Paper Microfluidic Diagnostic Devices

Final Documentation

Iowa State University

Senior Design May 14-26A

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1. Introduction

1.1 Background

Microfluidics is a field of science and engineering which aims to study and develop systems to manipulate small (µL to nL scale) volumes of fluid. The field was enabled by microfabrication techniques developed for integrated circuit fabrication. These techniques allow researchers to create micrometer-scale channels capable of manipulating fluids. Inkjet printers, the most ubiquitous example of microfluidic technology, rely on small chambers of fluid and integrated heating elements to propel droplets of ink onto the paper.

A large part of the rapidly developing field of microfluidics focuses on “lab on a chip” (LOC) applications. LOC systems integrate laboratory functions, like detecting biological compounds, on a single microfluidic device. Traditional microfluidic devices are fabricated using an elastic polymer, PDMS, bonded to a glass slide. The most sophisticated devices may have embedded electrodes for applying electric fields to the fluid flows. Recently, two alternative systems, digital microfluidics and paper microfluidics, have gained attention.

Paper microfluidics is a method of manipulating fluid flows using the wicking property of paper. Several systems have been developed for creating fluidic channels in paper, with wax printing being the most promising option. Melting the wax through the paper forms a fluid barrier, enabling channels to be created. Paper microfluidics can be used to develop more sophisticated lateral flow assays, or test strips, which are commonly used for point of care testing.

1.2 Executive Summary

Our project is a paper-based multifunctional saliva testing kit. The kit is designed for at-home use and serves as a pre-diagnostic tool for the detection of medical conditions. The kit includes two types of test strip devices: one for determining the pH of the user's saliva and the other for measuring the approximate concentration of amylase in the user's saliva. The fundamental property being taken advantage of with this design is the natural wicking property of paper. Wax channels that are printed onto the paper and melted through are used to control the flow of saliva across the paper.

1.3 Problem Statement & Solution

Problem Statement: Paper-based pH test kits currently include only a single type of test which limits the value the kit provides to the customer.

Solution: Create multi-functional paper-based test kit for human saliva and amylase to provide a preliminary indication of disease.

1.4 Goals and Deliverables

The current market for paper-based low-cost diagnostic devices is in its very early stages. While there are several small groups leading the market, there is still much room for growth. The goal is to, from research done on current technology in the market, create a multifunctional paper-based diagnostic device capable of being competitive with the current market. To achieve this, we hoped to first create a device to test the pH of saliva, and then take the project a step further to check at least one other analyte, such as amylase. In addition, our goal is to enhance the device by incorporating a downloadable smartphone app capable of reading the results of the saliva tests and providing a user-friendly output.

1.5 Intended Uses and Users

- Those people especially at risk from problems caused by poor dental hygiene such as in developing countries or the impoverished
- Those with genetic predisposition to oral disease
- Those in high stress environments
- Those at risk of schizophrenia

1.6 Current State of Market

In the current market, one of the leaders in the pH testing strip industry is Hydrion®. Hydrion's test strips work by use of a "dip" method, similar to our design. The test strips have a retail price of approximately $0.10 per test. As far as saliva amylase testing, there is currently no paper-based amylase test.

2. Project Design

2.1 - Functional Requirements

- Multifunctional device – The device should be capable of testing more than one characteristic of the saliva.
- 5 mL of saliva used – The volume of liquid should be sufficiently small to avoid cumbersome use by the user.
- No power needed – The device should not require the use of power. This extends its use to areas where electricity is not available.
- Colorimetric output – The device should give a colorimetric readout that is detectable either visually, by comparing to the reference strip included in the testing kit, or by use of the color detection mobile application.
- One-time use – Each device should be designed for a one-time use, after which the device may be discarded.

2.2 - Non-Functional Requirements

- Paper substrate – The paper substrate allows for taking advantage of the natural wicking properties of paper. Wax printing is also most feasible on paper.
- Portable
- Relatively quick – The test should provide output within approximately 1 minute for the pH test and approximately 15 minutes for the amylase test.
- Easy and safe to use
- Relatively inexpensive (~$.10/test)

2.3 - Constraints

- Set up interrupt structure to ensure when user are using the app while a text message or notification came in, user can switch to other function and came back with the reserved value.
- Develop the HSL (Hue, Saturation, Light) points that represent the color points in RGB (Red, Green, Blue) and make the comparison to make sure the read out of the app to be as accurate as possible.
- Debugging the code to make sure the app works at all conditions.

2.4 Detailed Design

- The iOS app is designed based on the basic theory of color comparison, We set up the calculation structure as HSL (Hue, Saturation, Light) points that represent the color points in RGB (Red, Green, Blue) and make the comparison with the photos can be taken by the smartphone's camera. Then, based on the auto generated the color matric table, the app will make the comparison and give out the result value.

2.4.1 - System Block Diagram

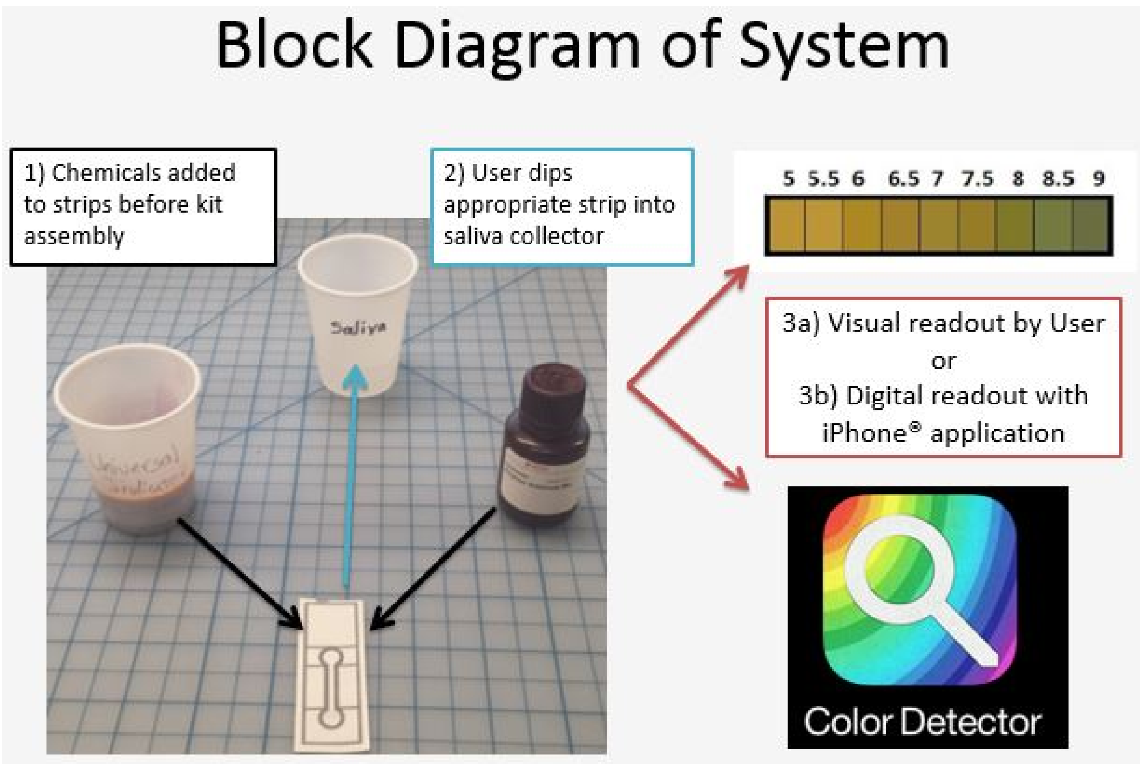

Figure 1: Block Diagram of the Test System

2.4.2 - Functional Decomposition

The paper device consists of several steps: saliva application at the source point, wicking of the saliva along the wax-defined channels, and colorimetric output.

The saliva is applied at a small paper opening at the end of the device. While most of the device will be coated with cold lamination to prevent contamination, this small area provides means of application. Saliva is applied to this source point by dipping the source point into a saliva "collection cup" which has been filled to the designated "fill-to line" by the user.

Once the saliva has been applied, it wicks along the paper through the wax-defined channels. Thinner channels are generally preferred because the reduced flow area requires less saliva per unit distance. Enough saliva must be applied to reach the pH indicator at the end of the channel in the "bulb" shape. Note: the pH indicator is applied in the bulb area and allowed to dry before the cold lamination and application of saliva onto the testing device.

The last step is the colorimetric output. Upon reaching the pH indicator at the end of the channel, the saliva reacts with the indicator, which changes to a color that is dependent on the pH of the saliva. The resultant color is compared to a standardized reference sheet of colors. The user matches the color with a color on the reference sheet, which shows each color's corresponding pH.

2.4.3 - System Analysis

The saliva testing kit uses paper as a substrate. The patient will deliver a sample of saliva and the saliva flows through the paper through the designed pattern. On the paper, we designed the pattern by using the wax. The wax barriers of the design can keep the sample within the boundaries of the pattern so that the saliva can flows through the paper through the designed pattern. With the pattern designed paper, patients will be able to do the test at home. The device will measure the pH and amylase level of the sample. For pH and amylase, the tests will provide colorimetric results that can be read either by visual comparison to the reference sheet included in the kit or by use of the color detection app, which determines the RGB values of the sample readout and assign an appropriate value, given either in pH or amylase concentration. The indicator is a recipe of four different indicators and is called "Universal Indicator". The indicator contains phenolphthalein, bromothymol blue, methyl orange, and methyl red indicators.

3. Implementation

3.1 Paper Well Plate Development

We first designed a paper “well plate” based on the standard laboratory 96-well plate that can be printed using a wax printer. Starting with the standard dimensions (Figure 2a), we modified the radii of the wells to account for the running of the wax when it was melted through. (Figure 2b) Keeping the center-to-center distance the same, the eventual product could be used with a standard 96-well plate reader, if more quantified RGB values were needed. Using this design, we were more efficiently able to test our pH and indicator solution.

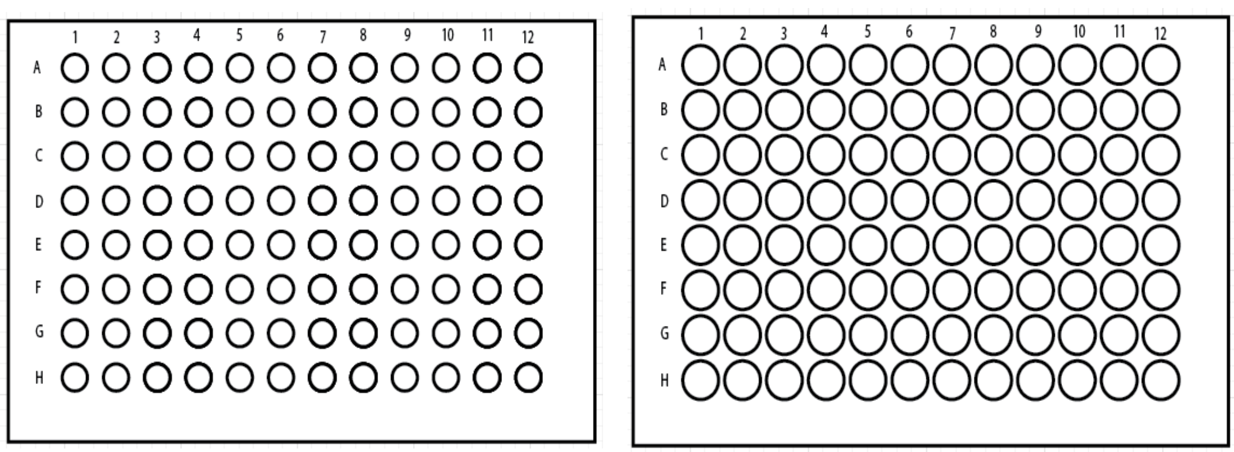

Figure 2: Paper well plate designs with a) Original Dimensions; b) Modified Dimensions

3.2 Development of pH Solutions

In order to eventually test the pH of an unknown solution, we knew that we had to develop solutions of known pH, in order to evaluate our indicators and create a reference. Our first attempt at acid-base solutions was the mixing of the three pH buffer solutions that were in our lab. These buffers are originally used for calibrating a pH meter. Attempting to make a standard range of solutions that would fit a statistical curve using the buffers, however, proved to be difficult and inconsistent. (Figure 3)

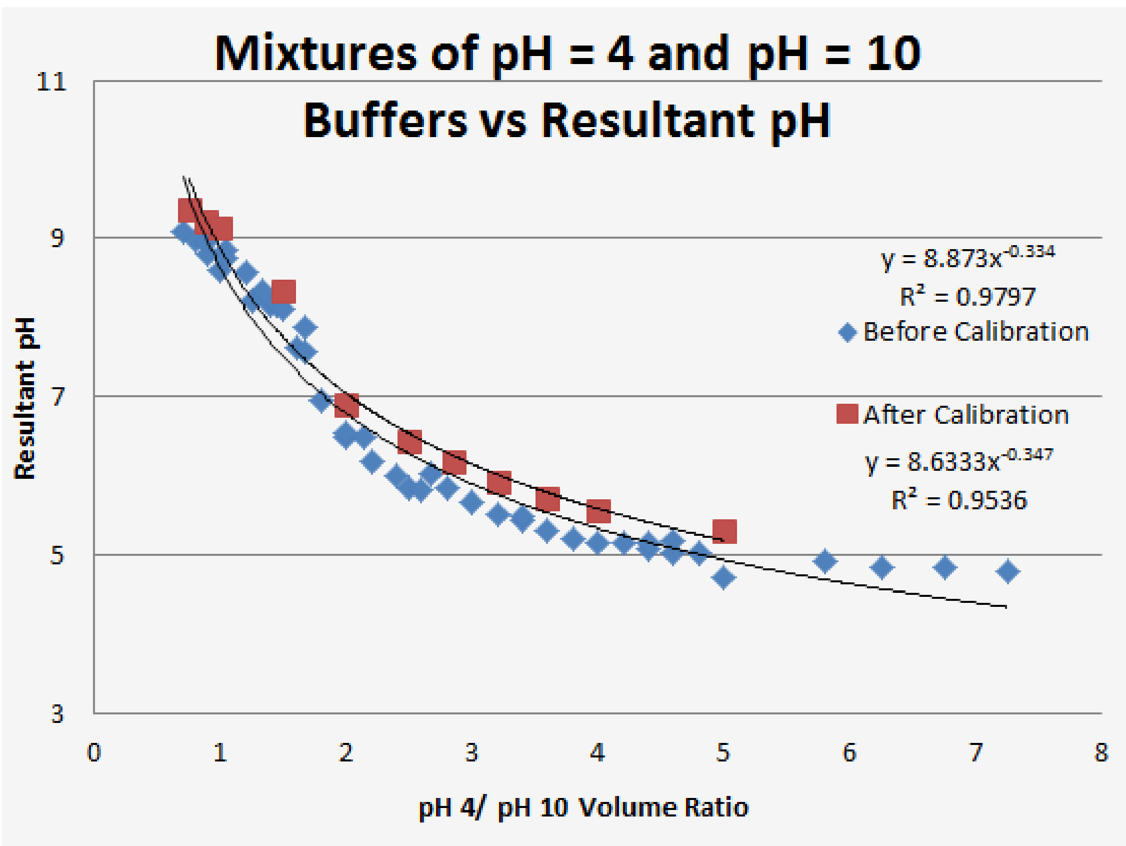

Figure 3: pH Buffer Mixing Curves

The next method that was attempted was a mixture of strong acid (HCl) and strong base (NaOH). Unfortunately, mixing of these two types of solutions results in a curve that does not change very much on the two ends of acid:base ratio and is not useful through the relevant range that we were aiming for. After consulting Dr. Joseph Burnett from the Dept. of Chemistry, we settled on using a combination of weak acid buffer solutions. Standard solutions of pH 4-10 were desired so McIlvaine's recipe of Na_2HPO_4 & Citric Acid ($C_6H_{12}O_6$) (Figure 4) was used to create solutions between pH 4 and pH 8. For the more basic solutions, a combination of Boric Acid (H_3BO_3) and 10% NaOH was used.

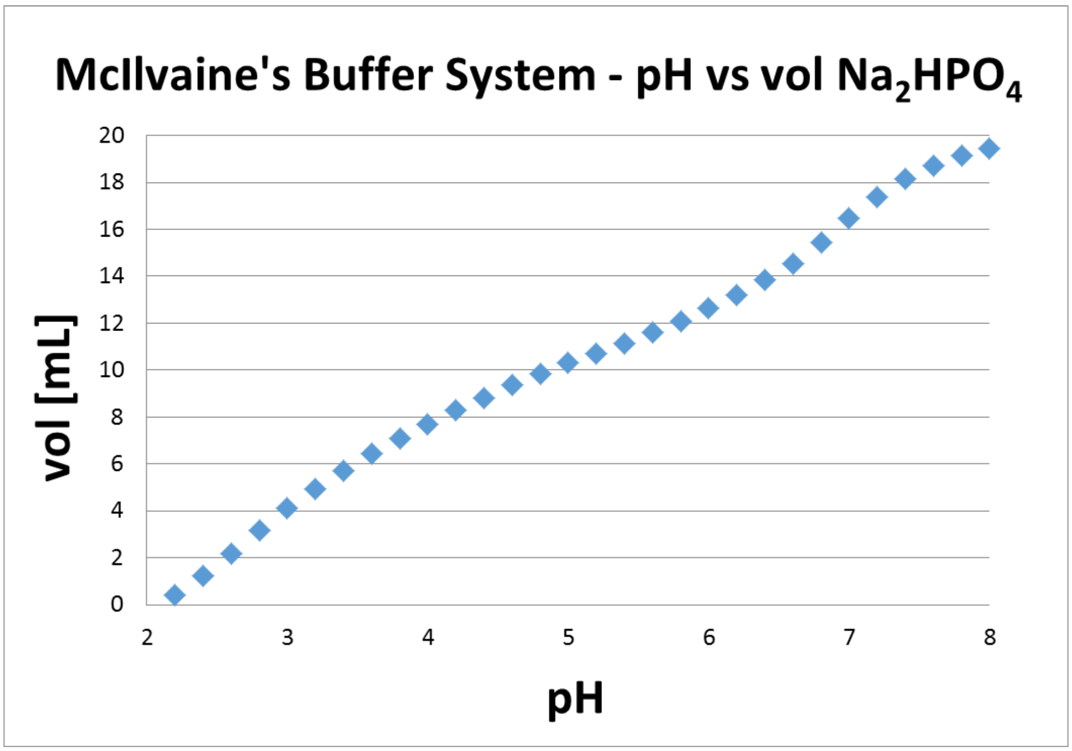

Figure 4: McIlvaine's Buffer Solution pH Curve

3.3 Test Strip Design/Creation Process

There have been several iterations of our test strip design that have changed as our project goals, product design, and general ideas have changed throughout the year. Before we decided to provide the user with a color reference strip, we considered having reference colors already placed on the strip. This was to be compared to the sample readout by the user and used as the pre-diagnosis. We soon realized that the colorimetric readout was going to be more complicated than one or two colors, and adjusted our strip to use a universal indicator for pH, or substrate for amylase. This change let us make one strip design that could be used for either test, depending on which chemical is added to the test spot. (Figure 5) Previous iterations of the design can be seen in Appendix II.

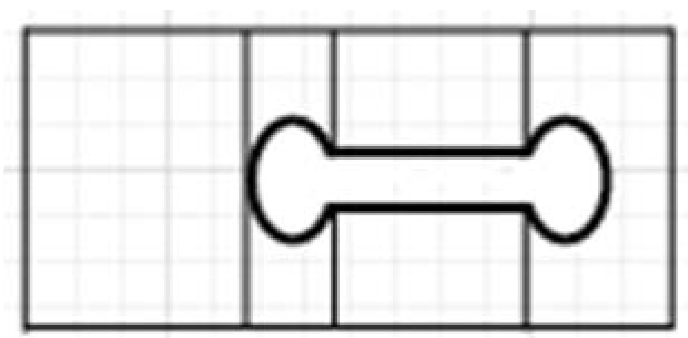

Figure 5: Final Test Strip Design

3.4 Color-Detection App

We have made a smart-phone app which will be able to compare image's color taken by the cellphone on the pH test stripe or selected in your photo album. The Color-Detection app is based on iOS operating system. The main programming language that we are using for the app is objective C, which is the programming language used to build applications for the Apple device such as iPhone and iPad. The whole development of the app is created by Xcode, which is an integrated development environment containing a suite of software development tools.

The Color-Detection app has four components. Take Detection, Detection History, pH table and Setting. In "Take Detection" part, users are able to compare the color based on the database. Users either can take the photo or select the photo in the photo album. In "Detection History" part, users can check the detection history. In the "Setting" part, users need to set the reference color before taking the detection and the app will automatically generate color reference table in "pH table" part.

4. Testing

4.1 Indicator development

Ultimately, a mixture of four indicators, collectively known as “Universal Indicator”, was decided upon. The Universal Indicator was chosen because it showed the greatest color variation across the relevant pH range, which for our purposes was 5.5-9. (Every person is different, so there is no exact definition of what an optimal pH for saliva should be. However, in general, a salivary pH of around 7 is considered healthy.) The “recipe” used to create the Universal Indicator is as follows: 0.7 ml of phenolphthalein, 15 ml of bromothymol blue, 10 ml of methyl orange, and 3 mg of methyl red. This was modified from the work of Dr. Robert Perkins of the British Columbia Science Teachers’ Association.

4.2 Amylase Testing

An amylase testing kit was purchased from Sigma Aldrich®. For a full assay, substrate is placed in several wells, and different volumes of a mixture of saliva and buffer is added to some of the wells, as well as a series of reference values to compare the colorimetric output to. Then, after the well plate is covered for 20 minutes to keep light from interfering with the test, the test is uncovered and the plate is placed in a well plate reader or chromatograph to read the difference in values. On paper, however, we did not have the convenience of several tests per plate. Instead, we attempted to optimize the volume of substrate placed on the paper in order to have the largest difference in color change.

4.3 Test Strip Construction

Adobe Illustrator® was used to design the test strip graphically. Several strips are printed on each piece of 200 mm x 200 mm Whatman® chromatography paper. The designs were printed with a Xerox® Colorqube wax printer. The wax then needs to be melted through the thickness of the paper. A hotplate may be used to melt the wax but the heat supplied over the area of the paper can be inconsistent. Instead, a laboratory oven was used at 180°C for 2 minutes per sheet. The individual strips are then divided using a paper cutter. Then the entire back side is covered with cold-lamination (contact paper). The plastic covering the backside of the paper was found to accelerate the wicking of the saliva through the device. The front side was then laminated in two areas as shown by the light blue color in Figure 6. The "Hold Here" Area is laminated in order to avoid any contamination by the User's hands. The bottom area is laminated in order to concentrate the uptake of saliva to a specific area. The appropriate chemical is then added to the test area depending on the type of strip desired. A pH test strip will have 5 μL of universal indicator added, while the amylase test strip will have 5 μL of substrate added.

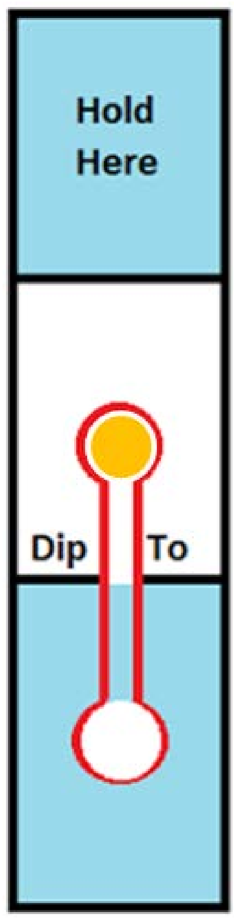

Figure 6: Sample Test Strip Schematic

4.4 Kit Components

The kit that the User receives will consist of the following items (Figure 7): a) saliva collector, b) test strips for both types of tests, c) color reference strip, and Saliva Testing Kit Instructions (Appendix I).

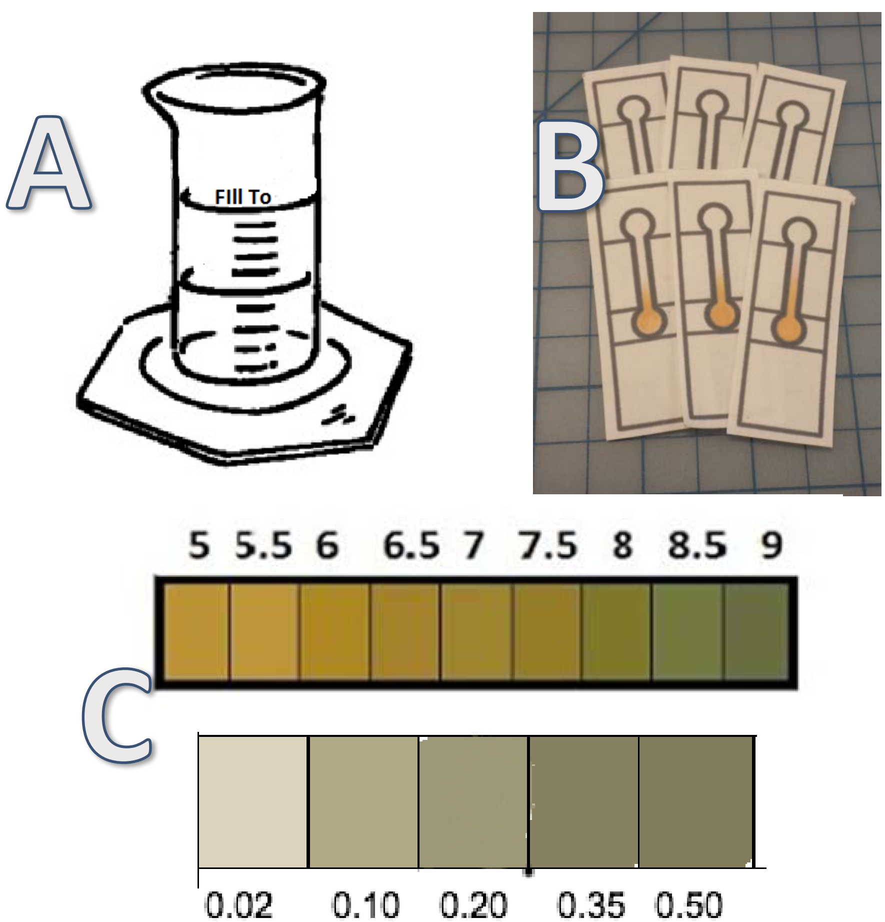

Figure 7: Kit Components

4.5 App Testing

The Color-Detection app will give more accurate results based on the color than human eyes. The following steps show you how to use the app:

Step 1: Open the Color-Detection app and tap the take detection button shown in Figure 8

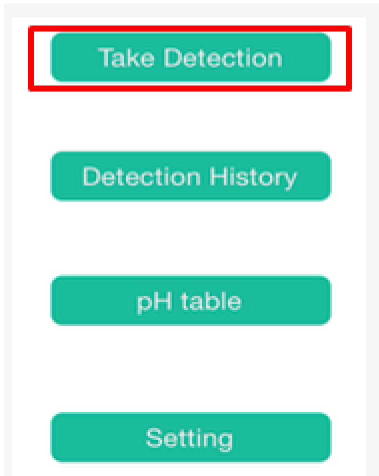

Figure 8: Step 1 - Take Detection button

Step 2: Choose the photo that you want to take the test shown in Figure 9

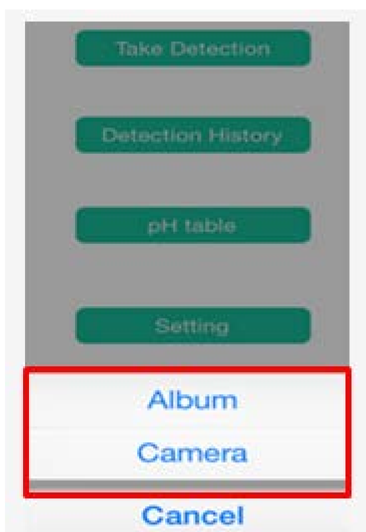

Figure 9: Step 2- Album or Camera Selection

Step 3: Set the testing zone shown in Figure 10

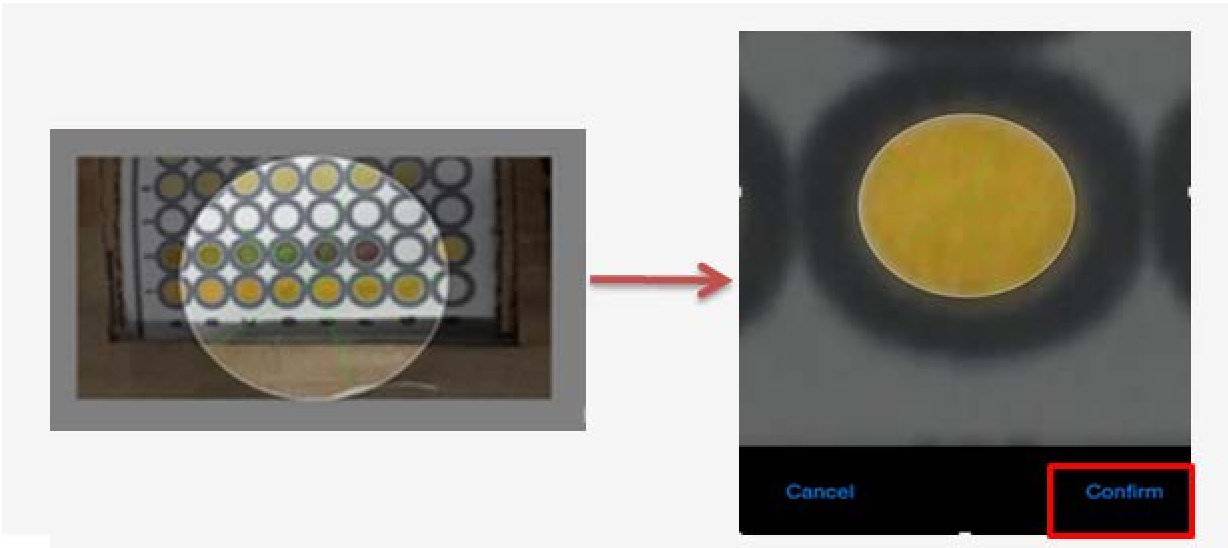

Figure 10: Step 3 - Testing Zone Selection

Step 4: Reading the result shown in Figure 11

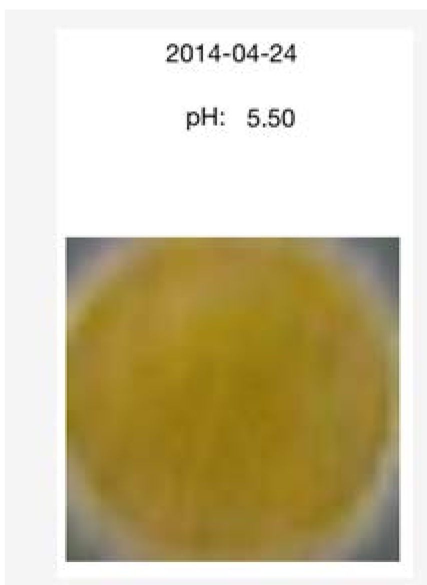

Figure 11: Step 4 - Resultant pH/concentration Readout

4.6 Technical Challenges & Solutions

- **Creation of pH Solutions** – As discussed above, several attempts were made to create solutions with known pH values. After inconsistent results from the first two methods, we solved the problem with the McIlvaine's buffer solutions, which allowed us to make reproducible solutions with static pH values.
- **pH indicator development** – Once we had solutions with known pH values, we needed a pH indicator that would effectively display a colorimetric readout to correspond to the pH of the solution. Our goal was to find or create an indicator capable of showing the 5.5-9 pH range with observable color differences for each 0.5 increment. We experimented with several common individual indicators such as methyl red, bromothymol blue, phenol red, and others. Some of these indicators were effective at showing distinct colors in a given range (i.e. 3.0-6.0), but we could not find an indicator that covered the 5.5-9 pH range with observable changes in every 0.5 step. We moved on to making mixtures of the indicators we had previously tested individually. Again, this resulted in mixed results, and none of the mixtures were deemed satisfactory for our requirements. Finally, during our research we came across a recipe for Universal Indicators, which was described in more detail in section 4.1.
- **Timing of Picture for App** – When using the color detection app, to ensure consistency, the pictures of the testing device need to be taken a set time after the test has been run. After our repeated observation of the color readouts, we determine that the optimal times for app pictures to be taken were 1 minute for the pH test and any time in the 15-20 minutes range for the amylase test.

5. Conclusion

5.1 Closing Summary

The goal of this project is to produce a paper-based multifunctional saliva testing device. Our final product is a paper-based test device capable of testing the pH and amylase concentration of a user-provided saliva sample.

5.2 Outlook of Future Functionality

5.2.1 Device

Our accomplishments with this project over the course of the past two semesters have led to what could be called a “proof of concept”. Our work and final design demonstrate that paper-based diagnostic devices are, in fact, a feasible method of detecting properties of human saliva. Paper-based pH testing has been around for a while and is a well-established testing method. Paper-based amylase concentration testing, on the other hand, is a novel process. As a team, we were unable to find any products or information online that test salivary amylase levels on paper. Our work, while far from perfect, shows that there may be promise for this application in the future.

5.2.2 Application

At the senior design stage, the Color-Detector app is able to tell the difference between two very similar colors based on the pH value. However, you have to set the reference color before you take the detection. In the future app development, we would like to show users the instant RGB values of the picture. In addition, we would like to add a circle on the camera screen to keep the distance between the camera and object constant.

5.3 Acknowledgements

- Zach Njus, Isaac Johns - Students in Research Lab
- May 14-26b (Tan CheeKang, Kong Taejoon, Jared Anderson, Riley Brien)
- Suhuai Song, Yifei Wang, Dr. Meng Lu - Department of Electrical Engineering
- Dr. Joseph Burnett – Department of Chemistry

Appendix I: Operation Manual

Saliva Testing Kit Instructions

Storage Instructions:

Keep the test kit in a clean, dark, dry, cool place (i.e bathroom cabinet)

Components:

- One (1) 5 mL Saliva Collector Tube
- One (1) Collector Tube Holder
- Ten (10) pH Test Strips
- Ten (10) Amylase Test Strips
- One (1) Color Output Reference Strip

Procedure:

- Fill the saliva collector tube with saliva until the "Fill to" line is reached.
- Choose the appropriate strip for the desired test
- Dip strip into the tube for 30 seconds; Be sure to align the " Dip to" line on the strip and the "Fill to" line on the tube
- Remove the strip and place Test Side Up on a paper towel or other cleanable surface
- Observe the color change and compare to the Color Output Reference Strip
    - For pH testing:
        - Color should manifest within 30 seconds
        - Compare as soon as color is seen
        - If the test shows a pH of 5.5 or lower, this may be a sign of more significant health issues. Please contact your physician.
        - If the test shows a pH of 6, test again in 2 days.
        - A pH of 6.5 or higher is considered healthy
    - For Amylase testing:
        - Color may take 15-20 minutes to appear
        - Compare to reference 5 minutes after first color is seen
        - If the result of .1-.35 IU, your amylase levels are acceptable
        - If a value above or below this range is seen, this may be a sign of a more significant health issue. Please contact your physician or seek additional medical advice.
- Discard used test strips and wash the collector tube in between test days
    - Wash with warm water and mild soap; or in the dishwasher on top shelf

Appendix II: Initial/Alternative versions of design

Saliva Application Decision

The method of saliva application onto the test strip was a design consideration that was debated throughout the year. There were three options: **1)** the user spits onto the saliva source point on the test strip, **2)** the user holds the saliva source point in their mouth for a few seconds to introduce saliva onto the strip, or **3)** the user spits into a collector tube until the designated level is reached and dips the saliva source point into the collector tube. The third method, the collector tube/dip method, was eventually agreed upon for the following reasons:

- The dip method allows the amount of saliva being applied to the test strip to be standardized. With methods 1 or 2, the amount of saliva transferred to the test strip can be highly variable from person to person and even from trial to trial for the same person.
- Method 1 could prove to be very messy and would require the user to spit onto a very small designated area. Method 3, the dip method, eliminates this problem, as the collector cup provides an easier "target".
- Some people may feel uncomfortable sticking a laminated paper test strip in their mouth.

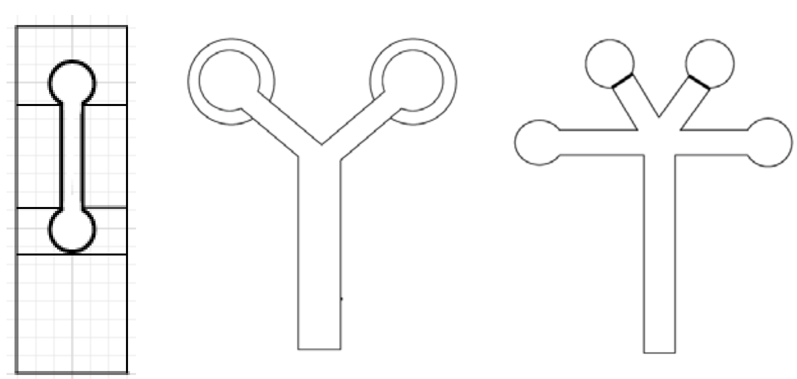

Figure 42: Old Iterations of Test Strip Design