A16 False Negatives and Decreased Sensitivity of Heme Tests on a Leather Substrate: Incidence and Causes

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After attending this presentation, attendees will be made aware of the potential for presumptive and confirmatory false-negative test results as produced by bloodstains on leather casework samples, and the possible chemical causes for the desensitized reaction.

This presentation will impact the forensic science community by showing how the possibility of substrate interference has significant implications. Although there are many publications devoted to the possibility of false positives when testing for blood (one of the most recent being a presentation at an AAFS meeting in 2011), comparatively little time has been spent investigating false negatives. Leather boots, jackets, and upholstery are encountered regularly as substrates during the investigation of violent crimes, and in this study, are identified as a possible source of false negative screening results. In turn, these results would mean lost opportunities for more discriminatory testing, such as DNA analysis. Field and serology analysts may be able to use the information generated during this study when deciding whether to include a "negative" sample for further DNA analysis.

Anecdotal reports from the New Orleans Police Department Crime Lab suggested that blood samples on Timberland® boot leather may produce false negative results when using phenolphthalein, a heme-catalyzed presumptive blood test. This claim was tested by acquiring boot leather samples and applying serially diluted human blood, using cotton cloth as a comparative standard. A general decrease in sensitivity over time, greater than that observed on control samples, was observed in the boot leather samples: in less than two weeks, blood dilutions of $1/10^3$ were still detectable on cotton cloth, but only 1/10 dilutions and whole blood were detectable on Timberland® boot leather. The result was then duplicated in upholstery leather samples when, after only two weeks, phenolphthalein sensitivity decreased from $1/10^3$ on cloth to 1/10 on two different types of upholstery leather. The common decrease indicates that the chemical inhibitor is most likely not specific to Timberland®-brand tanning processes, but is common to the leather industry as a whole.

A similar order-of-magnitude decrease in detection of blood on both boot leather and upholstery leather (as compared to cotton substrates) was observed with benzidine (another heme-catalyzed presumptive blood test); with Hematrace antibody/antigen test kits; and with traditional Takayama crystal testing. Heme-catalyzed color tests and Hematrace human hemoglobin detection tests showed a decrease in sensitivity in as little as two weeks; Takayama crystal tests showed a decrease in sensitivity in under a month.

The three tests utilize varying elements of the hemoglobin molecule. In heme-catalyzed color tests, the heme group central to the hemoglobin molecule behaves as a peroxidase, reducing hydrogen peroxide to water and, in turn, depleting the hemoglobin of electrons; these electrons are replaced from the test dye molecule, transforming the molecule from its colorless to a colored state. Although these tests are known to be pH-sensitive, phenolphthalein and benzidine have substantially different effective ranges (between 8 and 10 versus any pH above 4). The ABAcard Hematrace test uses anti-human-hemoglobin antibodies to produce a highly sensitive color-positive result. The Takayama crystal test relies on the formation of pyridine hemochromogen crystals when the reagent is reacted with hemoglobin and heated. As all three of these tests displayed reduced sensitivity and detection potential when performed on bloodstains exposed to a leather substrate, the question remains as to what chemical aspect of the leather substrate is affecting the tests, and whether the reaction can be easily reversed.

As the undesired effects appear not to be pH-dependent, and are common to multiple types of leather, the presence of residual tanning chemicals is the most likely source of the observed false negative effects. Further study on the effects of tanning chemicals on the iron atom at the center of the heme moiety is expected to reveal the source of the multi-test false negatives. Whether or not a simple counter-treatment can be identified, criminalists should be mindful of the age and environmental history of leather case samples submitted for serological screening, and make analytical decisions accordingly.

Blood Detection, Heme, Leather

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