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ARTICLE

Development of a Microfluidic Device (μPADs) for Forensic Serological Analysis

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In this paper, we describe a paper microfluidic device capable of performing a variety of presumptive tests for the presence of biological fluids at crime scenes. The device is multiplexed and permits the simultaneous detection of blood, saliva, semen and urine. This portable device utilizes a set of hydrophylic channels created with wax on chromatographic paper. On the terminal end of each channel, there are embedded colorimetric reagents that can be read by eye or detected using a cell phone camera. This portable device permits fast, simple and simultaneous screening of 4 different body fluids. It should prove useful in forensic analysis for individuals interested in sample collection for subsequent downstream laboratory analysis.

Introduction

In forensic science, determining the presence of biological samples involves the application of a set of chemical and enzymatic colorimetric tests.[1] This process, known as forensic serology, typically involves a two-step process.[2] The first step utilizes a presumptive screening test which indicates the potential presence of the biological fluid. The second step utilizes a confirmatory test which is human specific and permits identification of the body fluid at a higher certainty.[3] Presumptive testing is usually portable and capable of being used outside of the laboratory. Crime scene testing for body fluids can be critical for forensic investigations as such tests are used to assist investigators in the collection of evidence that may later be utilized in DNA typing. Proper collection of biological samples can be a critical influence in the outcome of a case. [4]

Unfortunately, traditional methods for identifying unknown biofluids and stains at crime scenes can be complex, destructive, and time consuming. When multiple tests are applied to the same evidence, this process can waste valuable sample. Thus, there is a need to develop methods which minimize time and limit sample wastage during evidence collection. In recent years, there have been a number of new procedures developed for forensic serology involving immunoassay and spectroscopic methods.[5,6,7,8] There also have been more advanced methods involving proteomic and epigenetic techniques.[9,10] While these new developments have advanced the field and in many cases have improved specificity, there is still a need for highly portable methods which can be quickly applied to suspect evidence at a scene without the need for expensive instrumentation or complex chemical devices.

One potential solution to the problem of developing fieldable detection methods involves the application of microfluidic devices.[11-14] These novel analytical tools known as “lab on a chip” have allowed researchers to analyse molecules and materials on chip-like devices using micro-scaled samples.[15] Using these devices, ultralow volumes of biofluids can be analysed in clinical applications, saving both time and precious samples.[16]

Microfluidic paper-based analytical devices (μPADs) were first developed at Harvard University by the Whitesides Group.[14,17-19]
These devices are being developed for a wide variety of applications in food,[20] environmental, [20] forensic

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and biomedical research. [16, 21] For example, microfluidic systems have been developed to detect various pathogens, additives and contaminants within food and water[20], nitrate for urinary tract infections, sugar and protein levels in blood, and biomarkers in cardiovascular disease, lung cancer, and transmitted infections.[21-25] μPADs are capable of both semi-quantitative and qualitative analysis.[26,27]

In this paper we propose the application of multiplexed paper microfluidic devices for presumptive testing in forensic serology. Because of its convenience, colorimetric detection has been used in both presumptive and confirmatory serological testing.[6,27] Modern applications include devices utilizing lateral flow immunoassays that can provide specific detection of targeted analytes.[26,28,29] However, these devices are more commonly used as confirmatory tests.[5] Instead, in this project, we are interested in the potential application of paper microfluidic devices as a more portable and more convenient method for presumptive detection of biological fluids at the crime scene. These systems utilize melted wax to define channels on chromatographic paper. The cellulose composition of paper is compatible with proteins and biomolecules and allows aqueous biological fluids to travel to the test wells via capillary action.

The hydrophilic channels created by the thermal wax direct liquid samples along a main channel to colorimetric sensor arrays. Incorporating colorimetric detection with microfluidic paper-based analytical devices (μPADs) can help to detect the presence of biological materials through a series of chemical reactions. Multiplex sample analysis occurs via the tree/branch structure of the device in which a single channel branches off into a series of detector pads. Previous work conducted within this laboratory has demonstrated the potential of paper microfluidic devices for the detection of explosives, seized drugs and toxicological samples.[30-33]

In this project we have developed a colorimetric sensing system that can be used in fieldable presumptive testing of body fluids. Modifications were made to the currently available colorimetric serological tests for blood, saliva, semen, and urine in order to permit multiplexed analysis on a single paper device.[5,34-38] The composition of the reagents on the paper matrix were adjusted and modified to permit stabilization and quick visualization.

Experimental

Chemicals

All chemicals used for this project were analytical grade. Sodium Perborate Tetrahydrate, Granular, 97% was purchased from Alfa Aesar™ (Tewksbury, MA, United States). Fast blue B Salt, Urease from Canavalia ensiformis (Jack Bean), α- Naphthyl Acid Phosphate Monosodium Salt, and Nessler's Reagent were all purchased from Sigma-Aldrich (St. Louis, MO, United States). Phenolphthalein, glacial acetic acid, and iodine solution were purchased from Fisher Scientific (Pittsburgh, PA, United States). Laboratory corn starch was obtained from Fisher Scientific (Pittsburgh, PA, United States). For each testing reagent, standard solutions were prepared bi-weekly for sample testing and placed on the chips.

Biological Fluids

Biological specimens, including urine, blood, saliva, and semen, were collected by volunteers with procedures approved by the Health Science Institutional Review Board of Florida International University, IRB-16-0279 and follows relevant federal regulations for the protection of human subjects in research (45 CFR 46). Written informed consent was obtained from all subjects. When conducting testing, dried and wet samples were tested. Two microliters of neat sample were diluted with distilled water to make a 4mL solution. Nine different concentrations of analytes were prepared including dilutions of 1:2, 1:5, 1:10; 1:20, 1:50, 1:100; 1:200, 1:500, and 1:1000. Once diluted, the samples are transported via capillary action up individual hydrophilic channels of the μPAD to a reagent location. When a blood, semen, saliva or urine sample contacts its designated reagent, a colorimetric reaction occurs, producing a readable response.

μPADs

A four-lane μPAD was designed as a testing device to detect, identify, and differentiate the following body fluids: blood, saliva, semen, and urine. The

device was prepared using a commercial wax printer (Xerox Color Cube, 8570DN, Xerox, US), which was used to print the four-lane pattern on chromatographic paper (Whatman No.1, GE Healthcare, UK). After printing, the paper was run through a temperature controlled laminator three times using aluminium foil as a heat insulation packing pouch to melt the wax into the paper. The laminator temperature was set at 160 °C at a motor speed of 2 cm/sec (Tamerica/Tashin Industrial Corporate, TCC-6000) to ensure that the wax printed would completely melt through each side of the paper, allowing for hydrophobic barriers to be created.

Each lane was designed to detect a specific type of biological sample that was present within a collected unknown fluid. When one or more of the analytes was present, a color response could be observed in each relevant lane. Figure 1 is an illustration of the microfluidic device, in which each channel is labelled with its respective body fluid type to indicate the location in which specific test reagents were placed.

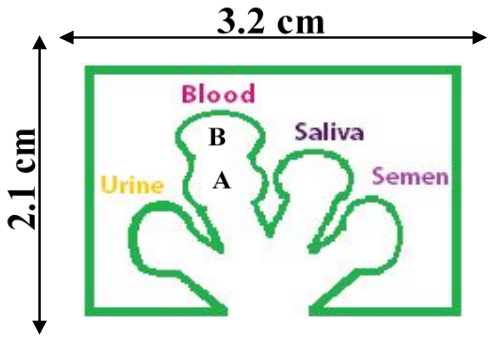


Figure 1: Multi-analyte paper microfluidic testing device developed for detecting body fluids. The device consists of 4 channels with 5 locations for reagents. Area labelled **A** is the location of where sodium perborate tetrahydrate is placed within a thin layer of water-based liquid glue. Area **B** is the location where phenolphthalein is placed.

A single sample is placed at the base of the device and capillary action transports the sample up into each individual branch of the device. A different colorimetric reagent is placed at the terminal end of each branch. The advantage of this layout is that both single analytes and mixtures can be assessed using one device.

Colorimetric tests

The initial procedures used for each colorimetric test were developed by examining traditional solution based forensic serological test methods. These included the Kastle-Meyer test for blood, the starch-iodine test for saliva, Nesslers reagent for urine, and the acid phosphatase (AP) test for semen.[34-38] These procedures were then modified to achieve compatibility with the paper substrate and the linear capillary flow. Reagent placement was critical for achieving proper mixing and sequential reactivity with the individual analytes.

Samples were prepared through dilution using a v/v ratio of: 1:2, 1:5, 1:10; 1:20, 1:50, 1:100; 1:200, 1:500, 1:1000, while dried samples are cut out to 3 x 10-3 cm3 and diluted with 20µL, 50µL, 100µL, 250µL, 400µL, 500µL, 700µL, 850µL, 1000µL of distilled water.

Initial tests were performed on single lane devices. In table 1, the µPAD design and colorimetric reaction test results can been seen for independent testing of each sample lane. Each body fluid specific reagents were spotted into the terminal ends of the hydrophobic channels. This includes **Row A**: 0.5 µL of Urease-Nessler's reagent (urine), **Row B**: the Kastle – Meyer test (blood) spotted in two separate locations with 0.5 µL phenolphthalein spotted in location **B** of the blood lane, and 3 mg sodium perborate tetrahydrate spotted in location **A** of the blood; **Row C**: shows the test for saliva involving a mixture of 0.5 µL starch solution and iodine for saliva; and **Row D**: 0.5 µL acid phosphatase mixture for semen. Each row demonstrates the design of the chip, the color of the prepared device and the color change that results from a positive test.

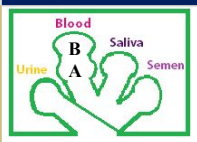
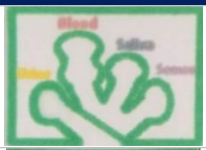
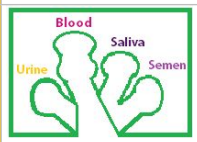
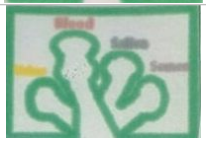
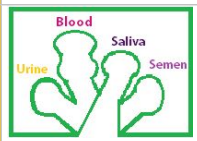
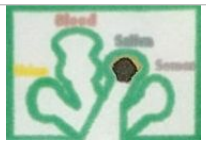
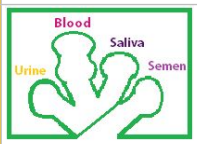
	µPAD Design	Prepared µPAD	Colored µPAD
A			
B			
C			
D			

Table 1: Each lane of the µPAD has been separated using a hydrophobic barrier lane for initial sensitivity testing. **A**. Urine **B**. Blood **C**. Saliva **D**. Semen

Next, the sensitivity and detection limit of each presumptive test was determined by performing serial dilutions of each analyte. Additional tests were performed for determination of the effects of sample age and matrix interference.

Test Development

Urine

In humans, urea is a generally used as a marker for urine due to its high concentration in this biofluid. [6] A useful test for urea takes advantage of the enzymatic activity of urease, followed by the detection of ammonium with Nessler's reagent.[3,37,38] The overall hydrolysis of urea is shown in equation (1):

$$\underset{\text{Urea}}{NH_2(CO)NH_2} + 2H_2O \xrightarrow{\text{Urease}} \underset{\text{Ammonia}}{NH_3} + \underset{\text{Ammonium Ion}}{NH_4^+} + \underset{\text{Bicarbonate}}{HCO_3^-} \quad (1)$$

In this experiment, crystalline urease from jack beans was used. [34,38-42] The hydrolysis involves an enzymatic reaction in which the metalloenzyme binds and degrades the urea into ammonium and carbamate.[39-44] The carbamate then decomposes into ammonia and bicarbonate.[40,43-45]

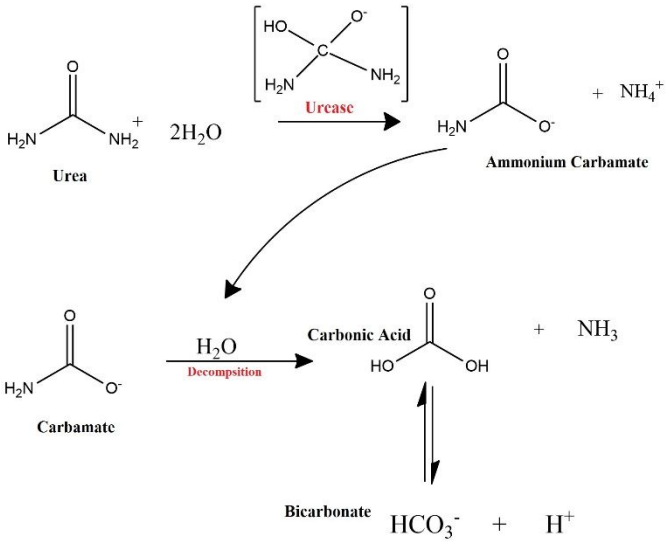


Figure 2: The detection of urea in urine is performed using urease to decompose urea into ammonia and bicarbonate ions.

To detect the presence of the ammonia that is formed from the hydrolysis of urea, a colorimetric method using Nessler's reagent is performed. The Nessler reagent contains mercury (II) iodide, sodium hydroxide and potassium iodide in water. Nesslerization occurs when the ammonia subsequently reacts with mercuric iodide to yield a mercuric ammonia complex. This reaction gives off a characteristic of a yellow-brownish color product.[45-48] When the presence of ammonia is low the color product is yellow, whereas when the presence of ammonia is high the colored product is brown. The Nesslerization of ammonia can be seen in figure 3. To prepare this reagent, powdered urease, 0.034g, was added to 45µL of purified water followed by the addition of 1 mL of Nessler's reagent. 0.5 µL of this solution was placed in the distal end of the urine channel.

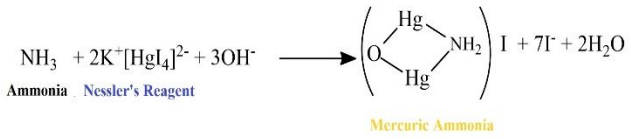


Figure 3: Ammonia that is produced following the hydrolysis of urea is mixed with Nessler's reagent to produce a colorimetric reaction and a mercuric ammonia product. This process is called nesslerization.

Blood

For blood identification, forensic laboratories typically utilize oxidation-reduction reactions when performing presumptive testing, followed by a more specific confirmatory test.[3] Most of the tests used in the detection of blood are based on the presence of hemoglobin.[3,37] A presumptive test that is widely used during criminal investigations when blood is suspected to be present is the Kastle-Meyer Test.[3,34,35,37] In the Kastle-Meyer test, the presence of hemoglobin in blood provides a peroxidase-like activity. Which in the presence of hydrogen peroxide hemoglobin can oxidize phenolphthalin into phenolphthalein, yielding a pink color.[3,6,34,35,37,38,49] For this reaction to produce stable results on paper we substituted the hydrogen peroxide with sodium perborate tetrahydrate because of its enhanced stability and oxidizing ability.[49,50] The overall reaction can be seen in Figure 4, in which the reduction of the sodium perborate simultaneously oxidizes the phenolphthalin. This test yields a fast and efficient result that can alert the user that blood may be present. To prepare the µPAD, (figure 1) 0.2-0.6µm of water based glue was placed in the A section of the blood channel. 3 mg of sodium perborate tetrahydrate crystals were added to water based glue, and 1.0 µL of the phenolphthalein solution was placed at the distal end of the blood channel (section B). In its solid form sodium perborate contains strong oxidizing properties while maintaining stability with the atmosphere. Mixing the crystalline form of sodium perborate tetrahydrate with small amounts of water based glue insures long term stability and avoids the problem of the reagent flaking off the chip.

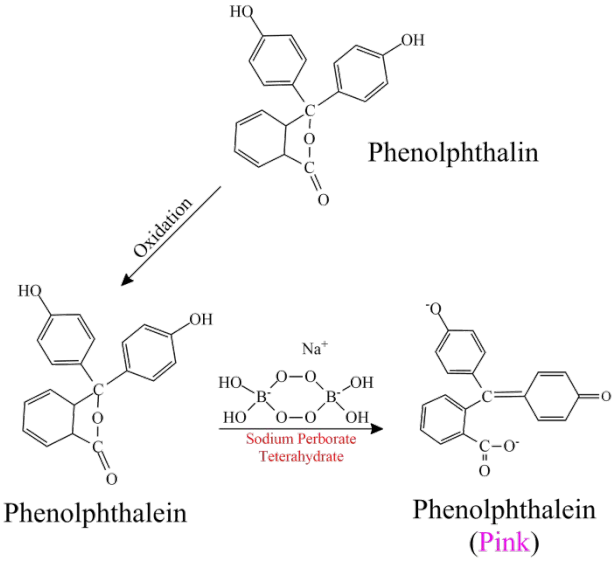


Figure 4: Blood can be detected by the Kastle-Meyer test by using the peroxidase-like activity of hemoglobin.

Saliva

There are several presumptive tests that are used to determine the presence of saliva in forensics.[6] One of these utilizes the presence of α-amylase in human saliva, which is used to break down starch. Starch from foods contains polysaccharides and when food is eaten, salivary α-amylase in saliva is used to help to digest these polysaccharides to produce maltose and glucose for the body.[51-55] A common presumptive test used to detect the presence of saliva is known as the starch-iodine test.[3,6,34,37] The starch-iodine test results are based on the fact that when starch and iodine react together, a blue/black color appears. During this reaction, iodine attaches to the helices of the polysaccharides. In the presence of α-amylase, saliva hydrolyses the starch by cleaving the α-(1,4)-glyosidic bonds of polysaccharides, causing the starch bond to break down and the loss of the blue color formed begins to disappear.[3,34,37] According to Gaensslen, this color intensity and shade formation is dependent on the helical chain length found in starch.[3] To prepare this test, 2g of laboratory grade starch was brought to a boil in 5 mL of water until it became a clear solution. Once cooled, 0.5 µL of the mixture was placed in the reagent location of the saliva lane, followed by 0.5 µL of 1N iodine solution. This created a blue/black color. Addition of saliva to this mixture produces the color change as the degraded starch releases iodine. 9 samples of saliva were diluted, 1:2 to a 1:1000

(v/v), using to determine the minimal amount of saliva that can be present and used for detection while using the paper microfluidic devices. Figure 5 demonstrates the starch-iodine test reaction that occurs when salivary amylase is present.

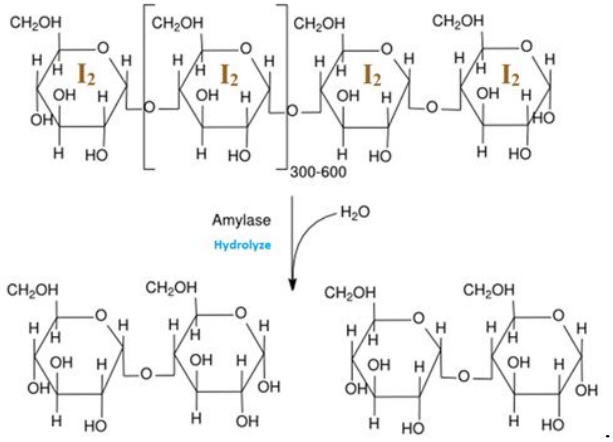


Figure 5: Saliva's amylase activity was measured through the hydrolysis of the starch-iodine.

Semen

In sexual assault cases, unknown biological fluids are many times left behind at a crime scene. One of these fluids is semen. Semen contains citric acid, lipids, acid phosphatase and other enzymes.[55-57] In male humans, semen contains high concentrations of acid phosphatase. A colorimetric test can be implemented in which the presence of acid phosphatase is detected based on its enzymatic function.[2,3,6,34,37,58,59] Acid phosphatase reagent at pH 5.0 can catalyze the hydrolysis of α-naphthyl acid phosphate to produce naphthol. [2,6,36,58,59] As seen in figure 6, the naphthol reacts with Fast Blue B dye, producing a purple azo dye.

Two solutions –substrate and dye solutions –were prepared using diagnostic test instructions and protocols provided by Arrowhead Forensics and the National Forensic Science Technology Center (NFSTC). The substrate solution included glacial acetic acid, sodium acetate, water and Fast Blue B.[37] A dye solution, including water and alpha-naphthyl phosphate disodium salt were also prepared.[37] The solutions were mixed and 0.5 µL of the mixture was placed within the semen reagent location.

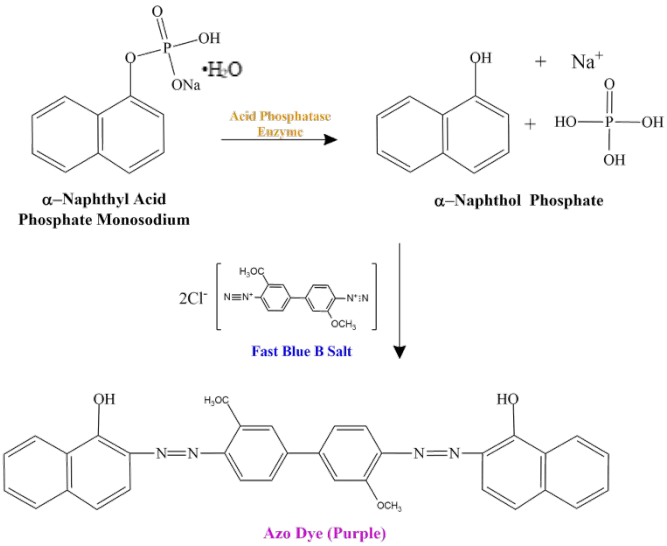


Figure 6: Semen was detected using sodium α - naphthyl phosphate with acid phosphatase and Fast Blue B.

Interference Testing

Presumptive tests are designed as screening tools, and are used mainly determining potential presence of body fluids. However, depending on the reaction mechanism, there are usually a number of potential interferences in these tests. Some of these contributors include oxidizing agents, thermal denaturants and certain environmental contaminants.

For example, when using the Urease-Nessler reagents to test for the presence of ammonia in urine; alcohols and ammonia based materials can create interferences.[60,61] The Sacramento County District Attorney Laboratory of Forensic Services suggested that some interferences to the Kastle-Meyer test for blood were those that contained chemical oxidants, other substances that contained similar peroxidase activity or materials containing peroxidase.[36,62] Additional interferences for the were also suggested by other laboratories. [34,36,63] For example raw potato may produce false positive results for the Kastle-Meyer test.[63]

When using the starch-iodine test for detecting the presence of amylase, it should be noted that animal and plant amylase can produce false positives when using the starch-iodine enzymatic test.[64] In addition, amylase can also be detected in breast milk, semen and perspiration of humans resulting in additional

false positives.[65] However, in general the levels of amylase in these other body fluids are low when compared to that in saliva. [66]

The acid phosphatase test has potential interferences which include male urine, saliva, and feces which, however, contain the enzyme acid phosphatase at lower levels than semen.[34,36,67] It has also been suggested that plants, fungi, and bacteria may contain enzymes similar to those of seminal acid phosphatase that produce a reaction to the AP test.[34] For example, teas that are made from *Camellia Sinensis* which display a colorimetric reaction when tested using the acid phosphatase-fast blue test.[36]

Based on a survey of the literature, the following substances were examined for interference testing: biological samples were donated by protected volunteers under the Health Science Institutional Review Board of Florida International University, IRB-16-0279 International University. Hydrastis Candensis (Goldenseal herbal supplement tea) was purchased from the Vitamin Shoppe (Miami, Florida); Camellia Sinensis (Green Tea), Bleach (Clorox), Glass Cleaner (Windex), Hand soap (Softsoap), Dish detergent (Palmolive), Corn starch (Great Value), Nail polish remover (Cutex Strength Shield Nail Polish Remover), and Potatoes were purchased from supermarkets in Miami, Florida.

Results and Discussion

The goal of this study was to prepare a multi-analyte µPAD method for the presumptive detection of body fluids. The design permits 4 simultaneous colorimetric tests to be performed, making the method highly specific, as interferences can be discriminated by their relative response in each colorimetric channel. The colorimetric tests used in this study were selected based on their previous application as solution tests and modified as necessary for use with paper microfluidic sensors

Samples were prepared through dilution using a v/v ratios for non-dried samples and volume flow rate of sample and distilled water for dried samples.

Body fluids are composed of various enzymes and proteins in a wide range of concentrations, the complexity of detecting these fluids in mixtures can increase and cross reactivity can become an issue [68] Therefore, initial tests were performed on single lane devices as seen in table 1. Each body fluid specific reagents that is spotted into the terminal ends of the µPAD is observed in the table 1. Urease-Nessler's reagent is used to test for urine, the Kastle – Meyer test is used to test for blood using sodium perborate tetrahydrate; saliva was tested using a starch-iodine solution; and acid phosphatase mixture was used to test for the presence of semen's enzyme Acid Phosphatase.

Individual fluid test were taken and 600 µL of the varying sample ratios were prepared and the µPAD was used for testing. The lane channels are separated to show the difference between the prepared reagent location and the locations of which each reagent reacts with each designated fluid. The µPADs in table 1, were only used to individualize each test for better observations during sensitivity testing. To see the demonstrated microfluidic device used for field testing, refer to table 2:

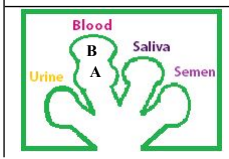
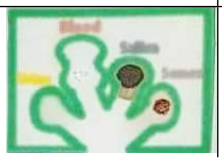
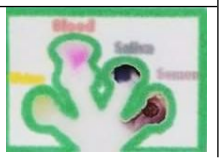
µPAD Design	Prepared µPAD	Simultaneous µPAD
		

Table 2: µPAD developed to detect four serological fluids simultaneously. The simultaneous µPAD column was used to detect all four fluids found in a mixed sample.

The time required for a positive response using the µPAD in table 1 and table 2 was estimated to require from 10 - 15 minutes. Where 150-300 µL of the varying samples were mixed to total a 600 µL prepared sample for the multi-analyte testing. The total time to detection varied depending on the quantity of sample present, the nature of the sample, the dilution of the sample and the nature of the biological fluid.[68]

Sensitivity Study

A study of the sensitivity of the device for each body fluid was performed using sample dilutions from 1:2 to 1:1000 for each body fluid type including blood,

semen, saliva, and urine. During preparation, 480 µL of each sample dilution was placed into a designated 1mL certified glass insert vial. The µPAD was placed into the vial and the fluid samples flowed up through the hydrophobic channels until they reached the terminal ends where the reagents were placed. The intensity and spot size of each analysis was recorded using a Samsung Galaxy Note 8 smartphone. To avoid external light interference and contamination, each microfluidic device was photographed using a white background with ambient lighting. A 15-cm distance was maintained between the camera and microfluidic device. The resultant images of the colorimetric reaction of urine at the various dilutions in water can be seen in figure 7. Figure 7 represents the dilution series for the colorimetric reaction for urine using Urease-Nessler's reagent.

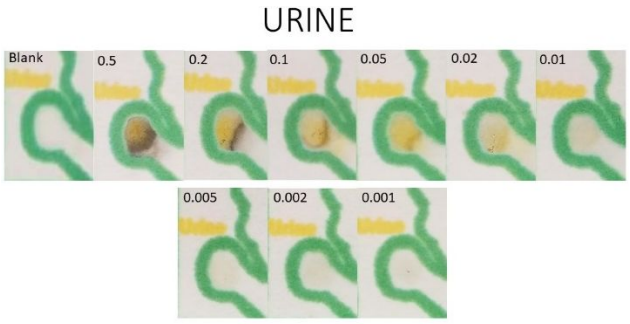
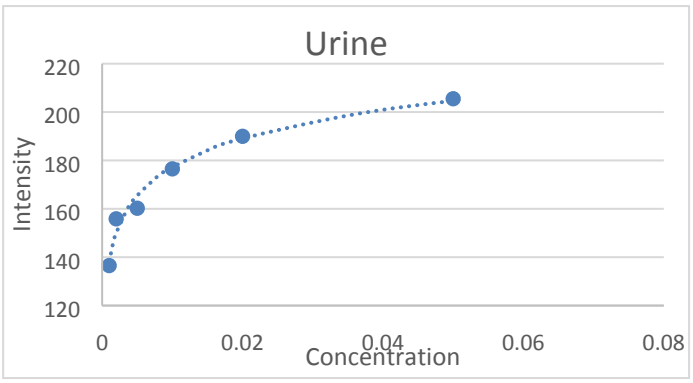
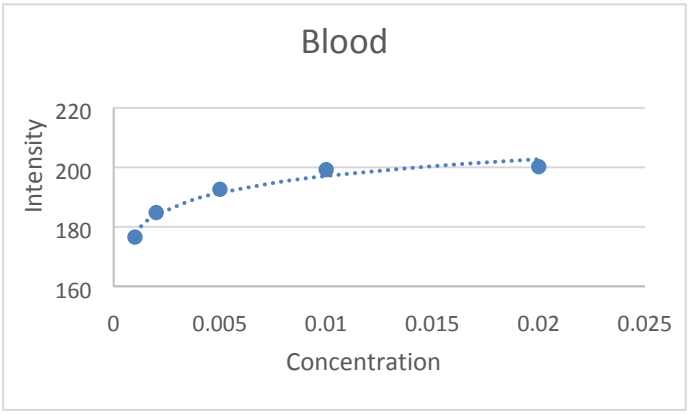
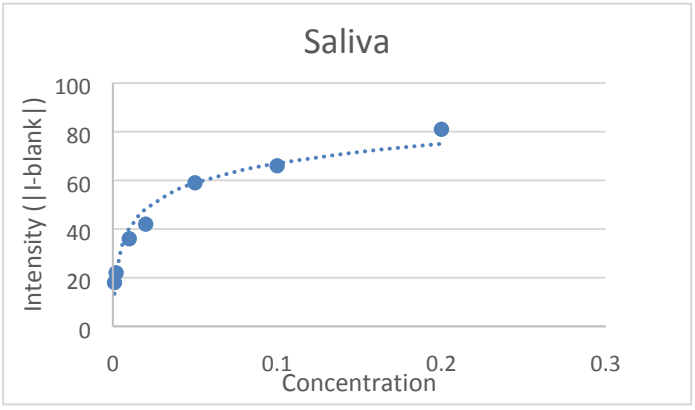


Figure 7: Illustrates the presumptive test results using colorimetric testing reagents for urine at dilution ratios of: 1:2, 1:5, 1:10; 1:20, 1:50, 1:100; 1:200, 1:500, 1:1000. Urease-Nessler test was used in in the above figure to detect urine.

The results were processed using the densitometry function of the image processing and analysis software- ImageJ. program and plotted using Microsoft excel. The data was fit to semilog plots of concentration vs color density. Data is shown in table 3.







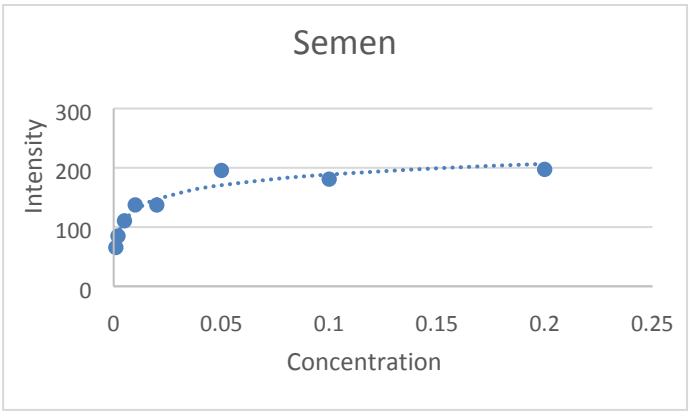


Table 3: ImageJ semilog plots of sample concentration vs color density.

A visual detection limit was determined by polling 33 individuals as to the lowest level of signal for which they could define a positive result.[69] The lowest level at which 90% of the participants detected a color change was deemed the visual detection limit.

For determination of the LOD based on smartphone data, an analysis of 10 replicate samples of each biofluid was plotted to a curve fit to determine the minimal detectable quantity. Urine was measured at 10 replicates of 0.01 v/v dilution. Saliva was measured at 10 replicates of 0.01 v/v dilution. Semen was measured at 10 replicates of 0.01 v/v dilution. Blood was measured at 10 replicates of 0.005 v/v dilution. The standard deviation, σ, of the signals obtained from the replicates were then fit into a curve to determine the minimal detectable dilution of each body fluid. This data is listed in Table 4.

Biological Fluid	Fit	R^2	Visual Limit of Detection	Cell Phone LODI
Urine	0.0604 ln(x) + 2.1687	0.9402	0.01	0.006
Blood	0.011 ln(x) + 2.2906	0.9748	0.005	0.003
Saliva	0.1715 ln(x) + 1.4598	0.8969	0.01	0.016
Semen	0.1799 ln(x) + 1.6071	0.9363	0.01	0.012

Table 4: Fit the calibration curves for each body fluid, R^2 values, the minimal concentration of each body fluid detected through visual observation, and the of body fluid based on the level at which (>90% agreement) and the minimal concentration detected by cell phone measured using ImageJ software.

Interference testing

Using known colorimetric test interference items and other household items that were available within the laboratory, interference tests were conducted under a blind study. Colorimetric reaction that occurred on the µPAD when a sample was tested were noted. While conducting the blind study, leafy herbal teas (Hydrastis Candensis, *Camellia Sinensis*, and other *Camellia Sinensis* varieties that were closely related to garden plants)[36] and vaginal fluid secretions [34] (only after a 2-minute waiting time period) were found to cause a purple color change in the Acid Phosphatase + Fast Blue B reagent location of the µPAD designated for semen detection but there were no other colorimetric changes to occur in neither the blood, saliva, nor urine testing locations using these reagents. It should be noted that low amounts of acid phosphatase may be present in vaginal fluids. This can result in the slow development of a weak color change for certain samples after 1-2 minutes [71]. Thus color changes which occur 1 minute or longer after the solution reaches the color pad indicate a potential interference and should be interpreted with caution.

10% concentrated bleach was tested for interference on the µPADs which resulted a color change in the in the Urease-Nessler's and Kastle-Meyer channels. The color change observed in the Urease-Nessler's channel was orange-brown. The color change in the Kastle-Meyer channel was the same color pink, as observed when blood was present. Glass cleaner from its commercial bottle produced a yellow and pink color change in the reagent locations with Urease-Nessler's reagent and sodium perborate-phenolphthalein.[60,61]. 10% hand soap and dish detergent was used to test its interference abilities. The alkali in hand soaps and dish detergent chemically reacted with the phenolphthalein causing an alkylation to occur and produce a slight pink colorimetric change when reacting with the sodium perborate- phenolphthalein reagent location.[70] Nail polish remover proved to be another interference with the Urease-Nessler's test, causing a yellow-colorimetric change to occur when there was no urine present due to its alcohol component.[60,61] The starch- iodine test showed false positives when tested against citrus fruits such as lime, lemon and oranges at whole fruit concentrations and a 1:10 diluted ratio with water.[64] The results for these experiments are detailed in Table 5.

Conclusion

In conclusion, we have developed a multi-analyte paper-based device (µPAD) that can rapidly and reliably detect serological stains on various substrates. This device should prove useful for presumptive testing of stains at crime scenes and in

the laboratory. Initial validation work has shown that the device can simultaneously detect mixtures of the biological materials. During the development we have also demonstrated that the multi-analyte device has up to a two-week shelf life when prepared with reagents and stored in a dry, dark location. The colorimetric response permits sample detection within a 10-15-minute time interval. This device has low sample volume input requirements and is inexpensive to make. The multi-analyte format increases the specificity of the detection method, preserves precious sample, and allows for users to distinguish if blood, saliva, semen, or urine is presumptively present prior to more specific confirmation testing.

	Urease + Nessler's Reagent	**Sodium Perborate + Phenolphthalein**	**Starch + Iodine**	**Acid Phosphatase + Fast Blue B**
Goldenseal (Hydrastis Candensis)	NCC	NCC	NCC	CC
Green Tea (Camellia Sinensis)	NCC	NCC	NCC	CC
Bleach	CC	CC	NCC	NCC
Glass Cleaner (Windex)	CC	CC	NCC	NCC
Hand Soap	NCC	CC	NCC	NCC
Dish Detergent	NCC	CC	NCC	NCC
Corn Starch	NCC	NCC	CC	NCC
Potatoes	NCC	NCC	CC	NCC
Granulated Sugar	NCC	CC	NCC	CC
Lemon	NCC	CC	CC	NCC
Orange	NCC	NCC	CC	NCC
Lime	NCC	CC	CC	NCC
Nail Polish Remover (Cutex)	CC	NCC	NCC	NCC

Table 5: Common test interferences that interfere with testing reagents while on the multi-analyte device CC: Color Change; NCC-No Color Change; Each color presented in the chart shows the colors that appeared when each item was tested.

Journal Name

ARTICLE

Conflicts of interest

None.

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