Sensabaugh *et al.* (1971) found that blood stored in a laboratory for 8 years lost a significant amount of its original biological activity, their focus was with regard to the enzymatic and immunochemical activity of specific globular proteins and not the peroxidase activity required for the presumptive detection of questioned matter as blood. This information may have been widely misinterpreted by archaeologists to mean that the *detection* of blood is affected the same way.

There is evidence suggesting that ancient blood can be detected. Loy (1983:1270) states, "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 (1991:379) claims that animal peroxidase is extremely stable to heat and time.

But the matter does not seem to be so simple. The vast majority of blood studies researched for this article used the "chemstrip" presumptive test as the initial step in these studies. The chemstrip method could not be evaluated at the time of this article based on a lack of technical information provided in any of the reports (i.e., sensitivity, chemical reactants, manufacturer, etc.). Add to this the manganese oxide contamination of chemstrips documented in Custer *et al.* (1988), and clearly, there is uncertainty in this realm. The issue of presumptive blood residue determination should first be settled before more studies are done to identify taxa.

Blood residue analysis is an aspect of analyzing prehistoric stone artifacts that is extensive in its science and expensive in its cost. Typical blood analysis studies include sending samples of stone tool assemblages to laboratories for in-depth analysis. These artifacts may have been chosen randomly, based on morphology, or other criteria. There is no prior knowledge as to whether the artifacts have been exposed to blood. Much time and energy can be wasted performing these tests on many artifacts, only to obtain negative results. With Loy's (1983) introduction to blood residue analysis, the primary step of testing for the presumption of blood was done using chemstrips. This was to provide an inexpensive method of screening items for the presence of blood before ascertaining taxa, but it still required sending the artifacts to a laboratory suited for such work. Using luminol as a presumptive test for blood in an archaeological context is a novel approach and does not require sending artifacts to a laboratory. We propose this test as a method to identify possible blood residue in an archaeological context, not to supplant any other method, but to add to the arsenal of tools used in these studies. Luminol's increased sensitivity, low cost, and ease of use, make it a

possible alternative to other presumptive methods. The benefits of luminol will add to the confidence researchers can place in blood residue analysis and ultimately, taxa identification.

LUMINOL

According to Yeshion (1991, 2001), 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. The peroxidase-like activity of hemoglobin is the basis of all catalytic presumptive blood tests.

Presumptive blood tests have been a critical tool for forensic scientists since the early 1900s. Although there are numerous tests available to the forensic investigator, the primary ones employed today are the reduced Phenolphthalein test and the luminol (5-Amino-2,3-dihydro-phthalzine-1,4-dione) test. Luminol differs from all other chromogen-based presumptive blood tests in that there is an emission of a blue-white light rather than a color change. As a result, luminol must be applied as a spray in near total darkness in order to see its reaction to trace quantities of blood otherwise invisible to the naked eye. Ancient buried samples bearing questionable stains, or suspected of possibly being bloodstained, will most likely not be responsive to the majority of the lesser sensitive chromogen-based presumptive blood tests. Luminol, however, is much more sensitive and has the ability to quickly screen many artifacts simultaneously and illuminate only those that give positive chemical indications for the presence of blood. If materials known to cause false-positive reactions are presumed to be absent (see

below), then these reactive samples can be further tested for species identification while screening out the negative samples.

False-Positive Reactions

It is important to recognize that there are serious limitations to using presumptive blood tests. First and foremost is the fact that no presumptive blood test is *specific* for the identification of blood to the exclusion of any other substances. C*onfirmatory* tests such as the Takayama or Teichmann tests are used by forensic investigators to absolutely prove that blood is present on a substrate. A major disadvantage of these confirmatory tests is that they do not work well with relatively old blood samples and likely impossible with ancient samples. Therefore, a negative finding would not necessarily mean that blood is absent.

Presumptive blood tests are indirect tests for blood in that the chemical reaction is based on the breakdown of an oxidizing agent such as hydrogen peroxide or sodium perborate by the peroxidase-like activity of hemoglobin. The problem is that peroxidases and other interfering substances exist in nature in matter other than blood. These interfering substances can be categorized into three groups that create false-positive reactions to presumptive blood tests:

- Chemical Oxidants: Even in the complete absence of blood, many household cleaners and antiseptics will cause a false-positive reaction to occur prior to the addition of hydrogen peroxide when using a multi-step method for presumptive blood testing such as the reduced phenolphthalein test. When using luminol, the inexperienced observer may have a difficult time differentiating such a reaction. Ordinarily, with evidence from a relatively current forensic investigation, the

phenolphthalein test would be used to backup positive results obtained from the luminol test. Although this technique generally works well in forensic cases, the lower sensitivity of the phenolphthalein test is more likely than not to react negatively on an archaeological artifact.

- Plant/Vegetable Peroxidase: Peroxidase naturally occurring in certain vegetables and plants typically gives a weaker reaction to luminol and acidic chromogen-based presumptive blood tests. Again, ordinarily in a forensic case the luminol reaction would be checked for this false positive with phenolphthalein as plant peroxidase does not cause false-positive reactions to phenolphthalein. This is most likely due to the fact that phenolphthalein, made alkaline with sodium hydroxide, reduces or eliminates false-positive reactions caused by plant peroxidase which prefers an acid substrate (Culliford 1971). The exception to this is when the *pulp* from horseradish, turnip, and parsnip are present (Quickenden and Creamer 2001). These vegetables have very high concentrations of peroxidase activity. In fresh cases one would be able to visualize the colors of the pulp material that make it look different from a bloodstain before luminol is applied. With regard to testing artifacts, plant/vegetable peroxidase, unlike animal peroxidase, is unstable to heat and time and therefore should not present a direct problem to testing artifacts.
- Chemical Catalysts: Luminol reacts directly and strongly to copper and its alloys, nickel, and iron. These reactions can be differentiated from a reaction to animal peroxidase from hemoglobin in that a reaction to blood will glow steadily, while a reaction to a chemical catalyst will burn and sparkle brightly, but fade away

rapidly.

It is strongly recommended that caution be taken when applying luminol to artifacts removed from areas where such materials are thought to be present. To increase the scientist's level of confidence in the test results, control areas consisting of surrounding soil, etc. should be sprayed and those reactions then compared to reactions observed directly on the artifacts. A positive control should also be run to ensure the chemicals are working properly. Forensic investigators will often use a copper penny for this purpose. Testing control areas is good science regardless of which presumptive blood test is employed. False positive reactions can occur with all presumptive blood tests, including chemstrips.

Cost

The cost of luminol is comparable to chemstrips, ranging from $20-$60 depending on the amount, though very little (4 oz.) is needed to test large quantities of artifacts. The low cost of luminol allows it to fit easily in any budget.

Sensitivity

The sensitivity of luminol is reported to be at least 1:1,000,000 (Yeshion 2001:77). Because of its sensitivity, luminol can be used at crime scenes to illuminate blood stains where attempts have been made to intentionally remove this evidence. Studies have also shown that the use of luminol does not adversely affect future DNA analysis (Yeshion 2001:77). The sensitivity of chemstrips, like most of the chromogen-based presumptive blood tests, is considerably lower at approximately 1:5,000 for dried bloodstains. One important note concerning the chemstrip method is that the procedure itself can decrease the sensitivity of the test. Adding water to a suspected stain only

dilutes the material further. The luminol method is conducted without any initial dilution of suspected blood residue.

Ease of Use

As stated earlier, to test artifacts with chemstrips, a solution must be derived directly from the artifacts and great care should be used to preserve suspected residues. This procedure still requires a relatively sophisticated lab and technicians to carry out the test.

Though artifacts to be tested with luminol should be handled in a similar fashion as the chemstrip method, luminol's sensitivity does provide considerably more freedom concerning this point. Application of luminol does not require a water extraction process. Luminol is applied as a spray directly to the surfaces to be tested. This spray is nondestructive to the artifact or to any further subsequent serological or DNA analysis. The disadvantage of using luminol compared to the chromogen-based presumptive blood tests is that it must be applied in the dark. It does not, however, require any artificial light source to visualize the reaction. It is a self-contained chemiluminescent reaction. This is the trade-off for employing a presumptive blood test with optimal sensitivity. Using luminol only takes an experienced eye to interpret the results. The set up and application can be done in any setting and does not require any particular laboratory environment.

LeeDecker *et al.* (1996), in their analysis of artifacts from the Two Guys site (7S-F-68), found a biface that tested negative in the presumptive tests using chemstrips. Later, the same artifact gave positive results when tested for specific taxa. One explanation given by the authors for the contradictory results is that the solution derived

for the presumptive blood test was not taken from the exact same area as that tested for species identification. This is a logical explanation, but one which presents a potential problem that should have caught the authors' attention. The use of luminol in this same scenario would have avoided these contradictory findings since the reaction can easily be viewed in direct relation to the artifact in real time. Thus, there would be no question about which part of the artifact was reactive and which was not.

FUTURE PROPOSALS

With controversies surrounding blood analysis, it appears researchers may have gotten ahead of themselves. Validating simple presumptive blood tests before proceeding to taxa identification should be an immediate priority. Collaboration is needed between the forensic sciences and archaeologists to weed out, through constant interaction and communication, any contradictions and falsehoods with presumptive blood tests that may be skewing the end results of taxa identification. With such collaboration, the research presented in this article will be followed in the future by systematic investigation of luminol. First, blind tests with replicated artifacts, randomly tainted with blood will be tested. This experience will continue to improve the outcome of identifying artifacts that have been exposed to blood by viewing the luminol reaction and becoming more comfortable with the process. Next, luminol will be tested in an archaeological context. Taxa identification on prehistoric artifacts is useless if archaeologists cannot carry out presumptive tests on artifacts with consistency and confidence. This fundamental principle is critical to furthering blood residue studies.

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Al Vish has a B.S. Degree in Geology from West Virginia University. He has worked as an archaeologist at Skelly & Loy, Incorporated for 11 years. His other interests have included studying thermally altered rock by replicating roasting features at the Harding Flats site in Tunkhannock, Pennsylvania, investigating alternative resources of Bald Eagle Jasper in the area of State College, Pennsylvania, and computer data management specifically geared toward investigating archaeological sites.

Ted Yeshion, President of ClueFinders, Inc., earned a Master of Science degree in Forensic Science from the George Washington University ('75) in Washington, D.C. and is currently completing studies at the State University of New York at Buffalo towards a PhD degree. Ted retired from the Florida Department of Law Enforcement in October 1999 and has a combined total of 30 years experience as a forensic scientist, crime lab director and special agent. He has testified for both the prosecution and defense in hundreds of criminal and civil litigation cases as an expert witness in the areas of Forensic Serology, DNA Analysis and Crime Scene Reconstruction. One specialty area that has earned Mr. Yeshion an international reputation as a leading forensics expert is in the application and interpretation of Luminol as a presumptive test for blood. Ted was a guest of Greta Van Susteren's Fox News show "On the Record" twice, talking about the application of Luminol in the Scott Peterson case. Ted Yeshion is a Fellow of the American Academy of Forensic Sciences and a member of numerous other professional associations. He is a noted author of peer-reviewed scientific papers, an international lecturer and an adjunct professor of forensic science and criminal justice.