THE EFFECTS OF ACID PHOSPHATASE MAPPING ON DNA RECOVERY

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Sexual assault cases comprise approximately 20% of the caseload at the Harris County Institute of Forensic Sciences Forensic Genetics laboratory. We endeavor to use the most effective methods to screen the evidence to locate possible bodily fluids. Acid Phosphatase (AP) mapping is used as a forensic tool to locate semen stains on clothing or bedding. It is customary to test stained areas indirectly by a method that transfers the dissolved enzyme onto wet filter paper applied directly to the fabric. AP activity is located on the paper using a serological AP Spot test reagent; a strong positive color change is indicative of the presence of semen at the corresponding location on the fabric. Many laboratories use an AP mapping technique but we were unable to locate studies of the amount of DNA that is lost via transfer using this method. This study focuses on characterizing the removal of sperm and non-sperm DNA from fabric samples after AP mapping treatment. The integrity of the DNA removed during the AP pressing process is also evaluated. The second goal of this study is to determine the amount of water, force, and time needed to obtain an effective AP test while minimizing or preventing the transfer of DNA to the AP paper.

Three different fabric materials, denim, cotton (bed sheet), and polyester, were tested in this study. In the first series of tests, an equal volume of human semen was deposited on 1 cm^2 fabric cuttings in triplicate and allowed to air dry at room temperature for approximately three hours. 1 cm^2 filter paper cuttings moistened with sterile water were laid over the fabric pieces and pressed with a grill press for 5 seconds. Two different subjects performed the pressings on the triplicate fabric samples. The filter papers were treated with the AP Spot test reagent solution, incubated for 5 minutes, and the results were recorded. All the AP filter papers were scored as AP +4. The fabric and AP paper cuttings were then extracted via a Qiagen differential extraction method without obtaining the non-sperm fraction. The AP pressing samples and controls were examined for spermatozoa and for the presence of PSA. The samples were quantified with Quantifiler Duo, amplified with Identifiler Plus, and then typed by capillary electrophoresis. The male and total human DNA was quantified for each sample and the percentage of human DNA removed was calculated. Samples with undetected amounts of DNA were not amplified.

AP paper pressings from the cotton bed sheet fabric samples removed an average of 17% of the sperm DNA. The AP paper pressings from the denim samples removed approximately six times less sperm DNA than the cotton samples at an average of approximately 3%. The polyester treated fabric samples lost even less sperm DNA to the paper at an average of 0.4%. A microscopic slide search of the AP pressings aliquots was negative for sperm; only paper sample, from polyester, was PSA positive.

The next study will vary the amount of weight used to press cotton fabric to determine the most effective force to acquire the highest AP results with the minimum amount of DNA removed. From the previous study, pressing cotton resulted in significant percentages of DNA removed in both trials. We will test 10, 15, 20, and 30 pound weights for 5 seconds. The cotton fabric will be pressed in triplicate and the AP pressings will be tested for AP. The fabric cuttings and AP pressings will be extracted and quantified. In a further test, pressing times will also be varied.

The final study will test mixtures of saliva and semen samples on the three fabric types with the ideal weight. The fabric and AP pressing samples will be then extracted and typed for DNA. Based on these studies attendees will be able to make an informed decision on what steps are needed to be taken in their own laboratories to prevent DNA loss due to acid phosphatase mapping.