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Abstract
Screening a substrate for bodily fluids is an extremely important step for locating areas that may contain DNA. Several different methods have been developed for saliva (1). The Phadebas® Forensic Press (PFP) test is a presumptive saliva test that utilizes a preloaded paper that will react with the enzyme amylase, a component of saliva (2-5). Because of its ability to screen for amylase while simultaneously locating stains, the PFP may prove to be an effective, rapid method for screening. However it is important to assess whether the PFP introduces any inhibitors (7) to downstream processing such as PCR amplification. Based on previous studies, we hypothesize that the PFP will provide a rapid and sensitive method for locating multiple saliva stains simultaneously, without introducing inhibitors to DNA profiling. To test the limitations of PFP as well as evaluated its effects on DNA profiling we first created a dilution series of saliva ranging from neat to 1:5000. After this we preformed sensitivity tests on an indirect method, UV degraded samples and washed samples as well as with bodily fluid mixtures. Once all sensitivity tests were done, cuttings were taken from the substrate and PFP paper and analyzed for DNA. Tests found that the sensitivity ranges of the PFP were between 1:10 and 1:1000, indirect tests were less sensitive than direct, all bodily fluid mixtures were detected, and UV degraded samples took more time to react. In addition our DNA results confirmed our hypothesis that PFP does not inhibit DNA and is a useful method for locating stains. This project was funded by NSFREU Grant DBI 1262832.

Keywords
DNA typing, saliva testing, PCR

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DNA Typing Compatibility With a Rapid, One Step Saliva Screening Test

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Abstract

Screening a substrate for bodily fluids is an extremely important step for locating areas that may contain DNA. Several different methods have been developed for saliva (1). The Phadebas® Forensic Press (PFP) test is a presumptive saliva test that utilizes a preloaded paper that will react with the enzyme amylase, a component of saliva (2-5). Because of its ability to screen for amylase while simultaneously locating stains, the PFP may prove to be an effective, rapid method for screening. However it is important to assess whether the PFP introduces any inhibitors (7) to downstream processing such as PCR amplification. Based on previous studies, we hypothesize that the

PFP will provide a rapid and sensitive method for locating multiple saliva stains simultaneously, without introducing inhibitors to DNA profiling. To test the limitations of PFP as well as evaluated its effects on DNA profiling we first created a dilution series of saliva ranging from neat to 1:5000. After this we preformed sensitivity tests on an indirect method, UV degraded samples and washed samples as well as with bodily fluid mixtures. Once all sensitivity tests were done, cuttings were taken from the substrate and PFP paper and analyzed for DNA. Tests found that the sensitivity ranges of the PFP were between 1:10 and 1:1000, indirect tests were less sensitive than direct, all bodily fluid mixtures were detected, and UV degraded samples took more time to react. In addition our DNA results confirmed our hypothesis that PFP does not inhibit DNA and is a useful method for locating stains. This project was funded by NSF-REU Grant DBI 1262832.

1.Introduction

DNA typing has become an important tool in forensic science. Steps in forensic DNA typing include screening for biological samples such as blood, saliva or semen, DNA extraction, quantification using quantitative PCR, PCR amplification of genetic markers such as autosomal or Y chromosome short tandem repeats, comparison to reference or database DNA profiles and then interpretation and reporting of results. Screening tests are often done with presumptive test methods. A test is considered presumptive because it only tests for a component of a substance (e.g. the component that is tested for when screening for saliva is amylase). Currently two methods for screening for saliva are being used in the Santa Clara County

Crime laboratory, the Alternate Light Source (ALS) method, and the Starch- Iodine test. The ALS method subjects a substrate to different wavelengths and if bodily fluids are present they fluoresce. However, studies have shown this test to give false positives (4). The Start- Iodine test works by reacting with an enzyme found in saliva, amylase, which breaks down carbohydrates. Multiple different swabs are taken from a substrate then the saliva is extracted from these swabs. Next the extracted sample is placed in a starch late well and left to incubate until Iodine is poured over the starch plate turning it blue. If amylase is present, then the starch plate will not turn blue. This method is time consuming, and imprecise. Because of these challenges, a new presumptive saliva screening test is being implemented in the Santa Clara County Crime Laboratory. This test is known as the Phadebas® Forensic Press (PFP) test. The PFP test is a paper that has been preloaded with an immobilized starch. If a substrate contains amylase, the amylase hydrolyzes the starch creating a color change (2,3,4). This method is believed to be a better alternative to the two current methods because it is easy to interpret, and can detect and map multiple stains simultaneously, therefore taking less time.

2. Methods and Materials

2.0 Materials

White cotton fabric was purchased from Jo-Ann Fabric then machine washed on the gentle cycle with Tide detergent and dried in a dryer on low to remove any water resistant chemicals that had been put on the fabric during manufacturing. The fabric was cut into 20 x 20 cm squares. Then 150ul of sample was used to make each stain placed on the fabric. Up to six stains were placed on one piece of fabric.

2.1.0 Determining the Sensitivity Limits of the PFP Test with a Direct Method

To evaluate sensitivity of the method, dilutions of saliva (from neat down to 1:5000) were stained on cotton substrates. Saliva dilutions from five different donors (NR, EB, BB, JW, and TM) were collected and used to create 1:2, 1:10, 1:100, 1:1000 and 1:5000 dilutions. After stains were placed they were left to dry overnight. The next day the PFP test was performed following the Protocol prepared by the manufacturer(2,3). The test was observed closely to detect visual color changes for 40 minutes.

2.1.1 Determining the Sensitivity Limits of the PFP test with an Indirect Method

A sample was prepared in the same way for an indirect test using JW's saliva dilution and the same protocol was followed, however, instead of pressing the PFP paper directly to the substrate a piece of filter paper was moistened and placed in between the PFP paper and the substrate to prevent chemical transfer from the paper to the substrate.

2.1.2 Determining the Sensitivity Limits of the PFP test with Degraded Samples

Donor NR's saliva dilutions were stained on a substrate and subject to UV degradation. Once dried, one sample was place in the back of a car window where it would be exposed to sunlight and hot temperatures for a period of one week. The other sample was placed in a UV cross linker for 8 minutes at 250,000 uJ. In addition to UV degradation one stained sample containing JW's saliva dilution was machine washed with laundry detergent on the gentle cycle with cold water and left to dry. All samples were tested following the PFP protocol (2,3).

2.1.3 Determining the Sensitivity Limits of the PFP Test with Bodily Fluid Mixtures

Mixtures of blood:saliva using TM's saliva and semen:saliva using JW's saliva were prepared (1:5, 1:2, 1:1, 2:1 and 5:1) and stained on cotton substrates. They were left to dry then tested with PFP using the manufacturers protocol (2,3).

2.1.4 Semen Testing: False Positives and Acid Phosphotase Testing Compatibility

Neat semen was stained onto a substrate and then tested with PFP to determine if the amylase levels in semen could result in false positives. Next a semen dilution series down to 1:128 was created and stained on a cotton substrate and tested with the PFP test to determine if any chemicals in PFP test interfere with Acid Phosphotase Testing , the presumptive test used for semen.

2.2 Testing for DNA Inhibition

Cuttings were taken from the just the fabric, just the paper, and of both the paper and the fabric and placed in 2ml microcentrifuge tubes (Costar® 3212) then subject to organic DNA extraction, DNA quantification (Quantifiler® Duo qPCR kit Life Technologies, Foster City, CA) and PCR amplification (Identifiler® Plus and Yfiler® STR kits Life Technologies, Foster City, CA) to evaluate whether any inhibition was observed. All processes followed the protocol developed by the Santa Clara County Crime Laboratory. Once a profile was generated it was compared to the known profile of the donor to determine how many alleles from the donor were present and if any contamination was present.

3. Results

3.1 Sensitivity: Indirect Test Less Sensitive, Dilution Series Detects Down to 1:1000.

Degraded samples exhibited delayed positive results taking approximately twice as long to detect. Control saliva was detected in just under 5 minutes whereas UV cross linked neat saliva was detected in just under 8 minutes as seen in Fig. 2. Samples that were placed in a car window also exhibited delayed reactions. The neat sample took almost three times a long to detect (just under 15 minutes) as seen in Fig. 2.

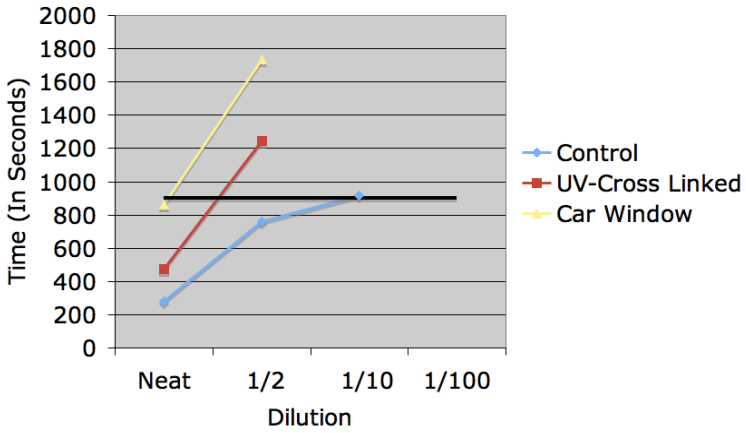

Fig. 2. UV exposed samples took longer to react than the controlled, un-degraded sample. Seconds indicate how long it took to see a visual color change. A black line has been added as a reference at 15 minutes.

Samples that were washed were not detected within the 40-minute observation time; however, after the PFP paper was removed, dye had been transferred to the substrate and once dry, a color change was observed on the PFP paper.

3.3 Sensitivity: Bodily Fluid Mixtures Demonstrate Expected Results

All ratios of blood and saliva were detected but reactions were delayed out to 30 minutes for the lowest ratio of saliva to

blood (1:5) Fig. 3. All ratios of semen and saliva were detected in less than 10 minutes (Fig. 3).

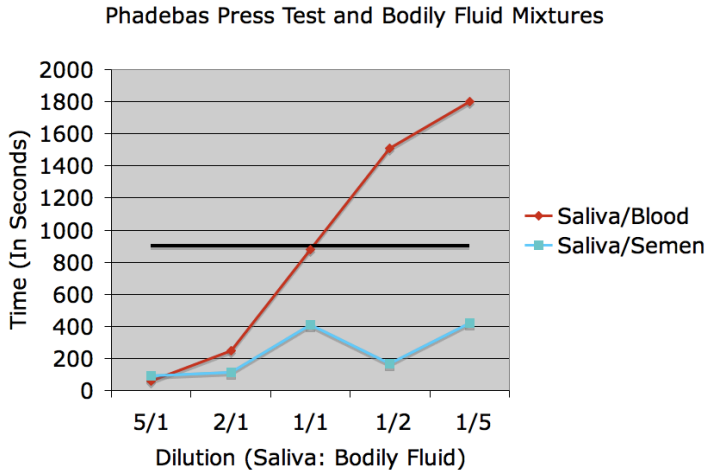

Fig. 3. All mixtures were detected in 30 minutes. Seconds indicate how long it took to see a visual color change. A black line has been added as a reference at 15 minutes

In addition to this, neat semen and diluted semen samples were not detected by the PFP test indicating that PFP does not give false positives when only semen is present. Acid Phosphatate testing results were positive for semen after being tested with PFP.

3.4 No PCR Inhibition Observed: Full Profiles Recovered Down to 1:100 Dilution

Full STR profiles for both Identifiler® Plus (Fig. 4.) and Y Filer® were amplified using DNA extracted from cuttings of the fabric or cuttings of the fabric and PFP paper for all saliva dilutions of EB and BB down to 1:100. Full profiles were only recovered for neat samples when using only PFP paper cuttings. For autosomal profiles 32 alleles is considered a full profile

where only 17 alleles are needed for Y chromosome profiles. Allele recovery was proportionate to the amount of DNA recovered during quantification (results not shown).

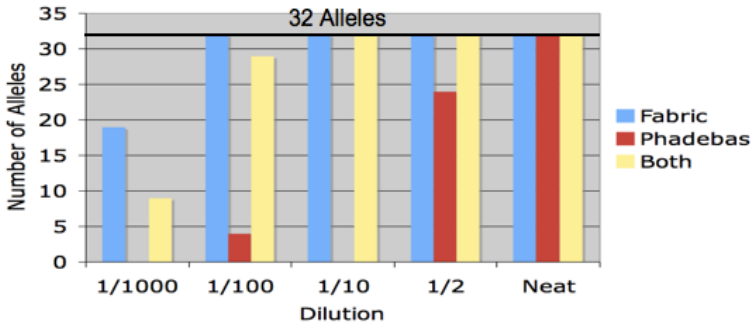

Fig. 4. Allele recovery from donor EB from either cuttings from just fabric, just PFP paper, or from both fabric and PFP paper. A black reference line has been added at 32 alleles.

3.5 No PCR Inhibition Observed for Mixtures: Full Profiles Recovered for all Mixtures

Full profiles were observed using DNA extracted from the fabric substrate for all mixtures of semen and saliva with no apparent inhibition for both the sperm cell fraction and the epithelial cell fraction. A full profile for the sperm cell fraction was considered to be 32 alleles from the male donor while a full profile of the epithelial cell fraction was considered to be 64 alleles, 32 from the male donor and 32 from the female donor. DNA extracted from the PFP paper provided full STR results only for the neat and 1:2 dilutions for only the sperm cell fraction.

4. Discussion

The PFP appears to have adequate sensitivity to generate full profiles for DNA profiling. The wide range in detection (between 1:10 and 1:1000) is most likely explained by the

natural varying amount of amylase in between every individual. Furthermore, a full DNA profile was generated for BB down to 1:100 dilution; however, the PFP test only detected amylase down to 1:10 dilutions. This indicates that PFP does not detect all possible stains that contain DNA. This result is expected, as the amount of amylase and the amount of DNA in a stain are not correlated (4).

No PCR inhibition seems to have been observed. Full profiles were recovered down to 1:100 with incomplete profiles at 1:1000. The incomplete profile is not believed to be due to inhibition because when compared to the amount of DNA recovered at quantification, a full profile recovery would not be expected due to low levels of DNA.

Preliminary results support the hypothesis. The PFP test appears to be a rapid, sensitive, method capable of detecting multiple stains simultaneously without inhibiting PCR. After comparing the number of alleles recovered to the amount of time it took the PFP test to detect a stain, the established cut off time for sensitivity is 15 minutes for most samples with a recommendations to check test again at 40 minutes for degraded or other compromised samples. Cuttings from only the fabric are necessary to recover full profiles while cuttings from only PFP paper generate incomplete profiles after the neat sample. This indicates that there is minimal transfer of substance between the substrate and PFP paper.

Further studies should include DNA results for blood:saliva mixtures as well as for degraded samples and assessed for inhibition and allele recovery should be compared to the amount of time it took to detect the stain using the PFP test. Additional tests and validations will be performed to determine the efficacy and limitations of the Phadebas® Forensic Press test even further

before it is implemented in the Santa Clara County Crime Laboratory.

5. Acknowledgements

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