



A90 Determination of the Sensitivity and Specificity of Six Presumptive Tests for Blood and Their Effect on the Recovery of High Molecular Weight DNA

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The goal of this research was to compare new presumptive tests for blood with each other and with traditional tests. It will show the benefits and drawbacks of the tests depending on their intended use and any subsequent evaluation of the evidence which may be required.

This research will impact the forensic science community by providing an unbiased analysis of the tests. By bringing together the most used presumptive tests and demonstrating their strengths and weaknesses of each, police forces and forensic scientist will be able to make informed decisions of which tests to use under different circumstances.

It is almost never necessary to apply presumptive test reagents directly to dried bloodstain evidence. However, with extremely small samples or when testing large areas it may be necessary to expose potential bloodstains directly to presumptive tests. It is therefore important to know that the tests being used will not destroy the sample for further serological testing. Luminol, leuchomalachite green, phenolphthalein, Hemastix®, Hemident™, and Bluestar® are all used as presumptive tests for blood. In this study, the tests were subjected to dilute blood (from 1:10,000 to 1:10,000,000 dilution factor) to determine the sensitivity of each test. Specificity was determined by reacting the tests with 14 different substances found previously to react with the tests or which could be mistaken for blood. The presumptive tests were applied to blood stains which were subsequently tested for DNA to determine if the presumptive tests damaged or destroyed the DNA.

Five dilutions of blood were prepared using whole blood and sterile water: 1:10,000; 1:100,000; 1:1,000,000; 1:5,000,000 and; 1:10,000,000. One hundred and fifty repetitions of each dilution of blood were placed on filter paper and allowed to dry prior to analysis. Each dilution of blood was then analysed 25 times with each of the presumptive tests.

Each presumptive test was also analysed 25 times for each of the 14 substances: saliva, semen, potato, tomato, tomato sauce, tomato sauce with meat, red onion, red kidney bean, horseradish, 0.1M ascorbic acid, 5% bleach, 10% cupric sulphate, 10% ferric sulphate, and 10% nickel chloride. The effect of each presumptive test on the recovery of DNA was determined by reacting blood with each of the presumptive tests followed by extraction and amplification. The largest and smallest of the SGMPlus™ loci were tested (D2S1338 and D19S433).

All presumptive tests were able to detect blood to a dilution of 1:10,000. Only luminol and Bluestar® were able to detect blood at a dilution of 1:100,000. Phenolphthalein, Hemastix® and Hemident™ did show a reaction at the 1:100,000 dilution level, however the color change reaction took over 1 minute to develop. None of the tests showed a positive reaction with dilutions of 1:1,000,000 or greater. Specificity studies showed that of all the substances tested, not one of the household items reacted with every test, however the chemicals did. Of those substances which did react most could not be mistaken for blood as the reaction proceeded at a different speed and color than what would be expected if blood was present. Results from each test were analysed using a chi-squared test to determine if they came from populations with the same distributions, which would indicate that they react in a similar way.

DNA was recovered and amplified from four of the presumptive tests. Luminol, phenolphthalein, Hemastix® and Bluestar® achieved DNA amplification at both loci tested, which corresponded to the alleles found on the positive control. Hemident™ and leuchomalachite green both produced negative results indicating that their use will destroy any DNA present. Phenolphthalein had a much reduced peak height compared with the other three tests indicating inhibition in the PCR or degradation of some of the DNA.

Presumptive Blood Tests, Sensitivity and Specificity, DNA Recovery