B115 Hematoporphyrin Fluorescence as a Test for Blood—Alternative Methods and Potential Interferences

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The goal of this presentation is to present the results of recent research in the area of hematoporphyrin fluorescence utilizing alternative methods of conducting this blood identification test with modern instrumentation. This presentation will have the purpose of illustrating newly recognized potentials of this currently dormant technique.

The research presented in this paper aims to clarify some of the questions currently surrounding the hematoporphyrin fluorescence test (HPF), and explores the possible advantages that the test can offer over catalytic presumptive tests for the identification of blood. The history and mechanism of the test will be presented, along with the conditions utilized, and details of problems that one may encounter when the fluorescence from a blood sample is examined instrumentally.

Hematoporphyrin fluorescence is a test with a long history. It has been under-utilized in forensic laboratories where it is considered to be merely another presumptive means of identifying an unknown material as blood. This lack of utilization can be attributed to the limitations of the standard method by which the test is normally conducted and uncertainty surrounding potential false positives. This is an unfortunate situation given the benefits the test may offer with regard to the small sample size required for an analysis coupled with the specificity that can be attained with it.

Historically, when this test was properly employed, a positive result was based upon visual detection of the orange red fluorescence that developed after a suspected stain was treated with a reagent, most commonly sulfuric acid, and exposed to long wave UV light. It has been argued that other porphyrin containing materials, namely chlorophyll, bile, feces, and meconium, will react in a similar manner and provide fluorescence that is identical to that obtained with blood when observed visually.

Other techniques utilizing different reagents and experimental procedures were evaluated along with the application of concentrated sulfuric acid on samples consisting of whole blood and protoporphyrin IX, the porphyrin found in blood. The resulting red fluorescence was examined instrumentally for spectral characteristics which could be used to distinguish blood from the other materials. Following this preliminary examination, porphyrin compounds similar to those found in the materials reported as false positives were tested and examined for differences in the fluorescence spectrum. Based on this spectral examination, all were differentiated.

All three techniques were then slightly modified and utilized on samples of dried blood, both alone and on a cotton substrate. This was accomplished with a fluorescence microscope equipped for microspectrophotometry. When carried out in this manner a small portion of a bloodstained thread was sufficient sample for analysis. With some methods, a result was even obtained from a single bloodstained fiber. The collective results of these trials will be presented along with a discussion of the advantages and disadvantages of each technique.

Blood Identification, Fluorescence, Microscopy

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