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Microfluidic Paper Analytical Devices

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Chemistry Senior Thesis

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Abstract

Microfluidic paper analytical devices (μPADs) are small, paper-based matrices similar to Lab-on-a-Chip (LOC). They are capable of semi-quantitative analysis, with applications ranging from medical to environmental to food and beverage testing. Important to improving point-of-care devices (POCs), various techniques have been integrated into μPADs to customize analysis and fit different clinical situations.

I. Intro

In December of 2011, Pakistani pharmaceutical company EFreeze produced both a blood-thinning medication called Isotab-20 and an anti-malarial drug called Maladar. One day during production, a batch of Maladar became wet and was mislabeled as inert starch filler. It was then added to Isotab-20, and the batch was distributed to thousands of clients for consumption. Over a thousand people become ill, and 213 patients died from ingesting the drug. This issue demonstrates quality control negligence of EFreeze (Pakistani Government). An easy way to test the drugs on a regular basis could have decreased the likelihood of this event occurring. Unfortunately, some companies deliberately add fillers to medications to decrease costs at the expense of patients' health. Cheap ways for outside sources to accurately test drugs could mitigate companies' deception.

Another present issue is foodborne pathogens, such as Salmonella, E. coli, and S. aureus. According to the World Health Organization, over 200 million foodborne related illnesses occur every year, as well as 420,000 foodborne related deaths. This is a global issue that disproportionately affects people from low-income areas. Low-income areas suffer three-fourths of all foodborne related illnesses that occur globally. Not only that, but the two lowest income areas, Africa and Southeast Asia experience the most years of life affected by foodborne related disability, whereas high-income areas (Europe and North America) experience the least years of life affected by foodborne related disability. An inexpensive, easy-to-use, fast, and accurate way to detect foodborne pathogens is greatly needed to reduce the incidence of illness and in turn, the foodborne pathogen caused mortality rate (Devleesschauwer et al.).

Along with foodborne pathogens, cancers such as lung, liver, prostate, breast, and colon cancer strike individuals, causing unnecessary tragedies. Early detection is key to increasing survival rates, however, according to the American Cancer Society. Unfortunately, people from lower socioeconomic situations tend to screen for cancer less than people experiencing socioeconomic security. To provide accurate diagnoses to the entire population, a fast, cheap, accurate device is needed (American Cancer Society).

Paper analytical devices could be a solution to drug quality control, foodborne-related illness detection, and cancer prevention. These areas are just a few of the countless applications research groups have established. Since around 2009, microfluidic paper analytical devices (μPADs) have caught the interest of numerous research groups that sought to detect all kinds of compounds, from environmental toxins to human biomarkers to pharmaceuticals. μPAD applications can be grouped into three main categories: healthcare, environmental monitoring, and food and beverage quality control.

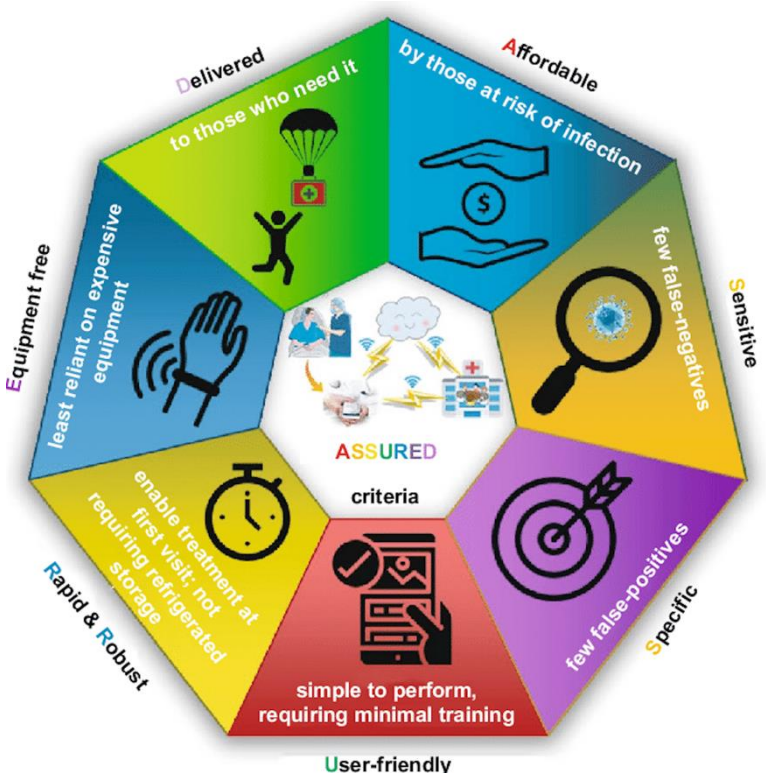
Health Diagnostics	Environmental Diagnostics	Food and Beverage Control
Biomolecules	Water	Pesticides
Small molecules	Soil	Foodborne pathogens
Nucleic acids	Air	Water quality
	Metals	Beverage quality

Although environmental monitoring is very important to scientific advancement, human health, and earth's preservation, for the purposes of this paper we will examine a few medical applications and food and beverage quality control examples. Healthcare analyses involve detection of biomarkers (such as proteins, hormones, neurotransmitters, etc.), biomolecules, (such as uric acid, glucose, etc.), and nucleic acids; this includes categories like blood tests, drug tests, and pregnancy tests. One of the most important applications is detecting infectious diseases and cancerous cells (Florea et al.).

Healthcare professionals all over the globe utilize devices and techniques for biomarker detection. Blood sugar tests, COVID tests, and pregnancy tests are all examples of biomarker detection. Physicians use the presence (or absence) and quantity of biomarkers to determine whether a patient has contracted an infectious disease, has a long-

term illness (such as cancer), or the presence and quantity of certain key molecules. For example, patients with high risk for diabetes monitor glucose levels in the blood to ensure they stay within a healthy range. Sometimes, rapid diagnosis can be the difference between life and death for patients. The American Cancer Society reports that, “Early detection of cancer through screening reduces mortality from cancers of the breast, cervix, colon and rectum, prostate, and lung” (54).

There are several platforms for detecting biomarkers, such as glass, polydimethylsiloxane (PDMS), poly(methyl methacrylate) (PMMA), poly(cyclic olefin), paper, and hybrid platforms, not to mention traditional methods using enzyme linked immunosorbent assay (ELISA), polymerase chain reaction (PCR), immune fluorescence, immunodiffusion, western botting, flow cytometry, and more. The traditional methods, however, are fairly complicated and equipment heavy. Healthcare professionals in the field would not be able to use them as point-of-care (POC) devices in rural, isolated, or underprivileged areas. Even if they were able to transport the required components, these methods require a large amount of sample and reagent. They are time-consuming when time is of the essence in medical diagnoses. Fast acting, inexpensive, easy-to-use, portable POC devices would help slow or stop the spread of infectious disease and provide faster diagnoses for health conditions, such as several types of cancer (Sanjay et al.). The World Health Organization has issued guidelines for POC devices. Using the acronym ASSURED, they stipulate that the devices must be Affordable, Sensitive, Specific, User-friendly, Rapid/robust, Equipment-free, and Deliverable to those who need it (Kettler et al. 17).

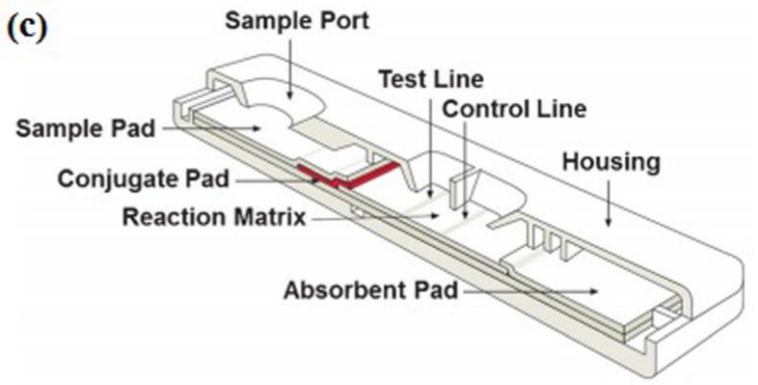


Similarly, four critical categories of "simple diagnostic" include non-skilled operators, inexpensive, accurate, and fast. When all four cannot be achieved, accuracy can be decreased in favor of simplicity and diminished cost.

II. History

To understand the current state of paper analytical devices, a brief history must be provided. Using paper for scientific analysis dates back to 79 A.D., during the life of Pliny the Elder. He devised a way to test purple dye and ferrous sulfate using papyrus. Thousands of years later, people began using litmus paper to test for pH thanks to Gay-Lussac's research during the 19th century. Radial paper chromatography came along later in the century, by the research of Runge (Santiago et al. 89-106). 1883 marked the idea of paper strips that could detect sugar and albumin when Feigl and Matthews created colorimetric assays that took advantage of filter paper's capillary effect. The1930s brought urinary diagnostic paper strip tests. This began a trend of "dipstick" analysis; dipstick tests are based on dipping the strip into the sample. It has expanded from urine detection to

ketones, proteins, hemoglobin, and more. Some simply detect the presence or absence of an analyte (indicates “yes” or “no” on the strip), while some incorporate a color change for detection. The latter method is considered a semi-quantitative because it provides the observer with greater clarity on the amount of analyte based on the intensity of the color. Pregnancy tests are another example of dipstick tests. Developed in the 1980s, they were the first commercial lateral flow assay (LFA) method used on paper strips. Soon, research groups realized they could be easily adapted to other analyses; samples like saliva, sweat, urine, plasma, serum, and blood could be added to the strips to detect biomarkers. These LFA strips provide a sample pad where the sample could be loaded, which lead to a conjugate pad, guiding the sample to the reaction matrix. An absorbent pad at the end of the strip collects excess sample, reactant, and product. Straightforward, inexpensive, and long-lasting, LFAs are a valuable asset to the medical, agricultural, biodefense, and environmental communities. They do not require power to operate, and they are relatively easy to manufacture (Altundemir et al.).



This figure demonstrates how LFAs function. First, the sample is loaded onto the paper matrix via the sample port. The sample travels through the conjugate pad and into the reaction matrix, where the first line contains the reaction for a positive or negative result, and the second line indicates the successful completion of the test. If an issue is suspected with the test, then the control line will not show a color change reaction. The absorbent pad at the end catches any excess sample, and the entire LFA is encased with housing, usually plastic (Carrell et al.).

Although simple and cheap, scientists are looking for improvements to LFAs because they do not deliver qualitative results. By only reporting "yes" or "no" outcomes, tests are limited in what they can accomplish (Carrell et al. 206). Additionally, only one analyte can be detected at a time, slowing down diagnoses. μPADs gained traction around 2009 as a way to solve the problem of multifaceted, qualitative detection. While retaining the rapid diagnostic speed, low cost, and easy use of LFAs, μPADs can detect multiple biomarkers at the same time, on the same device. Fabrication methods have evolved over the past 20 years, during which glass and silicon have been replaced by soft lithography, hot embossing, and wax printing techniques (Altundemir et al.).

III. Composition

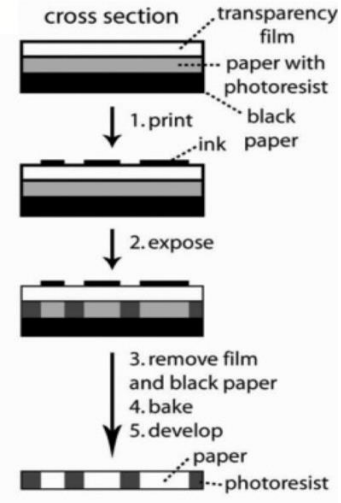
Paper analytical devices (μPADs) are incredibly thin, from hundreds to tens of micrometers thick. In order to be classified as a micro device, the technology must have at least one functional dimension at a micrometer scale (Santiago et al.). One reason μPADs are a promising solution to healthcare accessibility is their customizable nature. Easily adjustable to specific needs, groups can print, coat, or inject paper with various compounds to fit different situations. There are several materials that can be used to make micro analytical devices, such as glass combined with polydimethylsiloxane (abbreviated PDMS), poly(methyl methacrylate) (abbreviated PMMA), poly(cyclic olefin), paper, or a hybrid created from a combination of materials (Sanjay et al.). Although each material has its benefits, paper devices have unique properties that specifically complement WHO's ASSURED (Affordable, Sensitive, Specific, User-friendly, Rapid/robust, Equipment-free, and Deliverable) guidelines.

There are also other techniques that accomplish biomarker detection but have a few drawbacks that paper devices circumvent. For example, polymerase chain reaction is used in biochemistry to amplify bacterial signals, and it is routinely performed. Despite its frequency in use, it is not available in developing countries due to its high price and requirement for technical skill to operate. Other options include flow cytometry, which is a technique for sorting or counting cells, used to identify blood cancers and microorganisms. Although this approach provides a good deal of information, it requires five components: a

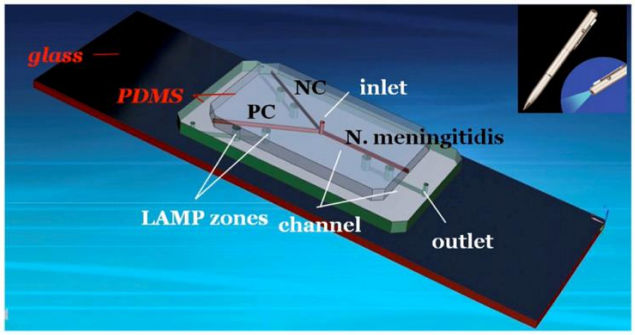
flow cell, a measuring system, a detector, an amplification system, and a computer for analysis of the signals (Cossarizza et al.). Western blotting is another method of biomarker detection, but it requires gel electrophoresis, transfer, staining, blocking, and overnight incubation (Yang and Mahmood). Each method has its place within the scientific community but are not the best for point-of-care analysis.

Paper analytical devices (μPADs) are made of cellulosic or nitrocellulose fibers that have been compressed into a certain shape. Paper can transport liquids without the need for external forces due to the capillary effect. Its microporous composition provides a high surface area environment for samples to react. Creating paper is a simple process; no cleanroom procedures are necessary for production, unlike PDMS fabrication. When considering design structures, paper is a flexible option because of its ability to be layered. By stacking several layers of paper, 3D structures can be created. Single layers can be utilized for 2D horizontal flow, and multiple levels can create 3D vertical flow (Sanjay et al.).

There are two main steps in the fabrication of μPADs: construction of channels and construction of hydrophobic or hydrophilic barriers. Within fabrication of channels within the paper matrix, there are two types of methods: photolithography and 2D cutting. The first technique, photolithography, is also referred to as fast lithographic activation of sheets (FLASH). Chromatography paper with light sensitivity acts as the substrate. It is initially covered by a transparency film and backed by black paper. Ink is printed on top of the transparency film, and the layers are exposed to UV light. Then, the transparency film and black paper layers are removed, leaving the chromatography paper layer with some areas developed (photoresist) and some areas as paper. This method of creating channels is relatively inexpensive and requires little equipment (Sanjay et al.).



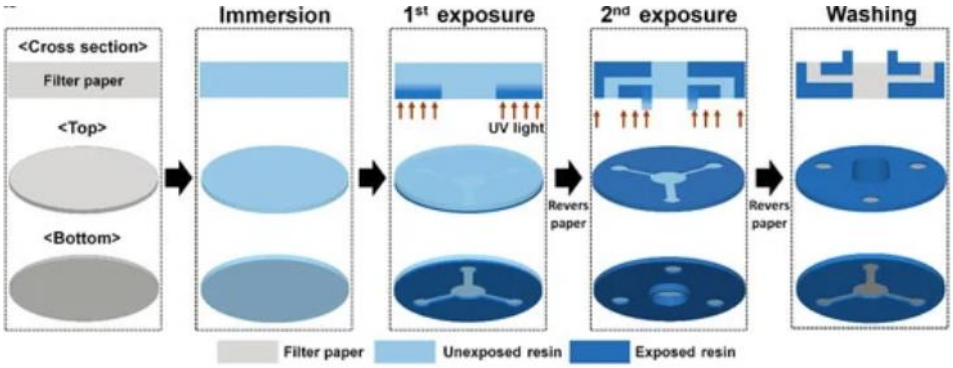
An example of this method was created by a research group led by Dr. Maowei Dou. Their group focused on meningitis detection, fabricating a PDMS/paper hybrid chip that can aid in the diagnosis of the bacteria using a UV light pen (Dou et al.)



The second method of producing pathways within the paper device is by 2D cutting. Channels are cut by programming x-y plots of where a guided knife should cut, or by using a CO2 laser cutter. In this method, nitrocellulose, chromatography paper, or regular photocopy paper can be utilized for 2D cutting, or it can be cut and stacked to create 3D structures (referenced as the “cut and stack” method) (Sanjay et al.).

While 2D microfluidic paper analytical devices (2D-μPADs) are the simplest to create, 3D-μPADs have the advantage of preventing evaporation of the sample. The “cut and stack” method is effective for creating vertical channels but does require either an adhesive spray or tape to combine layers (Baek et al.). One group, inspired by the ancient Japanese art of origami, researched the feasibility of folding paper layers to create a 3D

effect (Ahmed et al.). Another group led by Dr. Fereshte Gharaghani used intervals of UV light exposure to layer UV-curable resin. The channels created by lack of exposure to the UV-light were able to effectively detect eight biomarkers including: glucose (0–20 mmol/L), cholesterol (0–10 mmol/L), albumin (0–7 g/dL), alkaline phosphatase (0–800 U/L), creatinine (0–500 μmol/L), aspartate aminotransferase (0–800 U/L), alanine aminotransferase (0–1000 U/L), and urea nitrogen (0–7.2 mmol/L). All of the limits of detection related to concentrations in the normal to abnormal human range (Gharaghani et al.).



Hydrophobic barriers within a μPAD guide the sample to its target reaction point without soaking into the paper device. The paper matrix is by nature hydrophilic, so creating hydrophobic barriers forces reagents and samples to follow a certain path in the device. These barriers also help prevent contamination; a μPAD can have multiple channels of different compounds without mixing. Multiple multiplexed assays can be achieved. Although most devices require hydrophobic walls in hydrophilic paper, sometimes the desired effect is hydrophilic barriers while the channels are hydrophobic (Santiago et al.). There are several ways of fabricating channels. Methods include physical deposition, chemical modification, and wax deposition, with wax deposition being the most popular (specifically for hydrophobic barriers). Because wax is affordable, innocuous, and sets quickly, it is commonly used to create hydrophobic pathways. Wax is printed on the desired paper using a device similar to an inkjet printer. The wax is melted so that it seeps into the paper's pores, then it is allowed to solidify. When melted between 110-130°C, the wax is able to withstand temperatures below 60°C after it hardens (Sanjay et al.)

Channel size is another aspect to consider when fabricated a μPAD. Channels should be uniform in diameter and length in order to ensure signal consistency. Dr. Gharaghani's research included running an ink test through the channels to confirm equalized flow speed. To quantify the uniformity of the μPAD channels, his group recorded the speed at which the ink flowed through the channels and measured the length and diameter of the channels (Gharaghani et al.).

IV. Principles of Flow

When working with compounds in a μPAD, principles of flow must be considered since it is the primary means of sample movement, and the detection reaction usually depends on controlled compound flow. Current μPAD research includes two kinds of flow utilized in POC devices: wet-out flow and fully wetted flow. The wet-out process involves a fluid front travelling across dry paper or porous media of choice. This type of flow is described by the Washburn equation:

$$L^2 = \frac{\gamma D t}{4\mu}$$

"L" equals the distance the fluid front moves, "t" equals time elapsed while the fluid front moves, "D" equals the average pore diameter of the paper (or other media), "γ" equals the surface tension of the fluid, and "μ" equals the viscosity of the fluid. For fully wetted flow, the flow is observed through electrochemistry instead of a physical front. Electrochemical sensors detect the flow of samples that are already travelling along wet channels. Scientists observe that the Darcy Law is in effect in fully wetted flow:

$$Q = -\frac{\kappa W H}{\mu} \cdot \frac{\Delta P}{L}$$

"Q" equals the volumetric flow rate of the fluid, "κ" equals the permeability of the paper (or other media) in relation to the fluid, "μ" again equals the viscosity of the fluid, "W" over "H"

equals the area of the channel (perpendicular to the direction of flow), and $\frac{\Delta P}{L}$ equals the total pressure difference divided by the length of the channel (Fu et al.).

V. Detection

Detection in most cases depends on colorimetric assay results. Using the naked eye, results are semi-quantitative, as a change in color indicates a more concentrated sample. Furthermore, fully quantitative results can be obtained through digital image processing by documenting results with a camera (Kaneta et al.). Because an advantage of μPADs is their portability, adding the necessity for outside devices such as cameras and computers for analysis is unattractive. In certain cases, it might be worth the extra burden when improved accuracy in concentration determinations is necessary, but overall, the simpler the better. To solve this challenge, some groups have developed time-based detection methods. Similar to affinity liquid chromatography, the channels are coated with a specific reagent that binds to an analyte if present in the sample. This increases the retention time as compared to a control group. From there, concentration can be determined based on length of time spent in the channel (Roda et al.).

One method of detection is electrochemical detection (ePADs). These devices utilize tiny, highly sensitive electrodes, making the device portable, lightweight, and low-cost. Carbon is the most common electrode used, but electrodes can be other substances or even combinations of substances to created redox mediators. Gold is also used, but less common as it is more expensive. Some research groups have explored the possibility of gold nanoparticles to reap the benefits of gold electrodes without the high cost. Electrochemical detection works by describing and quantifying previously separated compounds. For example, ePADs have been used to detect the presence and quantity of glucose with the redox mediator, Prussian blue. In 2008, researchers paired ePADs with smartphones for controlling the devices. The smartphone provided potentiostatic control (Santiago et al.).

Other types of detection include chemiluminescence, which uses light to excite analytes, and the signal of emitted wavelengths is recorded. Another method combines

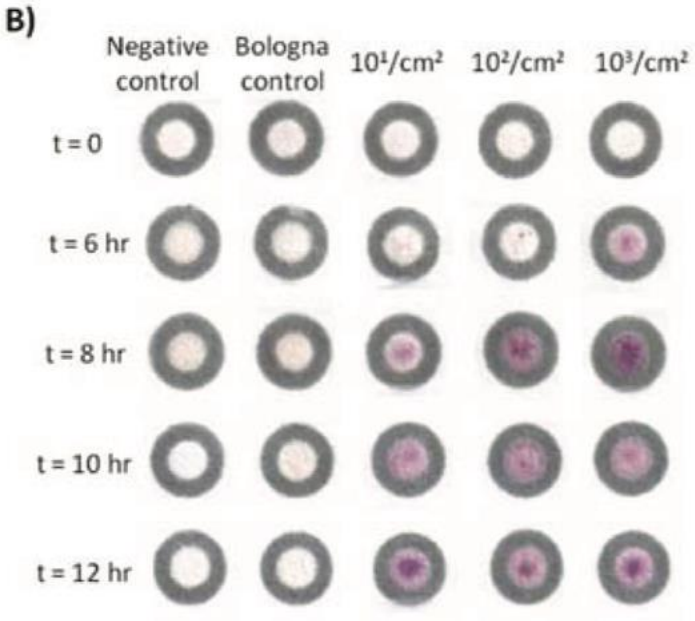
chemiluminescence and electrochemical detection to form electrochemiluminescent detection.

VI. Applications

There are many exciting advances in the field of paper analytical devices. Because paper is compatible with many biomarkers, one benefit of μPADs is its customizability. An example of a μPAD that detects biomarkers using multiple indicators is Dr. Weeseop Dungchai's research. His group designed and accomplished a μPAD that was able to semi-quantify glucose, lactate, and uric acid at medicinally relevant detection levels (0.5–20 mM, 1–25 mM, 0.1–7 mM respectively). Quantity of specimen present depends on color change; the darker, more intense the color, the greater amount of target compound present. Specifically, oxidase enzymes were used to decompose the target compounds, in turn producing hydrogen peroxide, which oxidized the indicator reagents. The oxidized indicators were what caused an observable change in color. The group used multiple indicators simultaneously for each analyte to improve the accuracy of the detection. (Dungchai et al.).

A second application utilizing the colorimetric analysis is seen in Dr. Jana Jokerst's research. Jokerst's group successfully developed a μPAD specifically for detecting foodborne pathogens. Their identification of pathogens depends on a color change within the device when a target enzyme of interest reacts with the chromogenic substrate. Examples they have tested include E. coli, L. monocytogenes, Salmonella, and others. To accomplish detection of the bacteria, three enzymes were used: β- galactosidase with

chlorophenol red β-galactopyranoside (CPRG), which binds with E. coli, phosphatidylinositol- specific phospholipase C (PI-PLC) with 5-bromo-4-chloro-3- indolyl-myo-inositol phosphate (X-InP) which binds with L. monocytogenes, and esterase with 5-bromo-6-chloro-3- indolyl caprylate (magenta caprylate), which binds with S. enterica. The colors change from yellow to red-violet, colorless to blue, and colorless to purple, respectively. The example below shows an analysis of bologna with and without spikes of Salmonella. The first two columns (negative control and bologna control) remain colorless, but the last three rows (spiked with 10^1/cm, 10^2/cm, and 10^3/cm bacteria) demonstrate a color change after 6-8 hours. The color change reaction in the highest concentration was also clearly visible sooner, indicating the ability to determine approximate concentration, not simply the presence or absence of the bacteria (Jokerst et al.).

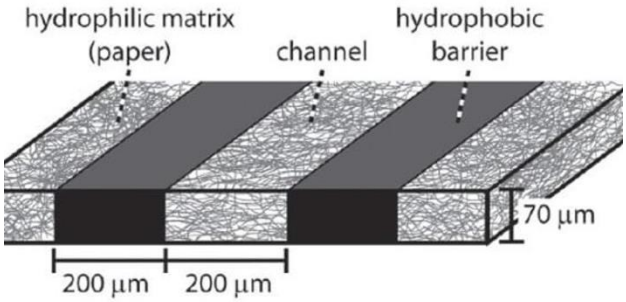


At Notre Dame, Dr. Marya Lieberman's group has developed a μPAD that tests the quality of a drug by using comparative analysis. The sample compound is ground into a powder and then smeared across the card. The card is placed in water, and similar to thin-layer chromatography (TLC), the water acts as the mobile phase. As the water travels up the paper, it carries the preloaded reagents up the sectioned channels until they come into contact with the drug. At this point, the drug either reacts with the reagent to form a color

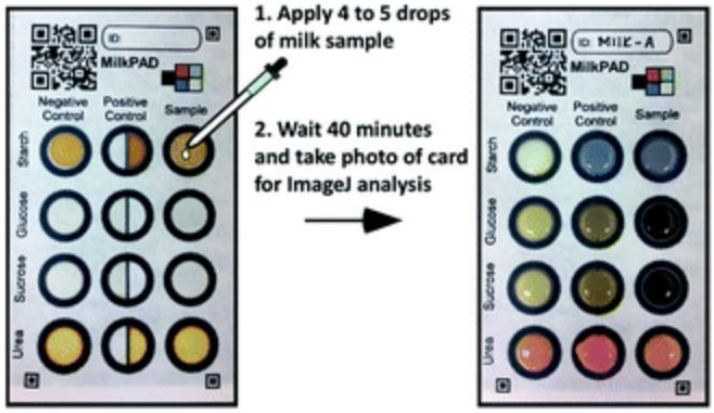
change, or it remains unchanged by the reagent. A pink spot at the top of column “A” indicates the completion of the test, at which point the observer can record the results by taking a picture of the μPAD. The results degrade within an hour of the initial test, so results must be recorded immediately.

A μPAD test must also be performed on a pure form of the tested compound in order to compare results. Once the pure compound’s results are in the database, future tests of the same drug can be tested against it. If the colors match, the test indicates drug purity. If the color is different from the original compound, then a technician has reason to be suspicious of the drug’s contents. From there, the compound must be sent to a lab capable of high-performance liquid chromatography for identification of the unknown pollutants (Lieberman et al.).

Another project Dr. Lieberman has developed is the detection of specific fillers added to milk. In some countries, such as India, milk is commonly diluted with water to increase the profit per gallon of milk at the expense of the consumer’s health. Starch, sugar, and urea are added as fillers to conceal alterations to the product. These fillers were difficult to identify without laboratory intensive analysis. This project demonstrates use of the wax-printing fabrication method. The wax printed on chromatography paper created hydrophobic barriers to keep the samples within designated areas.



The project employed a similar method to Lieberman's research on drug quality control, where a picture of the µPAD is taken after the completion of reactions. Their group named the µPAD, "MilkPAD."



Each compound analyzed required a color change reaction. Determining the presence of sugar was slightly more technical because milk naturally contains lactose. Lactose, a disaccharide composed of glucose and galactose, breaks down to form glucose through hydrolysis. Therefore, glucose is already present in unadulterated milk. Milk with added sugar has a higher glucose concentration than natural milk, though, once the sucrose breaks down to form glucose and fructose, and the change in glucose can be measured. The group used invertase to facilitate this reaction:

$$1. \quad \text{Sucrose} + H_2O \xrightarrow{\text{Invertase}} \text{Glucose} + \text{Fructose}$$

Then, glucose oxidase was used to react glucose with oxygen and water:

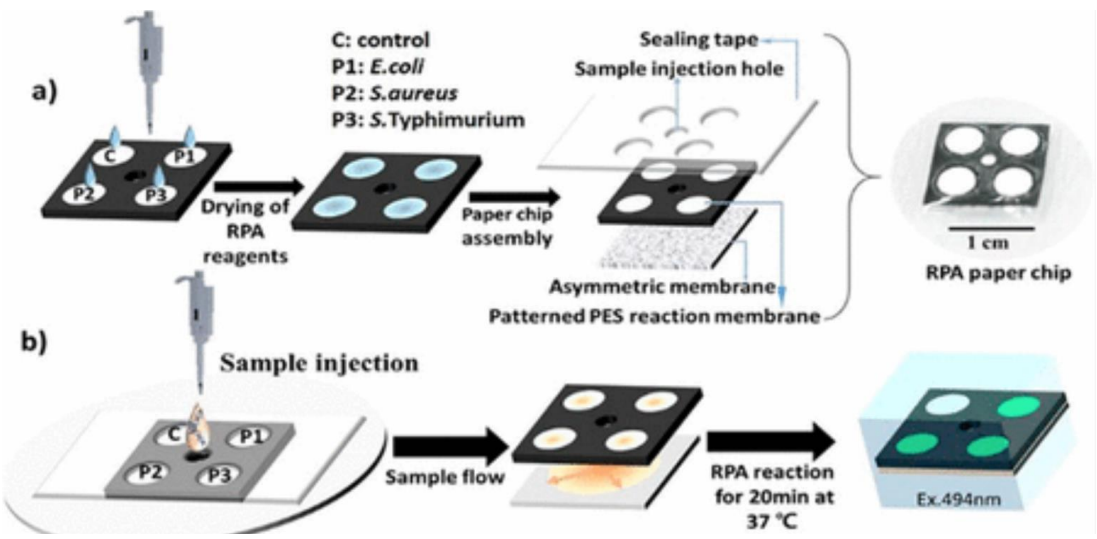
$$2.\quad \text{Glucose} + O_2 + H_2O \xrightarrow{\text{Glucose Oxidase}} H_2O_2 + \text{D-gluconic acid}$$

And finally, peroxidase and hydrogen peroxide promoted the oxidation of o-Dianisidine:

$$3.\quad H_2O_2 + \underset{\text{o-Dianisidine}}{\text{Reduced}} \xrightarrow{\text{Peroxidase}} \underset{\text{o-Dianisidine}}{\text{Oxidized}}$$

The group tested milk spiked with each of the three fillers, and their MilkPAD was able to detect the presence of the fillers (Luther et al.). However, urea is a non-competitive inhibitor of invertase, so this may cause the apparent concentration of sucrose to decrease. Their research did not discuss this, so it may or may not be an issue.

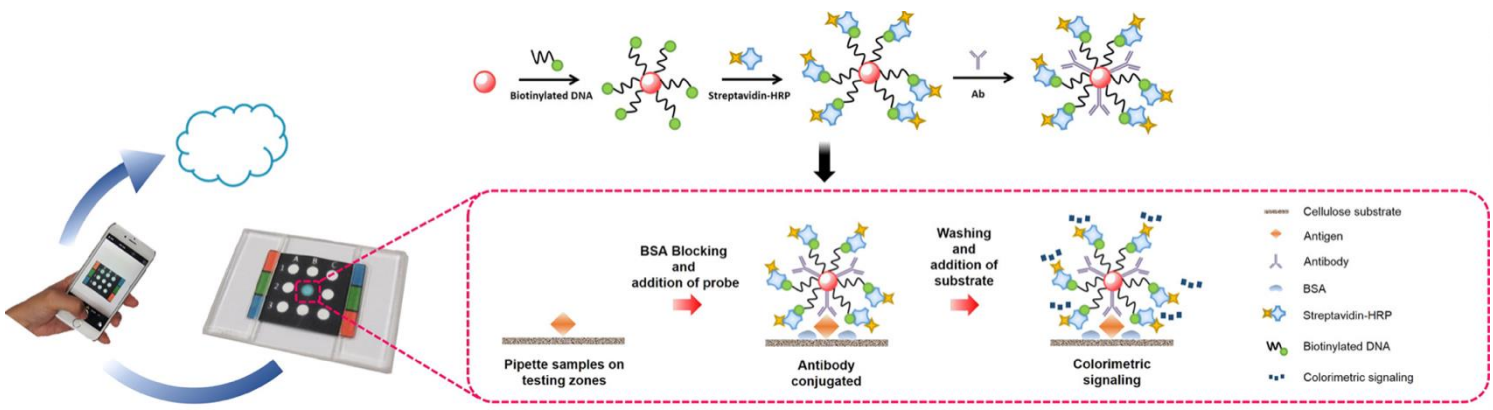
A second group has investigated milk analysis with μPADs. Instead of added fillers, Dr. Heeseop Ahn's group fabricated a device that detects the presence of E. coli, S. aureus, and S. Typhimurium in milk.



Section "a" illustrates the procedure used for creating the μPAD, and section "b" describes sample analysis. In this example, Dr. Ahn's group integrated a technique to amplify the signal of the bacteria known as recombinase polymerase amplification (RPA), commonly used in the field of biochemistry. This technique is well-suited for integration

with PADs because it does not require any special equipment while achieving sensitivity and selectivity. To begin fabrication, the matrix was developed with four compartmentalized reaction areas, and RPA reagents were added to each zone. RPA reagents included primers specific to the target pathogens, RPA-exo probes, and proteins for amplifying bacterial DNA, which were applied and then allowed to dry completely. The paper chip was then supported by an absorbent paper membrane underneath it to prevent any sample from leaking out of the reaction chip. The reaction membrane and absorbent membrane were covered with sealing tape, save for a sample injection hole in the center of the plastic to hold the layers together. Then, to analyze the bacterial contents of the milk, sample was injected into the hole in the sealing tape and distributed to all four sections of the paper chip. From there, the target-specific RPA reactions occur for ~20min at 37°C, increasing the amount of bacterial DNA exponentially (if target bacteria were present in the sample). Once completed, the zones can be observed using blue light to determine the fluorescence signal from the bacteria and the approximate concentration of the bacteria accordingly. Dr. Ahn's research demonstrates how chemical techniques can be integrated into μPADs. Because the paper matrix is customizable, processes can be combined with it to produce desired results. At the same time, the more complex the process, the more likely the costs and difficulty of use will be increased. With this μPAD, the question of disposal also comes up since the procedure grows harmful bacteria. According to the CDC, microorganisms grown must be autoclaved or incinerated before disposal, preferably before traveling outside the facility (Centers for Disease Control).

Another example of μPAD amalgamation with other techniques is illustrated by Dr. Jia-Yu Huang's research on cancer cell detection. His group developed gold nanoparticles probes, incorporating them into μPADs to quantify cancer cells in a sample.



First, the group developed gold nanoparticle probes, modified with ssDNA (biotinylated poly(adenine)), designed to bind to cancer cells. Once completed, the sample was loaded onto the μPAD, and the probes were added. The probes attached to the cancer cells, and any leftover probes were washed out. Streptavidin-horseradish peroxidase was added to bind to the remaining probes. Binding of the substrate to the probes created a color change, which could be observed and roughly quantified. The darker the color, the greater the concentration of cancer cells. In their research, they analyzed prostate cancer antigens, but mentioned that different antibodies can be loaded onto the gold nanoparticles to test other types of cancer. At a detection level of 10pg/mL, the device's sensitivity is competitive with other laboratory techniques. Not only is it sensitive, it also can be completed in just 15 minutes (Huang et al.).

VII. Challenges

Although μPADs have many advantages and are well-suited for point-of-care use, there are a few challenges. Firstly, they require pure samples for comparative analysis. Technicians must have a general idea of what the compound is before running a μPAD test. As of now, μPADs can help discern whether a sample is contaminated, but are unable to determine the identity of the impurities. When discussing the most commonly used technique (colorimetric assay), the most portable versions of detection are semi-qualitative but become fully qualitative if extra equipment (camera and computer) is available. This could increase the standard deviation of results for rural or low-income areas that do not have access to imaging equipment. In addition to this, colorimetric assays without equipment depend on color vision. Scientists who are colorblind may have difficulty interpreting results. However, several groups have researched methods of detection other than the colorimetric assay without the need for external devices, and these alternatives seem promising.

VIII. Future

Now that the groundwork is laid for μPADs, it is time for field experiments. Groups have already produced numerous models of μPADs that can detect biomarkers, bacteria,

and even cancer. The next step is to determine how well these devices perform after transport and the harsh conditions of the real world. Once the devices are field tested, modifications can be made to adapt to device weaknesses. Lab conditions facilitate easy detection, but success is determined by whether the μPAD holds up to outdoor elements. For example, a Red Cross tent might need a way to test for diseases in a crisis, but the clinic is exposed to sun and other weather. Rural, impoverished, or underdeveloped areas may not have access to ideal testing conditions, so it is important that devices can withstand various environmental climates. Once suitable models have been revised, mass fabrication methods can be arranged. Because μPADs are meant to be easily created and inexpensive, this step should be relatively attainable within a short time. With mass production, companies are able to decrease expenses, so ideally the already low-cost of these μPADs should decrease even further when produced in bulk.

Currently, there are several models of μPADs for different purposes. In the future, it is possible that research will move towards a few standard models that can be altered to fit various diagnoses. Another possibility would that a standard build would be developed that would encompass the most common detectors. Having a commonly used μPAD would also increase the confidence in color-change dependent μPADs. The greater the sample pool, the greater confidence labs can have in their results. For example, as of now, labs can test their own control groups with some degree of confidence. However, having a database of tested control samples to compare results with (similar to using a library of mass spectra) would be helpful and efficient. At some point, technicians should not need to run pure samples for comparison.

Some research articles projected that μPADs will expand into other products such as built into bandages for bacterial tests. Because of their paper composition, this makes them ideal for transport, easy use, and cost-effectiveness, but inadequate for extended use. The devices (at least with their current designs) could most likely disintegrate with day-to-day wear and tear. The point of paper devices is that they are meant for simple, POC analysis rather than recurrent usage.

Another take some groups have mentioned is incorporating μPADs with smartphones to utilize their display output. While this is a good idea, technology changes

so rapidly that it would be difficult to maintain compatibility. If the device paired with the μPAD, groups would need to ensure that it is simple enough to be used on most devices, and relevant enough not to become obsolete within a few years. Another consideration is that μPADs are meant to be very cheap and user-friendly, and the requirement for a smartphone increases costs while limiting who is able to use them.

This also evokes the question of how μPADs are discarded. None of the papers mentioned proper disposal protocol. This introduces possibility for contamination and compromised safety, especially when dealing with human disease detection. If μPADs became standards in clinical use, discarded μPADs could be sent to a sanitizing facility, or arrangements could be made on site to incinerate or auto-clave the used μPADs. For processes involving precious metals like the gold nanoparticle probes, research could be done to reverse the binding reaction to reuse the probes and decrease costs.

IX. Conclusion

Overall, the scientific community has developed numerous models of μPADs, integrating a wide variety of techniques ranging from biochemistry to electrochemistry and beyond. Colorimetric reactions allow for visually discernable results, although less accurate than traditional instrumentation. Their use as point-of-care devices will be an asset to labs and clinics around the globe, providing cheap, inexpensive, portable, and user-friendly methods of analyzing several compounds at the same time. Although field testing and plans for mass manufacturing and distribution have yet to be completed, much progress has been made in the field of microfluidic paper analytical devices.

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