DEVELOPMENT OF A COMPACT OPTICAL RAPID DIAGNOSTIC TEST READER AND A POINT-OF-CARE FLUORESCENCE LATERAL FLOW ASSAY FOR DENGUE DETECTION

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ABSTRACT

As the prevalence of febrile illnesses continues to increase, there is a growing need for reliable, low-cost means of diagnosing these diseases. To address this, a highly sensitive point-of-care fluorescence lateral flow immunoassay is being developed to diagnose dengue in its earliest stages. Through use of different fluorescent labels, multiplexed test strips will eventually be created to test for several diseases simultaneously. To quantify the resulting signals produced by the assay, a corresponding optical reader was designed, built, and tested in accordance with design thinking principles. The effectiveness of the design was verified through usability testing. The reader works in conjunction with software run on any internet-enabled device to take an image of a test strip and analyze the signal levels by comparing them to predetermined calibration curves. Through use of the fluorescence lateral flow immunoassay and corresponding optical reader, users in resource-limited settings can diagnose dengue rapidly and accurately.

BIOGRAPHICAL SKETCH

Jess Hohenstein grew up in Scotia-Glenville, a small town in eastern New York. She went to Northeastern University in Boston, Massachusetts to study Mechanical Engineering at the Bachelor level. While there, she worked on development of automation software at GE and designed and tested sustainable farming equipment for a nonprofit company in Cameroon. After earning her Bachelor of Science in 2014, Jess joined the Department of Mechanical and Aerospace Engineering at Cornell University. At Cornell, she worked with Dr. David Erickson on developing smartphone based mobile health and point-of-care diagnostics. In 2016, Jess will earn her M.S. from the Sibley School of Mechanical and Aerospace Engineering.

Some of Jess' research interests include mobile health in resource-limited settings, design thinking, human-centered design, and information visualization. In her spare time, Jess will likely be found hiking, traveling, taking photographs, or doing graphic design for various clubs and organizations.

“The Road goes ever on and on

Down from the door where it began.

Now far ahead the Road has gone,

And I must follow, if I can,

Pursuing it with eager feet,

Until it joins some larger way,

Where many paths and errands meet.

And whither then? I cannot say.”

- J.R.R. Tolkien

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LIST OF ABBREVIATIONS

ABS: acrylonitrile butadiene styrene

AuNP: gold nanoparticle

DENV-1, -2, -3, -4: Dengue virus 1, 2, 3, 4

DOL: degree of labeling

ELISA: enzyme-linked immunosorbent assay

FITC: fluorescein isothiocyanate

GPIO: general purpose input/output

HTTP: hypertext transfer protocol

IgA: immunoglobulin A

IgG: immunoglobulin G

IgM: immunoglobulin M

LED: light-emitting diode

LFA: lateral flow immunoassay

MAC-ELISA: IgM antibody-capture enzyme-linked immunosorbent assay

NASBA: nucleic acid sequence based amplification

NS1: non-structural protein 1

OD: optical density

PBS: phosphate-buffered saline

PCR: polymerase chain reaction

RBC: red blood cell

RT-PCR: reverse transcriptase-polymerase chain reaction

RDT: rapid diagnostic test

SBC: single-board computer

SEM: scanning electron microscope

SUS: system usability scale

VLSI: very-large-scale integration

CHAPTER 1

INTRODUCTION

Motivation

Contributions

Organization of This Document

MOTIVATION

Febrile illnesses, including dengue, are a prevalent problem worldwide with many cases misclassified and underreported[1]. Many regions where these diseases are endemic lack the laboratory equipment, trained personnel, and infrastructure necessary for efficient and accurate diagnoses. This presents an opportunity for rapid diagnostic testing using lateral flow assay technology. The LFA produces a detectable signal that is quantifiable through paired use with a reader instrument that can display the result to the user. Many existing readers are bulky and expensive, making them infeasible for resource-limited settings. There is a need for highly sensitive rapid diagnostic tests for febrile illnesses and portable, inexpensive optical readers for signal quantification.

CONTRIBUTIONS

The contributions in this thesis provide a foundation for new advancements in lateral flow technology and LFA reader technology. These contributions are the following:

- The design thinking process was used to create a novel optical reader device for quantification of LFA signals. The reader is portable, small, and inexpensive, making it ideal for use in resource-limited settings.
- Usability testing was performed on the new optical reader and improvements on the design were made. The foundation of the design thinking process has been established and will be continued to ensure a robust final product.
- The groundwork for a fluorescent LFA for dengue detection has been established. After experimentation with other detection labels, fluorescent particles were deemed the optimal label for this highly-sensitive disease diagnostic.

ORGANIZATION OF THIS DOCUMENT

The remainder of this thesis is divided into seven chapters, each of which is self-contained and can be read separately. The chapters can be decomposed into two complimentary categories: (1) development of a lateral flow assay for dengue detection, and (2) design and creation of a novel optical reader for LFA signal quantification. Chapters 2, 3, and 4 fall into the first category of LFA development, while chapters 5, 6, and 7 fall into the second category of the design of the optical reader.

Chapter 2 describes the burden of dengue along with current methods of detection and their limitations. Chapter 3 provides an overview of lateral flow assay technology. In Chapter 4, details of the reasoning behind and development of a fluorescent lateral flow assay for the detection of dengue are discussed. Chapter 5 details the design thinking process and the importance of usability in design. In Chapter 6, lateral flow assay reader devices are discussed, along with their shortcomings. Chapter 7 discusses how the principles of design were applied to create a novel solution to overcome the inadequacies in current lateral flow assay reader technology. Chapter 8 briefly concludes the work, provides some ideas for future directions, and discusses other work completed during graduate studies.

CHAPTER 2

DENGUE OVERVIEW

Introduction

Dengue Overview

Burden of the disease

Biology of the virus

Clinical presentations and underreporting

Current Detection Methods

Virus isolation

Nucleic acid detection

RT-PCR

Real-time RT-PCR

Isothermal amplification methods

Antigen detection

Serological tests

MAC-ELISA

IgG ELISA

IgM/IgG Ratio

IgA

Haemagglutination-inhibition test

Haematological tests

Shortcomings and New Directions

INTRODUCTION

According to the World Health Organization, dengue is the most rapidly spreading mosquito borne virus in the world[1]. However, the disease is often misdiagnosed or goes unrecognized due to a lack of available rapid, reliable, inexpensive diagnostic tests[2]. Therefore, a highly sensitive rapid lateral flow test to detect the dengue virus has is being developed.

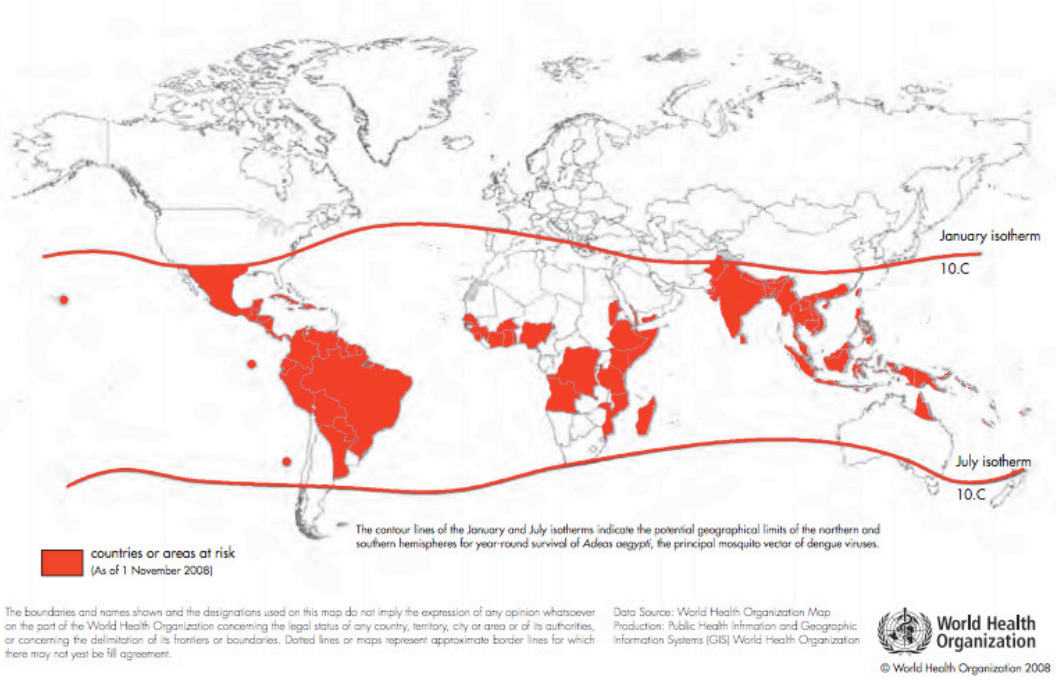

Figure 1: Countries/areas at risk of dengue transmission[9]

DENGUE OVERVIEW

Burden of the disease

Nearly half of the global population lives in countries where dengue is endemic[1], and the actual numbers of dengue cases are underreported and often misclassified[3]. Countries and areas at risk of dengue transmission can be seen in Figure 1. Recently, the number of reported cases has increased as the disease has spread to new areas. No treatment or vaccine currently exists for dengue fever.

Although dengue is rarely fatal in and of itself, potentially deadly complications include plasma leaking, fluid accumulation, respiratory distress, severe bleeding, or organ impairment[1]. Subsequent medical care can decrease mortality from these complications from more than 20% to less than 1%[1]. Because of this, rapid and accurate diagnosis is vital.

The cost of dengue was examined in eight countries (Brazil, El Salvador, Guatemala, Panama, Venezuela, Cambodia, Malaysia, Thailand) in 2005-2006. Combining ambulatory and hospitalized dengue patients with the risk of death, it was determined that the average cost of each dengue case is US$ 828. Taking into account the average annual number of reported dengue cases results in a total estimated annual cost of US$ 440 million[14]. However, dengue is widely misdiagnosed and underreported, so it can safely be presumed that the actual cost is much higher. This calculation also does not take into account the substantial costs associated with dengue surveillance and vector control programs[9].

Biology of the virus

The dengue virus has four distinct serotypes: DENV-1, DENV-2, DENV-3, and DENV-4[1]. When a cell becomes infected, the virus encodes a NS1 protein. The NS1 protein is then secreted from the infected mammalian cells, making NS1 an effective indicator when the patient has viraemia (high levels of dengue virus in the blood). IgM antibodies are the first immunoglobulin isotype to appear, usually 3-5 days after the initial exposure to the dengue virus. IgG antibodies against the virus are typically detectable at the end of the first week of illness[9]. The titres of the IgG and IgM antibodies will vary depending on if the infection is primary, where IgM levels are very high, or secondary, where IgM levels are lower and IgG levels are higher. These titres allow clinicians to determine whether a patient is experiencing a primary

or secondary dengue infection[2]. The onset of major diagnostic markers for dengue can be seen in Figure 2.

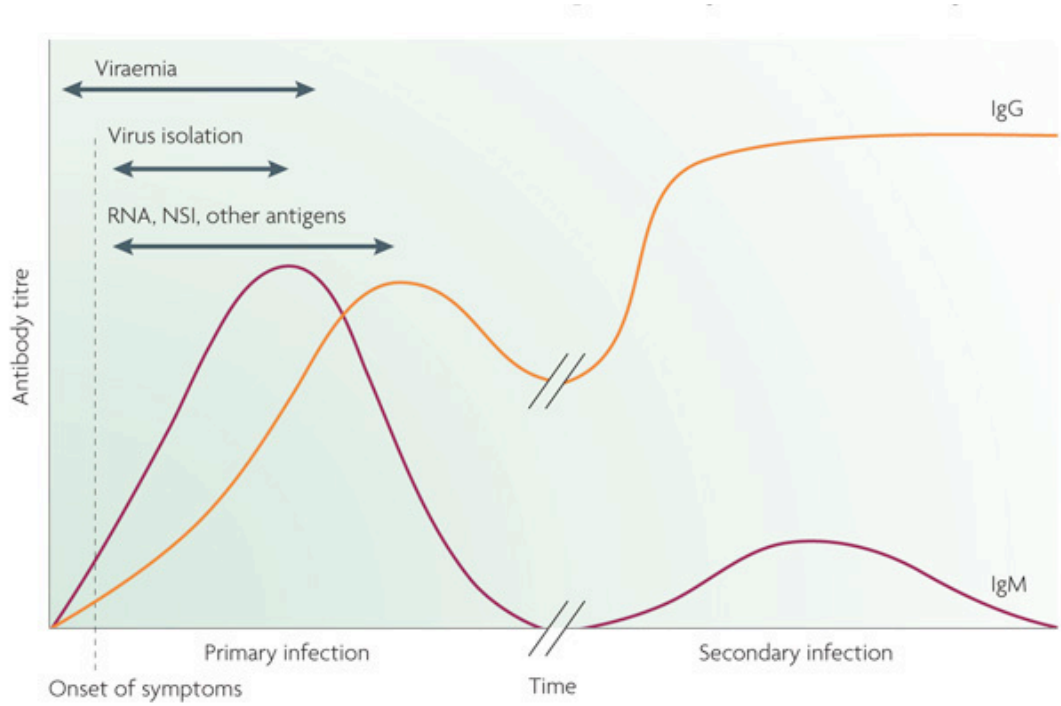

Figure 2: The major diagnostic markers for dengue infection include detection of the dengue virus, viral RNA, and viral antigens such as the NS1 protein when the patient has viremia. IgM and IgG antibodies against the dengue virus can be detected in most patients five days after the onset of symptoms. [2]

Clinical presentation and underreporting

Dengue and other common arthropod-borne diseases, such as malaria and chikungunya, have unspecific clinical and biological presentations and overlapping endemic areas[4]. Because of the time, human resources, and experienced lab technicians required, a parasitological exam is difficult to achieve for all febrile syndromes[5]. This results in frequent misdiagnosis of febrile illnesses, which causes a greater number of healthcare visits and associated costs[6], and an incomplete understanding of the burden of dengue[7]. Many studies point to the need for better diagnostic tests to fully assess the influence of and improve treatment for dengue[2,7,8].

CURRENT DETECTION METHODS

As discussed previously, dengue has non-specific symptoms, making a diagnosis based on clinical symptoms unreliable. A range of diagnostic methods is currently used for dengue diagnosis.

Virus isolation

Specimens (serum, plasma, tissue, and peripheral blood mononuclear cells) for virus isolation are collected during the period of viraemia and kept in a refrigerator for up to 24 hours or frozen for longer periods. In a laboratory, cell culture is used to isolate the dengue virus, including screening by an antigen detection immunofluorescence assay using serotype-specific monoclonal antibodies and flavivirus group-reactive or dengue complex-reactive monoclonal antibodies[9]. This method requires trained laboratory technicians, proper transportation and storage of the specimen, and about 1-2 weeks of time.

Nucleic acid detection

Nucleic acid detection assays involve three steps: nucleic acid extraction and purification, amplification of the nucleic acid, and detection and characterization of the amplified product[9]. RNA is heat-sensitive, so specimens for nucleic acid detection must be handled and stored in the same way described for virus isolation.

RT-PCR

RT-PCR offers better sensitivity (80-100%) and a faster turnaround time than virus isolation[9]. Typically, a nested RT-PCR assay is used, where the initial reverse transcription and amplification involves universal dengue primers targeting the C/prM region of the genome. A serotype-specific nested PCR amplification then takes

place[10]. Using electrophoresis, the reaction products are separated on an agarose gel and visualized as bands of different molecular weights using ethidium bromide dye, which are compared with standard molecular weight markers. Dengue serotypes can then be identified by the size of their bands.

Real-time RT-PCR

Real-time RT-PCR assays utilize a fluorescent probe to enable the detection of reaction products in real time without needing electrophoresis. The assays are either singleplex and detect only one dengue serotype at a type, or multiplex and can identify all four serotypes from one sample. Multiplex real-time RT-PCR assays are faster but less sensitive than RT-PCR assays[9].

Isothermal amplification methods

The NASBA assay does not require thermal cycling instrumentation. First, the single-stranded RNA target is copied into double-stranded DNA that serves as a template for RNA transcription. The amplified RNA is detected by electrochemiluminescence or by fluorescent-labeled molecular beacon probes in real time. The sensitivity of NASBA is similar to that of virus isolation[9].

Antigen detection

The NS1 antigen can be detected in patients with both primary and secondary dengue infections up to nine days after the onset of illness[2]. Commercial kits to detect the antigen are available, but they are not able to differentiate between serotypes. NS1 can be detected in acetone-fixed leucocytes and in snap-frozen or formalin-fixed tissues using fluorescent antibody, immunoperoxidase, and avidin-biotin enzyme assays[9].

Serological tests

MAC-ELISA

The IgM ELISA captures total IgM in sera using human IgM-specific antibodies coated onto a microplate. Dengue-specific antigens are bound to the captured anti-dengue IgM antibodies and detected by monoclonal or polyclonal dengue antibodies conjugated to an enzyme that results in a colored product, as shown in Figure 3. A spectrophotometer then measures the OD. The sensitivity and specificity are good when the test is performed within 5 days after the onset of the illness, with ELISA tests generally performing better than rapid tests[9]. False positives can result from cross-reactivity of dengue with other flaviviruses, such as past dengue infections and malaria[9].

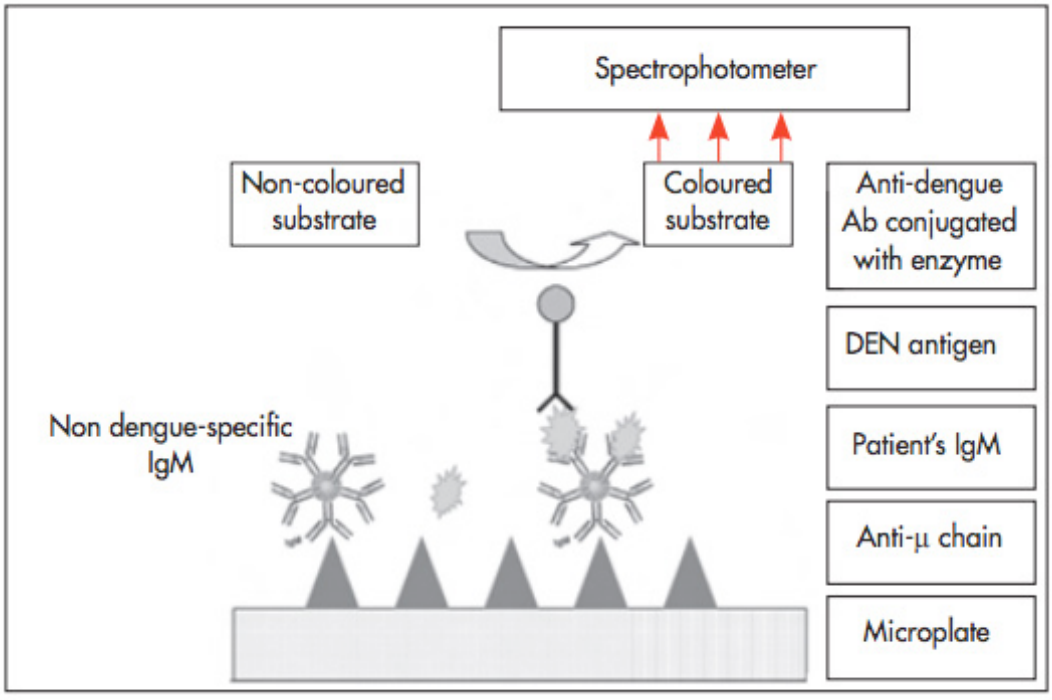

Figure 3: Principle of MAC-ELISA test[9]

IgG ELISA

This assay uses the same antigens as the MAC-ELISA to detect recent or past dengue infections. It can be used to detect IgG antibodies in serum or plasma and allows identification of a primary or secondary dengue infection[11,12,13]. Like the MAC-ELISA, the IgG ELISA lacks the ability to differentiate between dengue serotypes.

IgM/IgG Ratio

The IgM/IgG ratio can be used to differentiate between primary and secondary dengue infections, as shown in Figure 1. This ratio is usually achieved using IgM capture and IgG capture ELISAs. However, the cutoff ratios for each infection type vary between laboratories, and better standardization is necessary[9].

IgA

An anti-dengue virus IgA capture ELISA can be performed one day after that for IgM to detect serum anti-dengue IgA. This seldom-used approach can be used to help determine dengue serology[13] but requires further validation.

Haemagglutination-inhibition test

The HI test is based on the ability of dengue antigens to agglutinate red blood cells of ganders or trypsinized human O RBC. The test measures the level of which anti-dengue antibodies inhibit this agglutination, as shown in Figure 4.

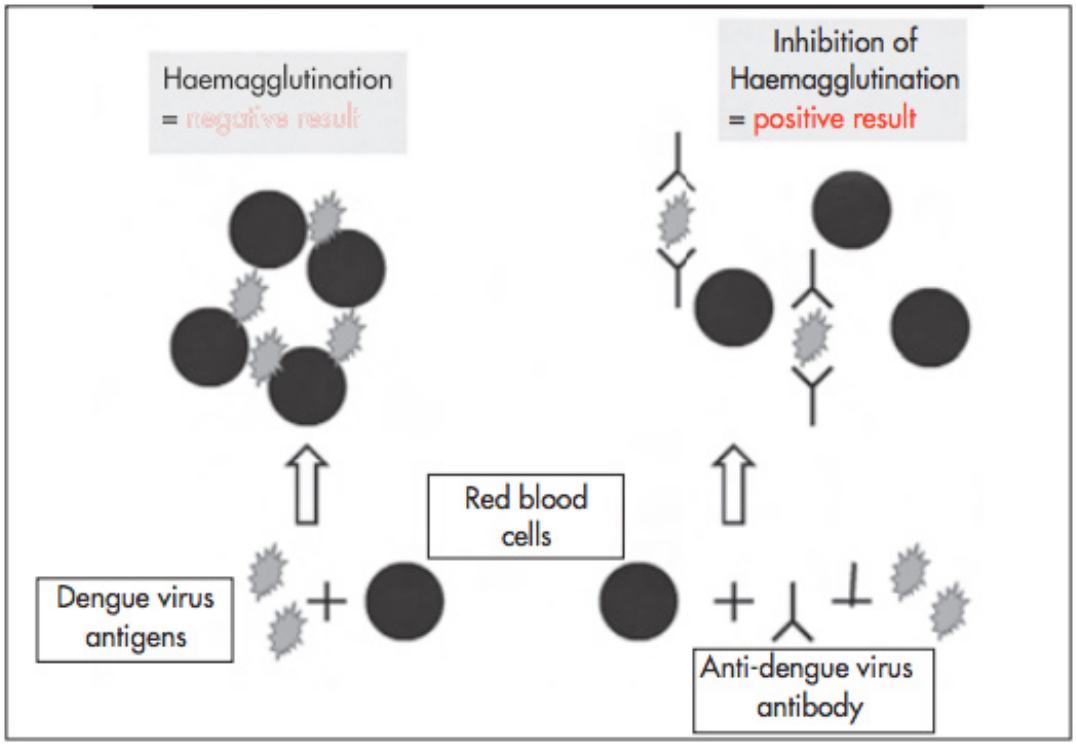

Figure 4: Haemagglutination-inhibition assay[9]

Haematological tests

During the acute stages of dengue infection, platelets and haematocrit values are commonly measured. One known feature of dengue haemorrhagic fever is a drop of platelet count below 100,000 per uL[9].

SHORTCOMINGS AND NEW DIRECTIONS

Because dengue is mainly endemic in developing countries, market-driven incentives rarely exist for the development of better diagnostics. Companies working in this area tend to be small and under-resourced, without the necessary research and development capabilities. In many endemic areas, regulations for diagnostic tests are lenient, and assays are often used without sufficient proof of effectiveness[3].

A range of diagnostic methods exists for dengue detection, but a lack of available resources in many dengue endemic settings often means that the tests with the highest sensitivity and specificity are inaccessible[9]. Another problem with current methods is the prevalence of false positives due to a cross-reactivity of dengue with other flaviviruses, such as past dengue infections, malaria, chikungunya, and zika. A diagnostic test that permits early and rapid diagnosis of dengue while being affordable, easy to perform, and able to overcome cross-reactivity problems is needed.

CHAPTER 3

LATERAL FLOW ASSAYS

Introduction

LFA Physical Components

Nitrocellulose membrane

Porous Pads

Sample pad

Conjugate pad

Absorbent pad

Configurations and flow

LFA Chemistry

Sandwich and competitive formats

Detection labels

Liposomes

Colloidal carbon

Colloidal gold

Fluorescent particles

Quantum dots

Upconverting phosphors

Bioluminescent markers

Paramagnetic particles

Latex particles

INTRODUCTION

The LFA is a popular tool for rapid diagnostics because of its ease of use, compact and portable nature, low cost, and quick time to results. Most do not require any external reagents, and a fluid sample is simply applied to initiate and complete the test. The most well-recognized lateral flow assay is the home pregnancy test, which detects human chorionic gonadotropin (hCG), a hormone produced during pregnancy. The LFA has a wide range of applications, including detection of diseases, vitamin levels[15], failure of internal organs[16], toxin presence[17], or the use of illegal drugs[18]. The LFA is primarily used to give a binary result, but quantitative results have been demonstrated with specialized equipment[15].

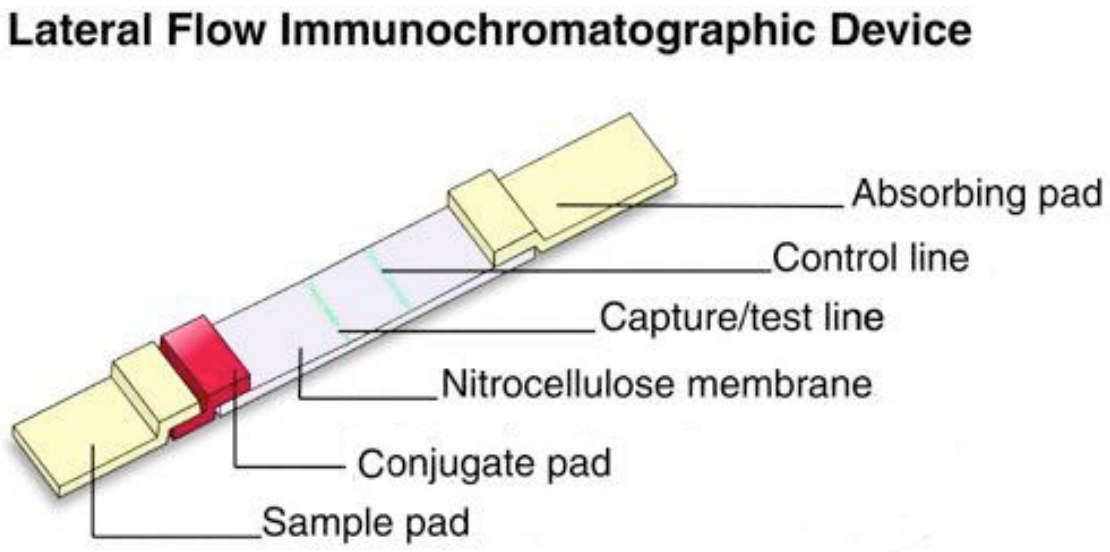

Figure 5: Typical LFA Layout[19]

LFA PHYSICAL COMPONENTS

A typical lateral flow assay layout is shown in Figure 5. This strip is usually housed in a plastic cassette that allows for easy transport and handling and obfuscates components besides the sample pad and test and control lines. The strip flow depends on flow through a nitrocellulose membrane and other porous materials.

Nitrocellulose membrane

The assay components are assembled on a nitrocellulose membrane. Nitrocellulose membranes are classified by various flow times, which is the time required for water to travel up and completely fill a 4 cm long membrane strip[21]. The flow time is directly related to pore size and typically ranges from 75s/4cm – 240s/4cm[20]. Membranes are specially manufactured to be consistent in structure, thickness, and capillary flow time[21]. Because of its hydrophobic nature, nitrocellulose has a high adsorptive capacity for proteins[20].

Porous pads

The sample, conjugate, and absorbent pads are made from porous materials and adhered to a backing so that they are in line with the nitrocellulose membrane. Usually, cellulose paper is used for the sample and absorbent pad while glass fiber is used for the conjugate pad. Other materials, such as various types of woven fabrics, are sometimes used[20]. The porous materials are characterized by bed volume and thickness[21], which dictate what sample volume is necessary to saturate the structure and how thick the plastic cassette housing needs to be, respectively.

Sample pad

The sample is applied to the LFA on the sample pad. If the sample contains the analyte of interest, it must be capable of binding to the capture reagents on the detection particles[20]. The sample pad should reduce any chemical variability in the sample so that if a signal is produced, is it proportional to the analyte concentration.

Conjugate pad

The conjugate pad holds dried detection particles that are resuspended by the sample flow during run time. It is important that the distribution of particles in the conjugate pad is uniform so that they are released from the glass gibers rapidly and quantitatively[20]. Because the detection particles will always move into areas where the flow is fastest, it is important to select materials with minimal variability in fiber density and distribution to avoid artifacts of non-uniform flow.

Absorbent Pad

The absorbent pad functions as a sink for the processed fluid at the end of the strip. The important consideration for the absorbent pad is the bed volume, as there needs to be sufficient pore volume to accommodate the full volume of the processed sample[20]. When the absorbent pad is full, fluid flow will stop.

After processing, test strips should not be stored with the pads on the strips, as liquid will evaporate from the exposed surfaces on the strip, and the absorbent pad will act as a reservoir and allow backflow onto the nitrocellulose membrane[20].

Configurations and flow

As the sample flows through the pads, it resuspends dried particles and carries them onto the nitrocellulose. The overlap between these materials must be configured properly so that flow is uniform and detector particles are completely transferred onto the membrane. The variation of the positioning and overlap of these components can affect the flow properties of the strip[22].

In strip manufacturing, it is also important to keep all cutting edges sharp and clean. Failing to do this can cause damage to the membrane edges and result in non-uniform flow.

Consideration must also be given to the interaction between the particles present in the flow and the membrane. First, the membrane pores must be large enough to accommodate the flowing particles. Second, the flow rate of the membrane must be sufficient to move the desired particles. As particle diameter increases, resistance to flow increases, and a slower flow rate will result in less force with which to move the particle[20].

LFA CHEMISTRY

Sandwich and competitive Formats

Two common formats for a LFA are sandwich and competitive. In a sandwich assay, the target analyte is sandwiched between the test line and detection conjugate, and the test line intensity is directly proportional to the target concentration[23]. In a competitive assay, the detection conjugate is pre-labeled with the target or target derivative. At run time, the target and reporter conjugate compete for open binding spots on the test line. A positive sample has no test line because the target analyte in the sample binds to the test line before the detection conjugate, and a negative sample results in a test line because the detection conjugate is able to bind to the test line. For the competitive format, signal strength is inversely proportional to target concentration[23].

Detection labels

Detection labels are used to illustrate the final LFA result to the user. AuNPs are considered to be the standard detection label for LFA, but many other labels are also used.

Liposomes

Liposomes are vesicles formed by a lipid bilayer and can contain signal-generating molecules including visual dyes, fluorescent dyes, enzymes, and electroreactive compounds. They range in size from 50-800nm and function as a detection label by being lysed and releasing the contained material[20]. Lipsomes are relatively unstable, and literature on the drying and reconstitution of liposomes is scarce[20], so they are not typically used in LFA.

Colloidal carbon

Colloidal carbon particles have been used in LFA since the 1970s[24,25,26]. Adsorption of protein onto colloidal carbon particles takes several hours[20], making its use more time consuming than other detection labels. Its advantages include its high stability and deep color contrast on the membrane. Currently, the use of colloidal carbon for lateral flow assays requires a licensing agreement from the vendors[20], making the barrier to entry higher than for other detection labels. A recent study comparing the limit of detection for various bioconjugates found a limit of detection of 0.01 ug/mL for colloidal carbon[33].

Colloidal gold

Colloidal gold is likely the most widely used detection label in commercial lateral flow assays[27]. Its popularity is probably due to its ease of use and relative inexpensiveness. It also results in an intense colorimetric result, usually without needing any special development processes. Its use and protocols are widely documented in the literature[20,27,33], and it is very stable in liquid or dried forms[20].

Silver enhancement is sometimes used to enhance the colloidal gold signal by one to two orders of magnitude. This results in the creation of silver shells around the

gold nanoparticles, which enhance the signal visibility[20]. The limit of detection has been found as 0.1 ug/mL for AuNP and 0.01 ug/mL for silver-coated AuNP[33].

Fluorescent particles

Fluorescence occurs when a particle absorbs light at one wavelength and emits it at a longer wavelength, with the interval between being the lifetime of the fluorophore[20]. Many fluorescent labels with various characteristics (excitation and emission wavelengths, stokes shift, lifetime, extinction coefficient, quantum yield, and photostability) are available commercially[28]. Fluorescent labels can generally be detected at very low concentrations and read by an optical instrument. The limit of detection for fluorescent particles has been found to be as low as 0.03 ng/mL[34].

Quantum dots

Quantum dots are another type of fluorescent label that have a comparatively high level of brightness, size-tunable fluorescence emission, narrow spectral line width, large absorption coefficient, and high resistance to photobleaching[29]. Because of these significant advantages, quantum dots are widely thought to have great potential for LFA and are being widely studied in the literature[20,29,30,31].

Upconverting phosphors

Certain substances produce a luminescent signal, called phosphorescence, after absorbing radiant or other types of energy. This is different from fluorescence because the signal persists even after the energy causing it is no longer present[20]. These particles are referred to as “upconverting” because they absorb two or more photons of infrared light and emit light at a shorter wavelength. For these labels, the background

signal is low and reaction conditions such as temperature or buffer do not have an effect[20].

Bioluminescent markers

Bioluminescent markers produce a signal as a result of a chemical reaction. An example of this is aequorin, a protein extracted from jellyfish, which has been used in LFA[32]. Aequorin is activated by divalent calcium ions and emits a blue light (about 470nm) and carbon dioxide. In the LFA created by Liotta et al., a photodetector was used to capture this light signal[32].

Paramagnetic particles

Paramagnetic particles are colloidal particles of iron oxide that become magnetic only when placed in a magnetic field[20]. When coated with polymers, they can be linked to antibodies or antigens and used in LFA similarly to other colloidal particles. The signal can be quantified by measuring the magnetic flux generated when placed in a magnetic field.

Latex particles

Latex beads can be conjugated to proteins in multiple well-documented ways including adsorption and covalent coupling through amino, carboxyl, or thiol groups. They frequently incorporate color or fluorescent dyes and paramagnetic media. The limit of detection for latex beads is comparatively high and has been found as 1 mg/mL[33].

CHAPTER 4

FLUORESCENCE-BASED LATERAL FLOW ASSAY FOR THE DETECTION OF DENGUE

Research Design and Rationale

Application of LFA to disease diagnostics

Detection label selection

Assay principle

Test Strip Preparation

Materials

Dengue-fluorescent particle conjugation

Test and control lines

Assembly

Testing

Further work

RESEARCH DESIGN AND RATIONALE

Application of LFA to disease diagnostics

For dengue, early case detection and treatment can reduce morality to less than 1%[1]. In many dengue endemic settings, laboratory diagnostic resources are limited, and RDTs that incorporate LFA technology provide an opportunity for inexpensive point-of-care diagnosis.

Detection label selection

In disease diagnostics, the ability to diagnose a patient in the first days of infection is a vital aspect of an effective RDT. It is therefore important to consider various detection labels and determine which allow for the most robust detection. Originally, latex beads were chosen for their ease of multiplexing, as shown in Figure 6. However, after further investigation, it was found that the limit of detection for latex beads is too high for the application of early disease diagnosis[33]. Fluorescent labeling was eventually chosen for its low limit of detection, ability for quantification, and multiplexing capability.

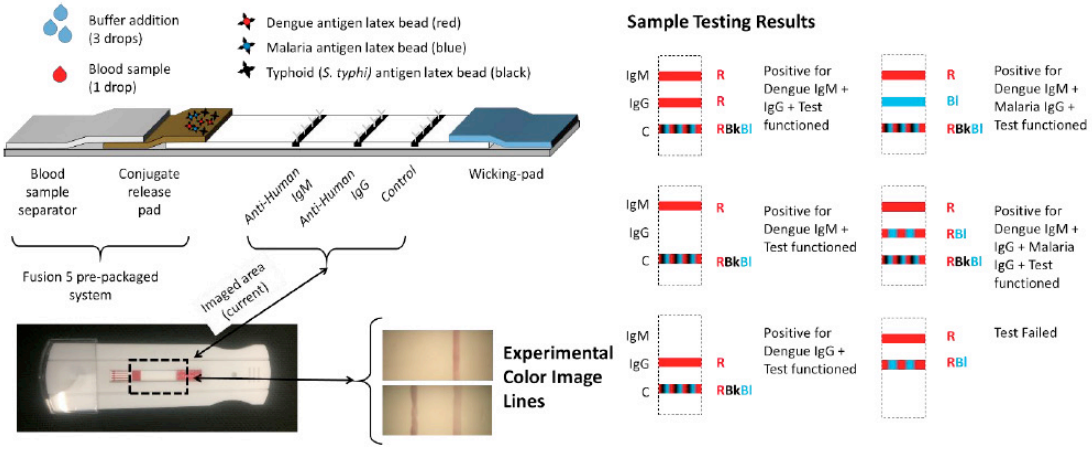

Figure 6: (Left) Multiplex assay for dengue, malaria, and typhoid and current imaging sample. (Right) Sample test outcomes showing color-coding to enable pathogen specific discrimination.

Fluorescent particles are more sensitive and used less often in LFA than colloidal gold and colored latex, while offering a significantly lower limit of detection[59]. Fluorophores can also detect wider dynamic ranges in analyte concentration and signal level[34], giving them a substantial advantage for detecting low levels in disease diagnostics[60]. However, the sophisticated hardware and software necessary to read the fluorescent signal can lead to higher expenses, and fluorescent particle conjugation chemistry is more complicated than other commonly-used particles.

Assay principle

The test strip in development will detect dengue IgG and IgM in whole blood and serum. Pairing the strips with an optical reader device, as discussed later, will allow quantification of the IgG and IgM concentrations. To decrease the limit of detection and allow multiplexing in the future, fluorescent particles will be used as the detection label. The test strip design is shown in Figure 7.

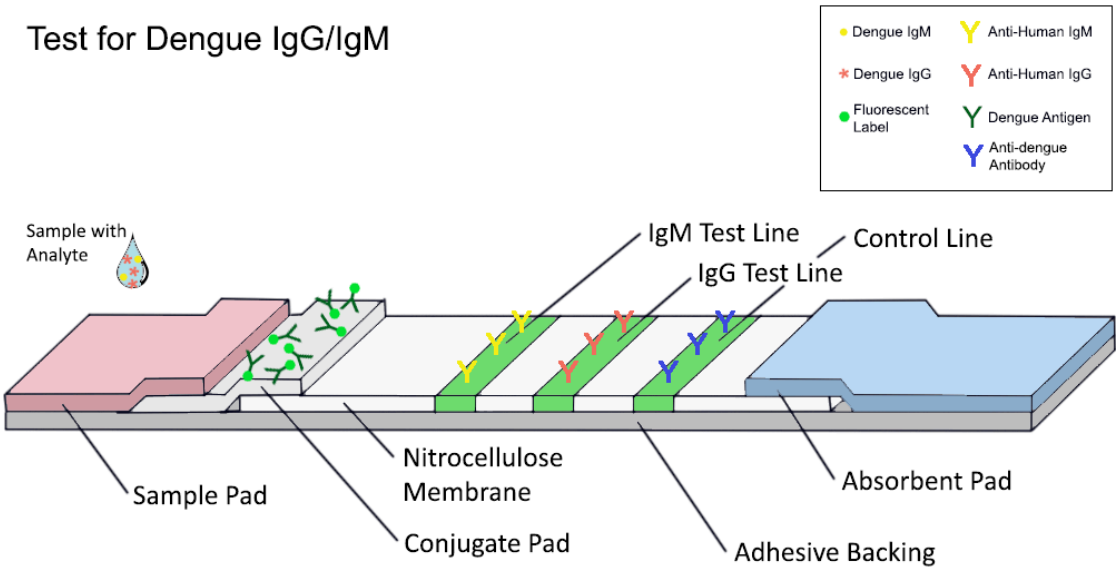

Figure 7: LFA design for dengue IgG and IgM

The LFA shown contains a sample pad, conjugate pad, absorbent pad, and nitrocellulose membrane on adhesive backing. The conjugate pad contains dengue antigen conjugated to fluorescent labels. The test and control lines are immobilized on the nitrocellulose membrane. The test lines are made up of anti-human IgG and anti-human IgM antibodies, and the control line consists of anti-dengue antibodies.

A positive sample will contain dengue IgG, dengue IgM, or both. Figure 7 shows a positive sample containing dengue IgG and dengue IgM. When flow begins, the dengue IgG and IgM in the sample will bind to the dengue antigen contained in the conjugate pad and continue to flow. At the test lines, the now-labeled dengue IgG and IgM will bind to the anti-human IgG and anti-human IgM, respectively. Any unbound antigen-fluorescent particle conjugates will then bind to the control line, and anything that remains will be captured on the absorbent pad. The LFA at the completion of the flow described is pictured in Figure 8.

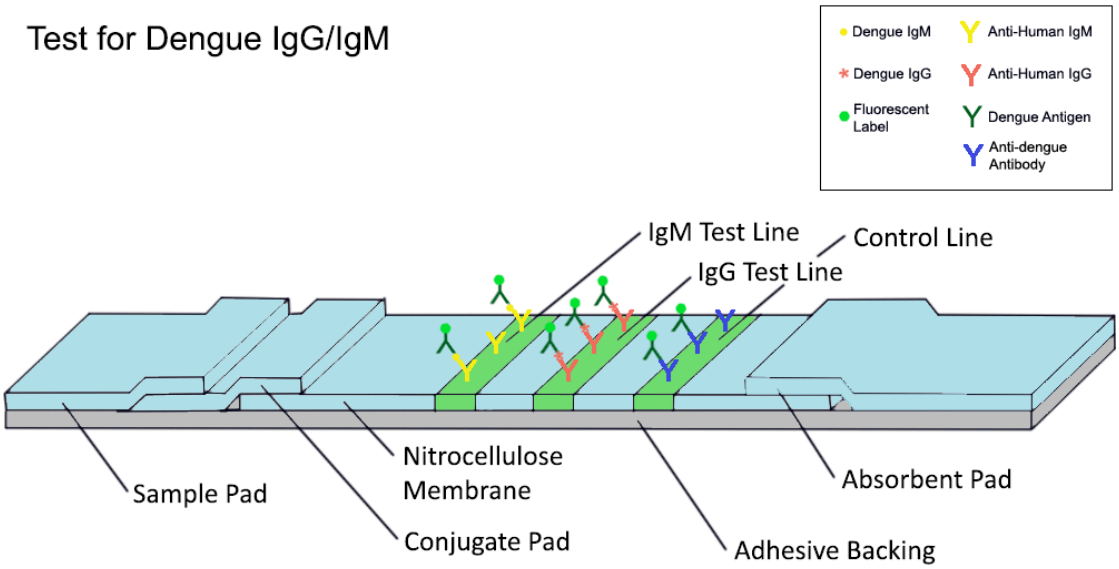

Figure 8: Completed positive test for dengue IgG and IgM

If a negative sample is applied, there will be no dengue IgG or IgM to bind to the test lines, and binding will only occur between the flowing fluorescent-labeled dengue antigen and immobilized anti-dengue antibody at the control line.

TEST STRIP PREPARATION

As discussed in Chapter 2, each LFA component shown in Figure 7 must be carefully chosen and prepared in order to make a robust, highly-sensitive LFA.

Materials

Hi-Flow Plus 180 membrane cards from EMD Millipore were used as the strip backing and nitrocellulose membrane. For the conjugates, recombinant dengue antigen was purchased from Ray Biotech, and Alexa Fluor 488 particles were purchased from Thermo Fisher Scientific. For the control line, anti-dengue antibody was purchased from Abcam. Anti-human IgG was purchased from Thermo Fisher Scientific.

Dengue-fluorescent particle conjugation

For conjugation to the dengue antigen, Alexa Fluor 488 was chosen. It is a bright green fluorescent dye that has a high fluorescence quantum yield and high photostability, allowing for detection of low signals with high sensitivity[61]. The recombinant polyvalent dengue antigen contains all 4 dengue antigen subtypes, with 25% from each subtype. This composition means that the antigen is capable of binding to all dengue subtypes, which is necessary for a highly sensitive LFA. The dengue recombinant antigen is a 22kDa protein produced in E. Coli and fused to a 6xHis tag. The dengue antigen was supplied at a concentration of 1mg/mL, purified by proprietary chromatographic technique, and in PBS, pH-7.4[62].

The conjugation of the dengue antigen to the fluorophores was performed according to the procedure supplied by Thermo Fisher[63]. During the conjugation, fluorophores were bound to primary amines on lysines present throughout the dengue antigen. One of the most important aspects of this conjugation is making sure that enough binding sites are available after the conjugation for use in the LFA flow.

Absorbance (A) can be used to determine the protein concentration (c) with the Beer-Lambert equation:

$$A = \varepsilon cL$$

where L is the cuvette length and ε is the molar extinction coefficient. According to the conjugation procedure[63], absorbance measurements at 280nm and 494nm were used to determine the protein concentration, which was then used to determine a degree of labeling (DOL) for the conjugates. The optimal DOL for ~20kDa protein is 1-2, and the resulting dengue-fluorophore conjugates had a DOL of 0.649. This implies that the dengue antigen was underlabeled, which is a promising result, as sufficient binding sites remain for use in the LFA.

Because the resulting conjugates were light sensitive, it was important to store them in aluminum foil to protect them from ambient light whenever possible.

Test and control lines

For the control line, monoclonal and polyclonal anti-dengue were tested. The monoclonal anti-dengue was produced in mouse and reacts with all 4 dengue subtypes. It was supplied at a concentration of 1mg/mL, Protein A purified, and in a solution of PBS and 0.05% sodium azide, pH-7.2[64]. The polyclonal anti-dengue was produced in rabbit and reacts with all 4 dengue subtypes. It was supplied as whole antiserum[65]. The polyclonal anti-dengue was the focus of most experimentation, as polyclonal antibodies generally work better as a control line than monoclonal antibodies.

Because the polyclonal anti-dengue is supplied as antiserum, purification is necessary to make a highly sensitive LFA. Antiserum contains various antibodies and likely contains less than 10% of the target antibody[66]. This means that nonspecific binding can occur. Of the commonly-used purification proteins, Protein A has the highest binding capacity for subclasses of IgG from rabbits[67], so Protein A purification was performed on the polyclonal anti-dengue. The purification procedure[67] resulted in 3 elution fractions of purified anti-dengue. The Beer-Lambert equation was then used to relate the absorbance of the samples at 280nm to protein concentrations. The resulting concentrations were 0.78mg/mL, 0.99mg/mL, and 1.7mg/mL.

The test lines will consist of anti-human IgG and anti-human IgM, but this stage of development has not yet occurred.

Assembly

The dengue-fluorophore conjugates were diluted to 1 and 2 OD with conjugate buffer before being pipetted onto a glass fiber pad. Conjugate pads were incubated at 120°C until dry. During this process and afterwards, it was important to protect the fluorescent particles from light whenever possible

Before being applied to the test strip, the anti-dengue was diluted in PBS, resulting in an excessively low concentration. Control lines were created by pipetting the diluted anti-dengue onto the nitrocellulose membrane. The membranes were the incubated at 120°C until dry.

Test strips were prepared by layering components as shown in Figure 7. The glass fiber conjugate pad was placed at the base of the nitrocellulose membrane with a bit of overlap to allow continuous, even flow. The sample pad was then placed at the very beginning of the strip, overlapping the conjugate pad. Similarly, an absorbent pad

was placed at the very end of the strip, overlapping the end of the nitrocellulose membrane.

Testing

For full testing of the LFA, the necessary fluorescence capabilities must be fully integrated into the optical reader device discussed in Chapter 7. For initial testing, SEM with a FITC filter was used to image the strips. A control line was not seen during these tests, likely because the concentration of the anti-dengue control line was too low for a readable signal to be generated.

Because the nitrocellulose membrane has background fluorescence, it was important to determine what concentration of fluorescent label could be detected against the paper. For this reason, various dilutions of Alexa Fluor 488 were created and imaged with the SEM filter setup. As shown in Figure 9, a clear boundary with the nitrocellulose can be seen even when the fluorescent particles are diluted 100 times.

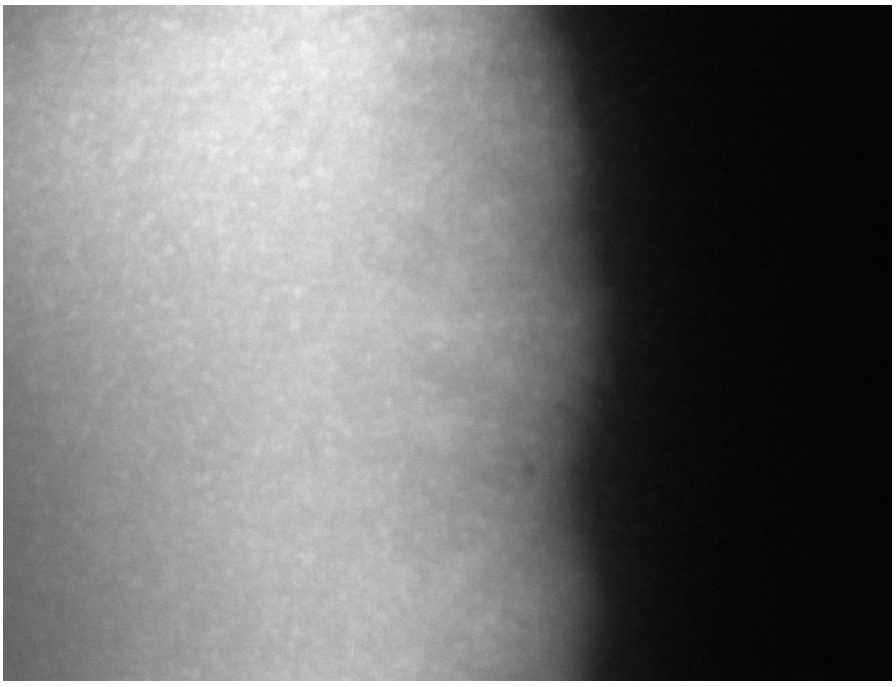

Figure 9: A clear boundary can be seen between the 100x diluted fluorophores (left) and the blank nitrocellulose membrane (right).

FURTHER WORK

A few things still need to be done to complete the development of a fluorescent LFA for dengue IgG and IgM detection. First, another batch of strips should be created and tested by directly using the elution fractions of purified anti-dengue as control lines without dilution. This could potentially yield control line signals, as 0.78mg/mL, 0.99mg/mL, and 1.78mg/mL concentrations are much more reasonable control line concentrations than what has previously been tested.

Further optimization can be accomplished by optimizing the degree of labeling of the dengue-fluorophore conjugates. It might be possible that a higher of lower degree of labeling will result in a better signal, and the conjugation procedure can be refined accordingly to produce these results.

If the conjugation continues to be suboptimal for the intended application, another option could be to bind to a different amino acid, such as cysteine. The current conjugation procedure attempts to bind to lysines, which are present throughout the dengue antigen. Cysteine has a smaller observed frequency in proteins (3.3% versus 7.2%)[68] and could leave more binding sites open during LFA run time.

CHAPTER 5

OVERVIEW OF DESIGN AND USABILITY

Introduction

Design Thinking

Design thinking process

Characteristics of successful design thinking

Design and Usability

Designing for usability

Human factors and emotion

INTRODUCTION

The design of a new product or experience is integral to its success. Even the most brilliant ideas are prone to failure as a product if they are badly designed. To prevent this, it is important to implement a cycle of design thinking and take consumer usability into consideration.

DESIGN THINKING

Design thinking is a methodology that is based on a thorough understanding of what people want and need and what they like or dislike about the way a product is made, packaged, marketed, sold, and supported[35].

Historically, design has been an afterthought. Designers have come along late in the development process to make the product aesthetically pleasing. Now, increasing numbers of organizations are realizing the importance of involving designers early in the development process to incorporate the wants and needs of the end consumers. Unlike the previous role of design, its new involvement in the development process is strategic and results in dramatic added value[35].

Design thinking process[36]

The steps of the design thinking process are shown in Figure 10. These steps are used as a repeated cycle so that designers can continue to improve on their original ideas. “Empathize” means working to fully understand the experience of the user through observation, interaction, and being immersed in their experiences. “Define” involves compiling the previous findings to form a user point of view that will be addressed with the new design. “Ideate” means to use divergent thinking to come up with as many possible solutions, realistic and far-fetched, that could solve the previously-stated problem. “Prototype” involves transforming the best idea(s) into

physical form so that they can be physically interacted with. Prototypes do not need to be expensive or complicated, as they are only needed to gain useful feedback to evolve the original idea. “Test” means using the prototypes with real users to make observations, get feedback, and refine original ideas. Through repetition, a robustly designed final product can be created.

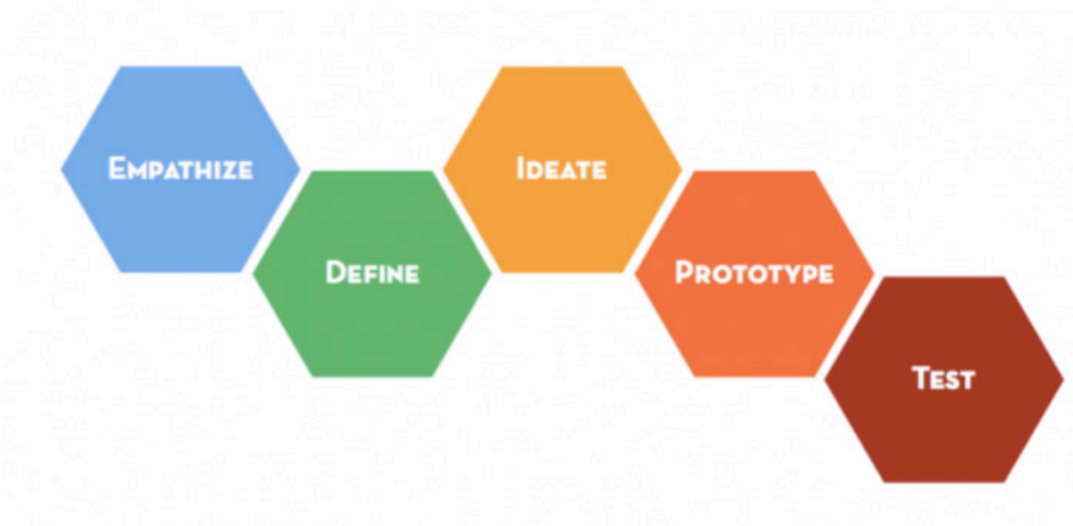

Figure 10: The steps of the design thinking process[36]. The linear representation of the steps is misleading; design thinking is actually a cycle that involves continually revisiting these steps so that a design is always improving.

Characteristics of successful design thinking

Success in design thinking requires a specific way of thinking about synthesizing solutions to problems, which can be adopted by thinkers of every profession. Possibly the most important aspect of design thinking is empathy. This is the ability to thoroughly understand multiple perspectives and take a “people first” approach[36]. Through careful observation and empathy, design thinkers are able to realize new problems and solutions that would otherwise go unnoticed. Another characteristic is integrative thinking, where the designer embodies an ability to see all aspects of a problem and generate new solutions that improve upon existing alternatives[37]. A design thinker is also optimistic and understands that no matter how

challenging the constraints may be, at least one solution must exist that is superior to the existing alternatives. Another aspect of successful design thinking is experimentalism, where the designer explores entirely new directions to solve a problem and goes outside the realm of small tweaks to current solutions. The last important characteristic is collaboration. The most useful design thinking occurs when people with completely different backgrounds and ways of thinking work together to look at a problem in distinctive ways and synthesize novel solutions.

DESIGN AND USABILITY

"In analytical fields that pride themselves on scientific basis and experimental rigor, the hidden danger is to neglect areas that are not easily addressed in the framework of science and engineering"[47]. Engineers and other technical workers who make products and experiences for users often forget that they are not typical users. They have a unique extensive knowledge of all facets of the product that the final consumer will likely not have. This makes understanding the design process and testing the product's usability integral pieces of fabricating a successful product, as the designer needs to thoroughly understand how the customer will actually respond to it.

Designing for usability

The International Standard Organization defines usability as "…the effectiveness, efficiency and satisfaction with which specified users can achieve specified goals in particular environments"[38].

The process of designing for usability involves an early focus on the users and the task to be accomplished, where the designer needs to understand exactly who the users will be and the nature of the work. A sample of these intended users should use the prototype and be analyzed, where the designer pays special attention to aspects of

the design that the user struggles with or does not like. Specific questions are often asked of the users to rate the product's overall usability. Many examples of such usability surveys exist in the literature[40,41,42,43,44,45]. Any problems found during user testing should be fixed, and new iterations of the design should be continually tested for usability[39].

Human factors and emotion

Understanding the interaction between humans and the system is essential to the usability and overall success of a product.

An interesting study on human factors in product use asked respondents to give feedback on extremely good and bad products that they owned[46]. It was found that specific product attributes are associated with pleasure and displeasure. The product aesthetics, including both style and color, were found to contribute strongly to pleasure. Alternatively, some people found a product to cause displeasure for no reason other than it was "ugly". The product's features need to be appropriate and function efficiently, and any unnecessary features or missing features cause displeasure. In terms of usability, a pleasurable product must be easy to understand, have a helpful layout, and not be too complex. The size of the product should enhance its performance or suit its use context. If a product was too expensive, users expressed negative feelings. However, low cost was never mentioned as a contributing factor for a pleasurable product.

From these findings, it can be seen that various aspects of a product provoke emotional responses from users. In fact, most users mentioned more than one emotion in connection with each product that they discussed[46]. The connection between design and emotion must be understood to facilitate creation of a successful product.

It is fundamental for designers to understand that “usable designs are not necessarily pleasurable ones”[47]. The affective system is very judgmental, and as soon as a user sees a product, they will rapidly begin to assign positive and negative valence[47]. When a user assigns positive valence to the appearance or functionality of a product, they will be more tolerant of minor difficulties and problems. In other words, the experience of using the product will seem to go more smoothly if the design is pleasing to the user, and “attractive things work better”[47].

CHAPTER 6

LATERAL FLOW ASSAY READER DEVICES

Background and Related Technologies

The NutriPhone System

Operation Principles

Customer Needs

Problems with Current Solutions

BACKGROUND AND RELATED TECHNOLOGIES

As previously discussed, LFA technology allows for medical diagnostic tests that are informative and easy to use without the need for expensive laboratory equipment and trained technicians. In the presence of the analyte of interested, the LFA produces a detectable signal that is quantifiable through paired use with a reader instrument that can display the result to the user[48]. Traditionally, quantification of a LFA signal has been performed in a clinic or research laboratory using benchtop research-grade instruments, such as a microwell plate reader[49,50,51]. Such instruments offer very high performance, but the associated high costs and large sizes make these instruments infeasible for many potential applications.

For diagnostic purposes such as point-of-care, personal, and resource-limited settings, a low-cost reader system for LFA quantification is needed. Several research groups and companies have investigated smartphone-based reader systems which take advantage of the universal familiarity and ubiquity of smartphone technology[15,52,53].

To improve LFA sensitivity, some companies have moved from the classically-used colloidal gold label to paramagnetic particles and fluorescent dyes. Because these particles cannot be seen by the naked eye, the need for reader devices has been further established.

THE NUTRIPHONE SYSTEM

The NutriPhone system, shown in Figure 11, is a smartphone-based system for at-home blood work and nutrition monitoring, and it was developed and is currently used by my research group. NutriPhone consists of a small, plastic reader accessory that clips over a smartphone camera, a custom LFA in a plastic cassette, and a smartphone app that guides the user through the testing process to get a result.

To use the device, the NutriPhone app is started on an iPhone or iPad and step-by-step instructions are shown for whichever analyte is being tested. This involves a finger prick and collection of a single blood droplet that is placed on the supplied test strip. The software then takes a picture of the result region of the test strip using the smartphone camera and processes it to display a result. This process takes about 10-15 minutes.

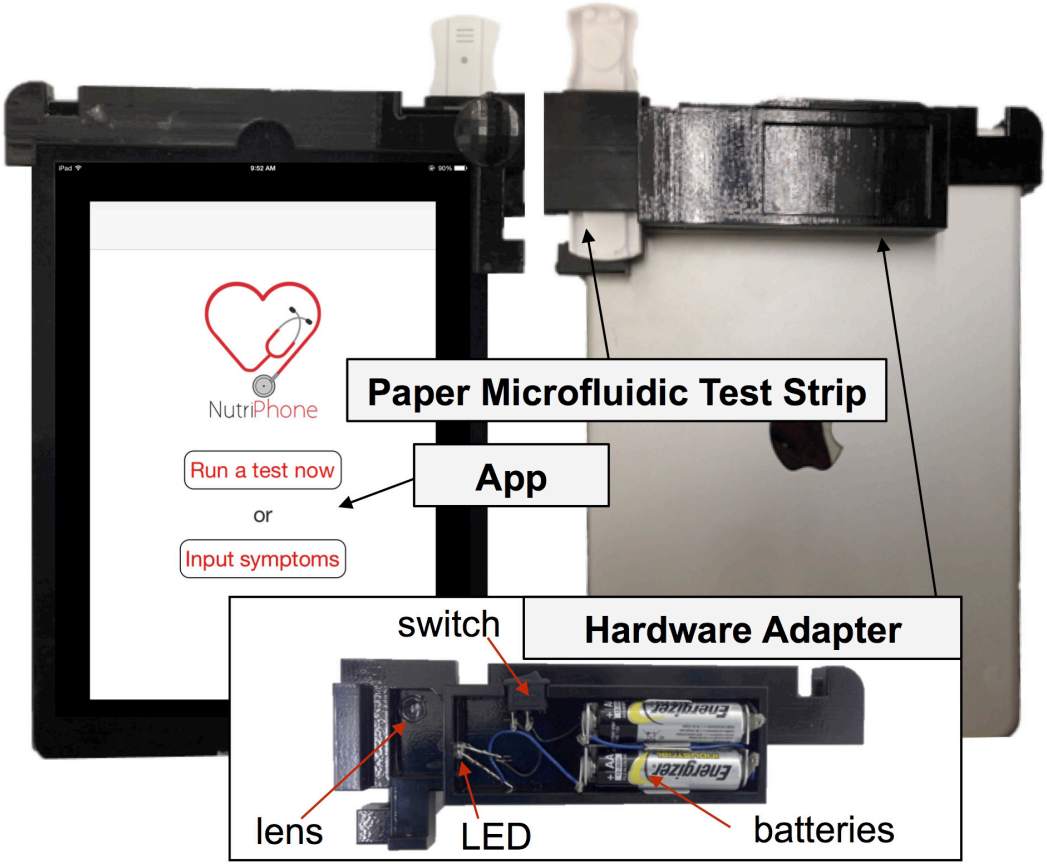

Figure 11: The NutriPhone system consists of a plastic accessory that clips around the iPhone/iPad camera, a disposable LFA, and software to process images and display results.

OPERATION PRINCIPLES

Current LFA reader devices operate on the same basic principle to read the most commonly-used gold nanoparticle labels. The test strip is placed under or over a light source, and a detector receives the light. Absorption and reflection of light are proportional to the AuNP density on the test and control lines, allowing for quantification of the results. In reading fluorescent particles, a sensor excites the fluorescently labeled control and test lines and records the increases in fluorescent signals. A typical example of results from a colorimetric LFA using 40nm AuNP[20] is shown in Figure 12. This graph shows a good correlation between signal intensity and THC concentration.

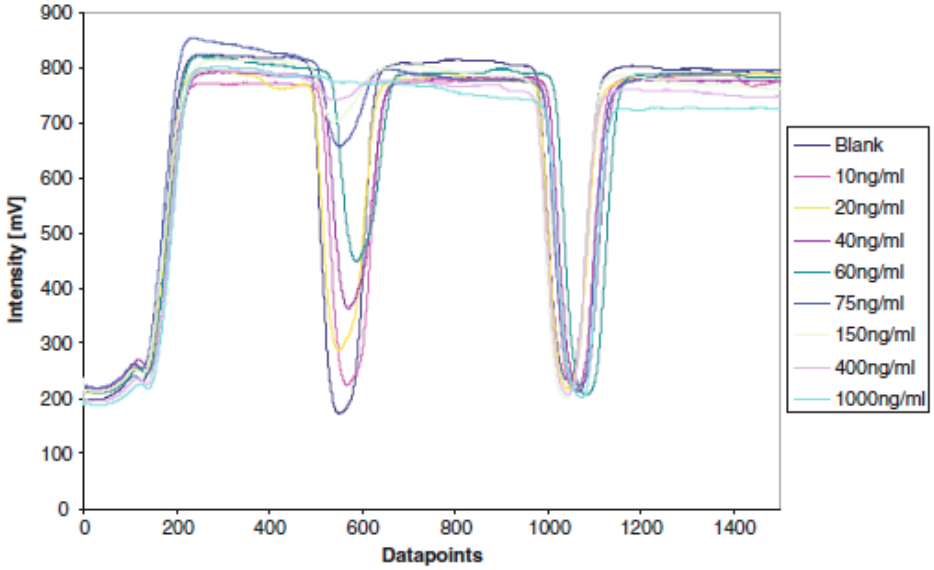

Figure 12: Raw data of LFA with AuNP and different concentrations of THC. Test and control bands, respectively, are visible. At the far left is interference from the cassette housing[20].

CUSTOMER NEEDS

Customers and LFA users repeatedly report the importance of certain features that should be included in a LFA reader, besides accurate performance. Examples of the most common requests[20] are shown in Table 1.

Table 1: Most common customer-requested features for a LFA reader device[20]

Customer request
Ease and convenience of operation
Quantitative read out
Automatic electronic documentation of results
Higher sensitivity
Objective interpretation of results
Use of reader in QC for test strip manufacturing
Handheld reader format or mobility
Operational robustness
Physical robustness
Audio/visual display of results
Connectivity to PC, printer, barcode reader, or other data management system
Hard copy of test results
Compatibility to clinical workflow and systems
Standalone reader without use of computer
Batch and calibration data management system
Low price
Appealing design
Self-test/self-calibration of reader
Fast read out
Wireless data transfer
Compatibility to customer's unique cassette format
Compatibility to customer's unique label
Availability of professional software
Compatibility to different tests and test formats
Multiplexing
Full customization

PROBLEMS WITH CURRENT SOLUTIONS

Current LFA reader devices do not fulfill all of the features shown in Table 1, especially those regarding simple operation, mobility, speed, and low cost[20].

Besides unmet customer needs, these devices also have problems providing accurate quantitative results, as various factors need to be overcome to do this successfully. One of these is positioning error, an example being when the test and control bands can vary in their placement location on the LFA. Another positioning error involves the cutting of the membranes, which can lead to variability in strip width. The strips can also be placed in slightly different locations in the plastic cassettes. Positioning errors can be detrimental to the accuracy of the test, as small differences can cause a large error in the result. For example, a 4% shift in the position of the control line can result in a 50% signal decrease[20].

Difficulties also arise in trying to achieve uniform illumination of the strip. Even with good background subtraction, accurate quantitative results cannot be achieved when insufficient or dissimilar lighting results in a dark image or shadows. It is difficult to find a balance between closeness to the strip and sufficient distance to provide uniform lighting.

There are also inherent issues specific to the NutriPhone system. First, the system is only compatible with iOS devices (iPad and iPhone). In 2014, Android was over twice as pervasive as iOS, with 1.9 billion Android devices in use versus 682 million iOS devices[54]. This results in a limited market for NutriPhone among worldwide users of smart devices, especially in resource-limited settings. The NutriPhone accessory also will not fit over a case or cover that may be on the smart device, and a different accessory is needed to fit the various physical constraints of each iPhone/iPad version. Custom accessories are also needed for each plastic cassette shape. Finally, there is also an issue of contamination because the user is required to

place bodily fluids in close proximity to a smart device. There is a need for a new device that meets all customer requests for such as device (Table 1) and overcomes the issues with the current NutriPhone accessory.

CHAPTER 7

THE “TIDBIT”: DESIGN OF AN OPTICAL READER DEVICE FOR LATERAL FLOW ASSAYS

Introduction

Design Process

Problem definition

Specifications

Ideation

A tray to hold all strips

Design of the new reader device

Prototypes

Testing

Current Design

Hardware

Reader case and pullout tray

Optical components

Electronics

Testing procedure

Quality control

Usability Study

Procedure

System usability scale

Use and scoring

Participants and results

In Progress Design

Future Improvements and Alternate Versions

Modifications for future designs

Small form factor design

Alternate control devices and functionality

Conclusion

INTRODUCTION

In order to address the shortcomings of currently available LFA reader devices and the problems with the NutriPhone accessory, the Tidbit, a new optical LFA reader system, has been created. The Tidbit is a standalone device that is wirelessly controlled by any internet-enabled device. Unlike offline reader systems, internet-connected smart devices allow for easy software updates and rapid deployment. The incorporation of a familiar mobile platform results in a system that is easy to use without training and allows greater processing power and network connectivity.

In addition to the benefits of using mobile technology, the standalone device has a dedicated camera separate from the smartphone camera and is connected wirelessly. The optical properties of a smartphone camera, such as focal depth, white balance, and resolution, can vary significantly between different manufacturers and devices. Through use of a static, external camera, these variations do not affect the performance of the LFA reader. Smartphones and tablets vary extremely in hardware and physical dimensions, with new versions often making old accessories obsolete. The Tidbit is not limited to any physical smart device constraints and can be run from any internet-enabled device that has the necessary app installed. A single device can also be used by multiple users asynchronously, with each user only having access to the data stored locally on their smartphone or tablet.

DESIGN PROCESS

Taking into account the problems with current LFA reader devices, the steps of the design thinking process (Figure 10) were followed in order to synthesize new solutions. This process included defining the problem, making a list of specifications, ideation, creating prototypes, testing, and refining.

Problem definition

The problem is to create a low-cost reader device that can be used with any smart device and test strip cassette to provide accurate quantification of LFA results. The use of the device should require minimal training.

Specifications

Based on problems with current readers, customer-requested features (Table 1), and the problem statement, it was decided that a new device should meet certain characteristics. Paramount among these was simple operation, mobility, and low cost. These aspects are particularly important in resource-limited settings, where there is a lack of trained technicians and infrastructure (i.e. electricity, transportation). It was also important that the new reader be compatible with any smart device and have the ability to read any size test strip cassette and LFA format (i.e. multiplex, different detection labels). To read different detection labels, the incorporation of various filters will eventually be necessary, which was also taken into account during the design process. The creation of a unique and appealing design that provokes positive affect was also addressed.

From this information, certain decisions were made immediately about the format and components of the new device. In order to allow total mobility, it was decided that the device should be battery-powered and wireless. The need for compatibility with any device led to a decision to make a standalone reader that wouldn't physically attach to the smart device being used. To keep cost to a minimum, ideas needed to use a minimal amount of materials and parts. Based on this, the first step was to determine all of the components that would be necessary for the new reader, along with their physical dimensions. The device would need to hold an internal camera and be light tight. The camera would need to be driven by a SBC,

which would need power from a rechargeable battery pack or an AC adapter. For imaging, lighting would also be necessary, requiring a breadboard and LEDs. After acquiring the camera, a testing of various focal depths showed that the optimal height difference between the LFA surface and camera was 0.85”. There would also need to be sufficient room in the reader to house the inserted test strip, so its size was also taken into account. The dimensions of the necessary internal components are shown in Table 2. The specific parts shown were chosen because of their combined low cost and small size.

Table 2: Necessary components for the new reader device

Component	Length [in]	Width [in]	Thickness [in]
Raspberry Pi A+	2.5625	2.25	1
CMOS camera and LEDs	1.4	1.4	0.75
Focal depth	0.85	-	-
Breadboard	3.25	2	0.75
Battery	2.5625	2.1875	1
Large test strip cassette	3.75	1.25	0.25

Based on these dimensions, it was determined that a height of at least 4”, a width of at least 3”, and a depth of at least 5” would be necessary to completely contain all of the components.

Ideation

With the minimum necessary dimensions known based on the desired reader characteristics, various ideas were generated. First, the desire for the reader to be able to hold any size test strip was addressed. The simplicity of use was also considered, along with a goal of making the design as attractive as possible.

A tray to hold all strips

To address the challenge of reading any size LFA cassette, the idea for a simple pullout tray was imagined. The tray would be big enough to hold any plastic cassette, which vary significantly in length and width. The thickness, however, is typically quite uniform for various cassettes and usually doesn't deviate significantly from 0.25". This means that the focal depth will be essentially the same regardless of cassette size, which is important for the image quality and signal quantification.

Several tray designs were examined, all of which included a rounded handle for easy pulling in and out of the reader, as shown in Figure 13. The design of the inner bed of the tray was similar for all ideas, as shown in Figure 14. All LFA cassettes include a rounded cutout to allow sample placement, so a similar rounded cutout was placed on the far side of the pullout tray to give the user a clue about which way the test cassette should be inserted.

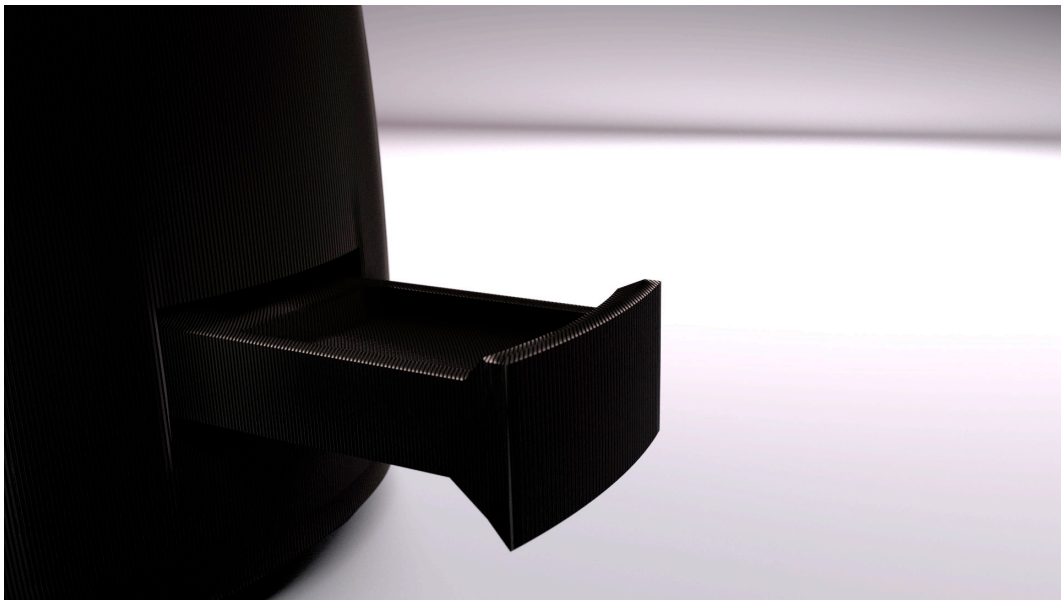

Figure 13: Rendering of pullout tray with rounded handle.

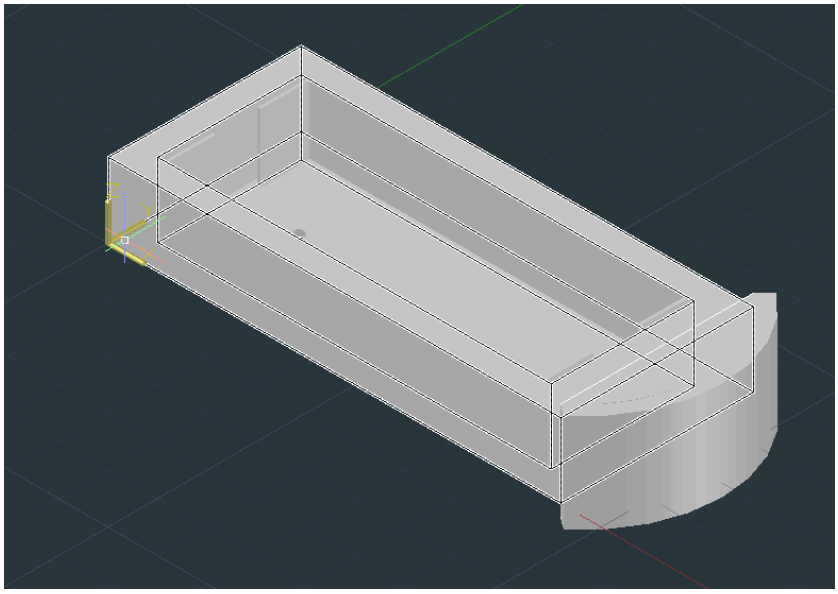

Figure 14: One tray design showing basic shape of inner bed where test cassettes are held.

The first design featured small compression springs attached to the inner side, allowing various test cassette sizes to fit. Another design used metal clips at the inner end to hold various-sized cassettes in place.

Design of the new reader device

To make the reader as simple as possible for users, it was decided that the internal components should be completely enclosed inside the reader and inaccessible to the final consumer. The user's only task should be what they set out to do: place a test strip into a reader for signal quantification. Indicator LEDs on the outside of the device were used to let the user know that the device was functioning correctly. Based on these criteria and the necessary dimensions, various designs were chosen. The first of these was an hourglass shape, as shown in Figure 15.

Figure 15: Rendering of hourglass-shaped reader with pullout tray and indicator LEDs.

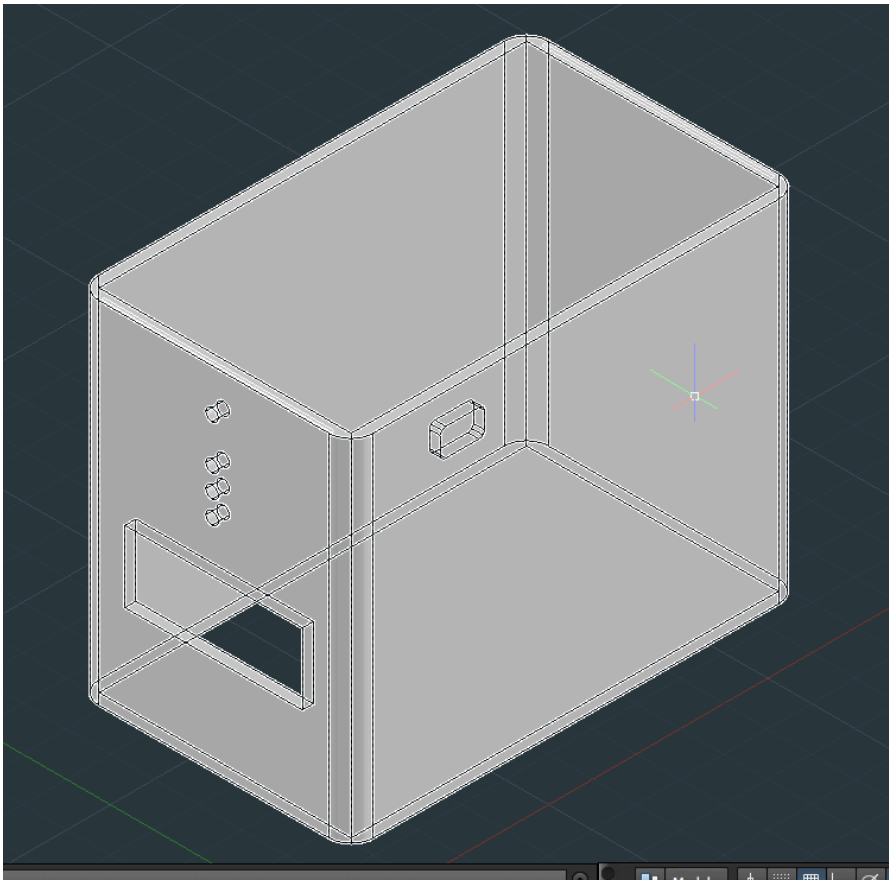

Figure 16: CAD of rectangular prism reader design with holes for pullout tray, indicator LEDs, and power cord.

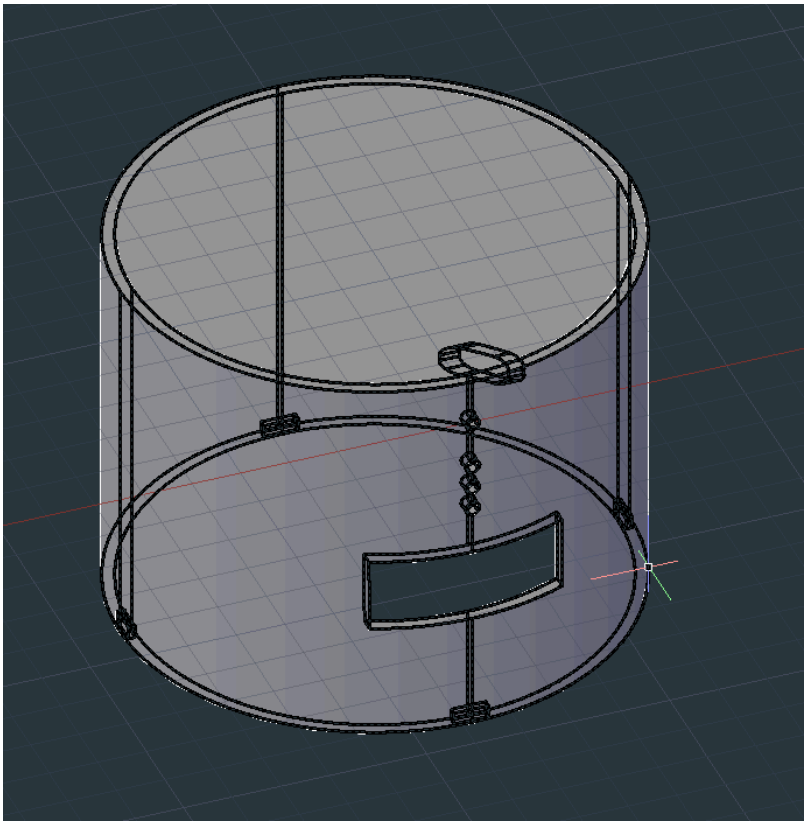

Figure 17: CAD of cylinder reader design with holes for pullout tray, indicator LEDs, and power cord.

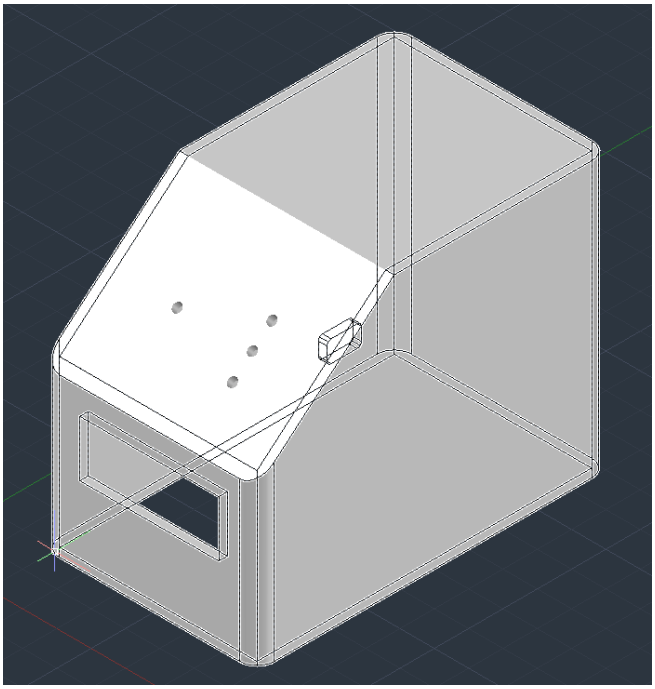

Figure 18: CAD of rectangular prism with slanted front face design with holes for pullout tray, indicator LEDs, and power cord.

Although an attractive design, the hourglass reader idea was quickly abandoned upon realization of its impracticality with regards to the necessary internal components. The camera, illumination LEDs, and any necessary filters would need to be placed at the center of the hourglass height, while the test strip cassette, SBC, and battery would be placed at the bottom. This results in an extremely inefficient use of space, with the top half of the hourglass being completely empty.

Other design ideas included a simple rectangular prism, a cylinder, and a rectangular prism with a slanted front face, as shown in Figures 16, 17, and 18, respectively. All of these designs are about 4" tall. The rectangular prism was the most simple, and the shape was not very unique. The rectangular prism with a slanted face also had a simplistic shape but was more visually interesting. Because it was asymmetrical, this design eliminated the risk of users attempting to place the device upside-down. Both of these designs had a footprint of 3" x 5". The cylinder had the largest footprint of the designs, with at 5" diameter. It was also judged to be the most attractive, as feedback about it was most promising compared to the other devices, and similar shapes have been used in recent tech designs including Amazon Echo, Mac Pro, and Google OnHub[55,56,57].

Prototypes

3D printed prototypes of the tray and reader designs were printed in white ABS plastic at the Cornell Rapid Prototyping Lab and spray painted black. Compression springs and metal to assemble the trays were purchased from McMaster-Carr and Amazon, respectively. A hole for an AC adapter was incorporated into the prototypes so that battery power would not be wasted during initial assembly and testing of the readers, and so that the battery could be charged for real use cases.

Testing

Upon testing the tray designs, it became clear that when cassettes were placed into the tray, they were very difficult to get back out. After discovering this problem, a new design was created with sloped inner edges that would allow different cassette sizes to fit while also allowing easy insertion and removal. This design is shown in Figure 19.

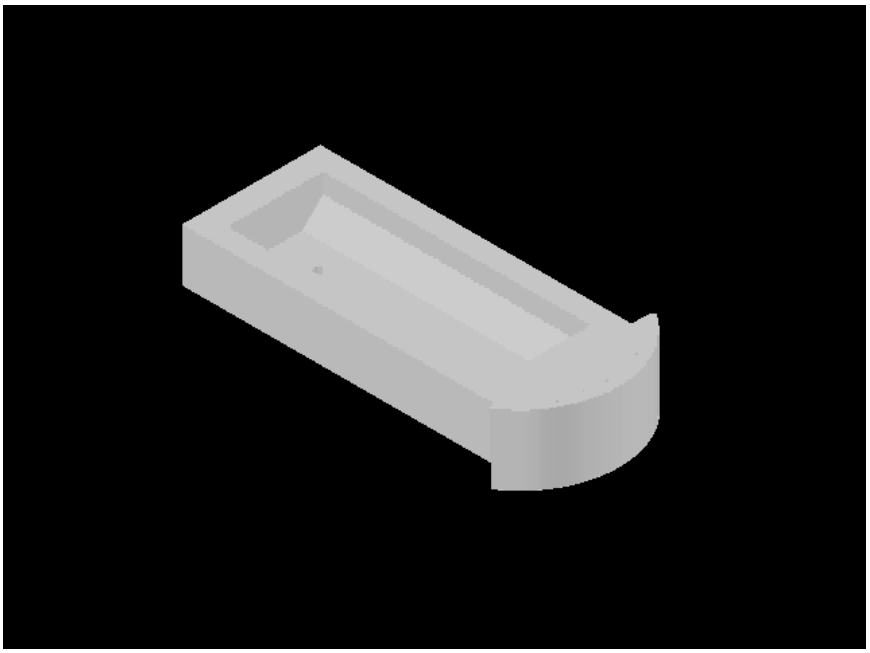

Figure 19: Pullout tray with sloped sides to allow different sized cassettes to be held securely in the center for imaging.

Based on user feedback, it quickly became apparent that the cylinder design was preferred to the rectangular prisms because of its unique attractive shape. This popular opinion motivated design refinements, including the placement of internal components, and future usability testing.

CURRENT DESIGN

The Tidbit is designed to allow repeatable image analysis of any size LFA strip using a smart device app as a wireless controller for running the test and receiving and storing results. The current design is shown in Figure 20.

Figure 20: Current Tidbit device design pictured with iPhone for size reference.

The reader is made of opaque, light-blocking plastic. Its compact size and rechargeable battery make it portable and easy to use, especially in remote or resource-limited settings. It is controlled via Wi-Fi or Bluetooth connectivity with a smart device (e.g. phone, tablet, computer) that is running the corresponding app. Because only Wi-Fi or Bluetooth is required, a wide range of potential devices can be used in conjunction with the Tidbit. Through the app on their device, the user is given detailed instructions on how to perform the desired test, and commands are sent from the device to the Tidbit. The reader then takes an image of the completed LFA and sends it back to the smart device for analysis and interpretation.

Hardware

The Tidbit is designed to perform repeatable imaging of biomedical test strips in a simple, user-friendly way.

Reader case and pullout tray

To allow repeatability, the case is opaque plastic, which isolates the internal components and LFA from variable external light. This allows the device to be used in any lighting conditions, from bright sunlight to total darkness, without any loss of image quality. Individual test strips can be placed into the pullout tray and inserted into the reader.

The tray (Figure 19) is designed so that hardware modifications are not necessary to accommodate various test cassette designs from diverse manufacturers. The edges of the tray are sloped so that different size cassettes can be held securely in the center for repeatable imaging.

Optical components

To obtain high quality LFA images, a 5-megapixel 1920x1080 CMOS camera is used. A custom plastic camera holder, shown in Figure 21, was designed that also incorporates a lens and a ring of white LEDs for illumination. The ring of LEDs surrounding the camera mimics a ring flash, like that used in macro photography. This lighting setup provides uniform illumination of the test strip without creating shadows. Because the CMOS camera is fixed-focus, a lens is necessary to minimize the optical path length needed for a sharp image and reduce the overall device size.

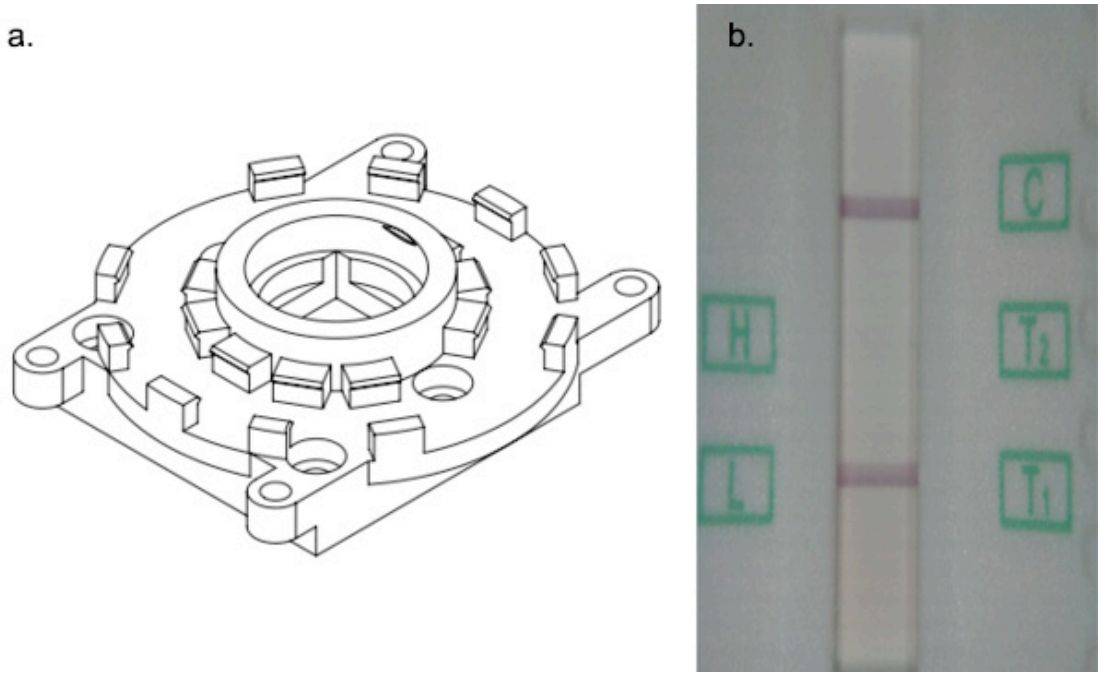

Figure 21: (a) A custom plastic enclosure holds the camera, lens, and LED ring. (b) A representative image of a LFA using this optical configuration shows consistent, flat illumination.

Electronics

The components of the Tidbit are powered and controlled by a Raspberry Pi A+, a low-cost Linux-based SBC. The SBC is powered by a rechargeable battery or AC adapter. Besides the camera and LED ring, colored indicator lights on the front of the device are also powered by the SBC. These lights indicate that the reader is powered on and continue to light in succession as the test progresses. To minimize the size and complexity of the device, the reader does not have its own display and instead generates a local wireless network to communicate with the user's own internet-connected device.

To supply the appropriate current and voltage to power the various LEDs, a custom circuit was designed, as shown in Figure 22. An image of the rectangular prism prototype running from the battery power supply is shown in Figure 23. The LED ring is only switched on when an image is being taken, as shown in Figure 23(b).

With the LEDs off, the battery life of the reader is greater than 16 hours, allowing for great portability, especially in resource-limited settings where electricity can be limited.

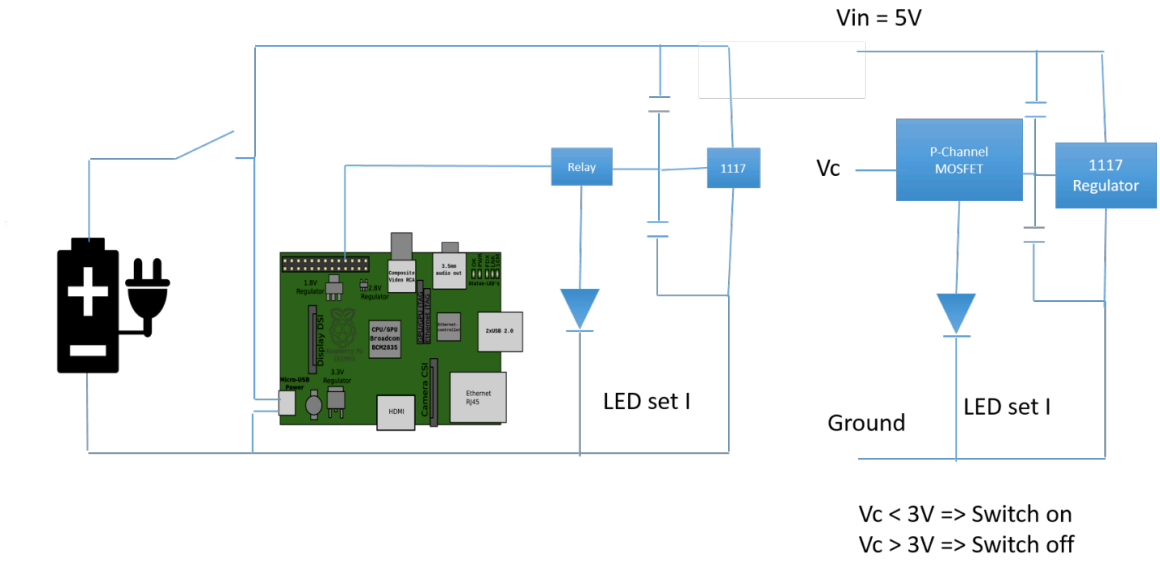

Figure 22: The circuit board design to power the LEDs. The voltage is regulated by the SBC through the GPIO ports using a script that is triggered wirelessly.

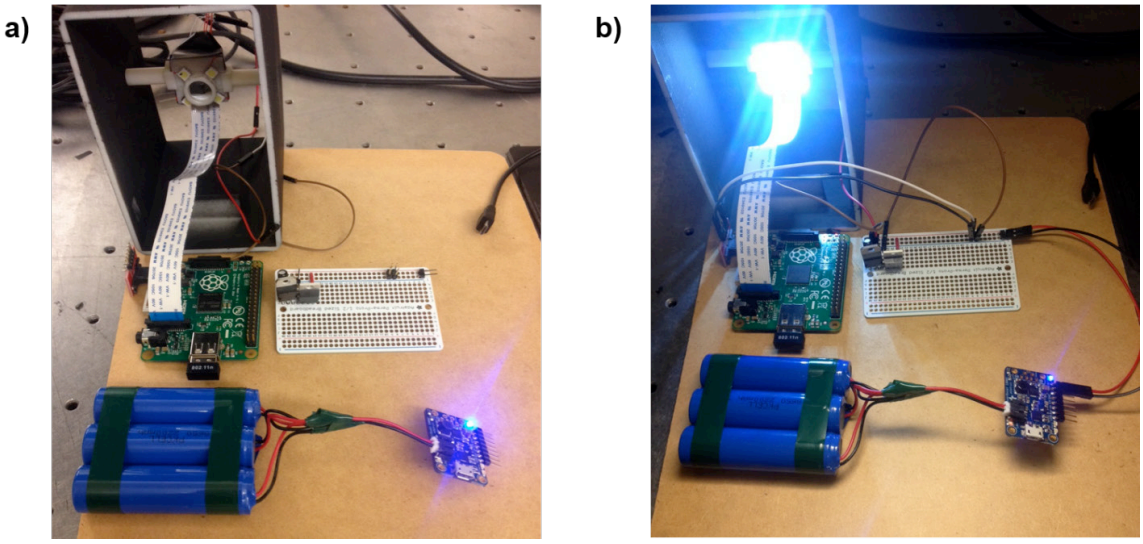

Figure 23: (a) The battery powering the SBC in a disassembled reader. (b) The voltage is switched to turn on the LED ring for illumination.

Testing procedure

To begin the test, the user first powers on the reader device, running on either an AC power supply or the battery. The internal SBC then runs a customized Linux distribution with a local Apache web server that broadcasts a pre-configured wireless network. The Tidbit is then connected to the network, allowing for direct wireless transfer of commands and image data. This local network allows for additional security, as the Tidbit can only be accessed by a internet-enabled that is in close proximity and has the necessary credentials. In addition, the wireless network is protected with WPA2 encryption, which further prevents unauthorized access.

Alternatively, peer-to-peer Bluetooth connectivity can be used to connect the Tidbit to a smart device. After pairing, Bluetooth transfer protocols can be used to send information to and from the device.

After the Tidbit is connected to an internet-connected device, the user can begin performing a test using the corresponding app. Because the reader works with LFA of various formats, the user is first asked to select the desired test from a list of options. This selection will load the corresponding procedure, which varies between test type in time required between steps, the region of interest for image analysis, and the calibration curve used to quantify analyte concentration.

The app then shows a series of steps that instruct the user in the appropriate sample collection and processing steps for the chosen test. The steps shown have been carefully edited and include specific pictures to aid the user in correctly performing the test. The usability of these instructions has been verified through human trials. The user is first instructed through the process of performing a blood draw and placing the sample on the test strip using a capillary tube. Sample instructions from the app are pictured in Figure 24. The strip is then inserted into the Tidbit for analysis.

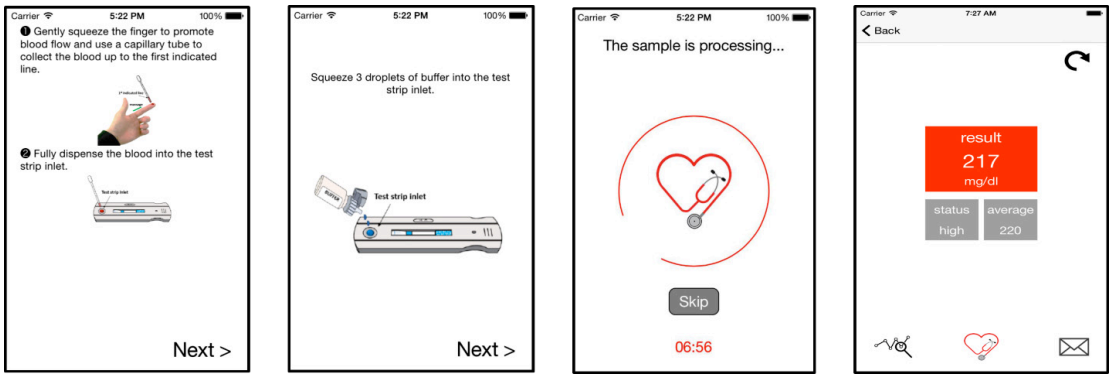

Figure 24: Instructions from the LFA app running on a tablet. The individual pages provide explicit step-by-step instructions and pictures to maximize usability. After the test is inserted into the device, a timer is started to allow the LFA to develop. When the reaction is complete, the reader takes an image of the LFA and sends it back to the smart device, where the app processes it and provides a result.

After the processing steps are completed, the app sends an HTTP request to the Tidbit to control the colored indicator LEDs that show the analysis progress. Upon receipt of the request, the web server triggers a script that supplies current to the specific indicator LED from the Raspberry Pi serial GPIO connection. The red and yellow LEDs are turned on at 50% and 100% of the LFA development time, respectively. After the strip is fully developed, an HTTP request is sent to the Tidbit to start the imaging process, which takes less than 5 seconds to complete. The LED ring is turned on to uniformly illuminate the LFA, and the camera captures an image. This image is returned via HTTP to the smart device, and the last green indicator LED is lit to confirm that the image has been received.

Once the image is received by the smart device, the image analysis takes place within the app. First, the image is cropped to the region of interest, and the analyte concentration is determined through image processing techniques that are specific to each individual test. The processing for a representative AuNP LFA is shown in Figure 25. The intensity of the test and control lines on the LFA are determined from a graph of pixel intensity, such as in Figure 25(c). The ratio of these intensities, the T/C

ratio, is compared to a corresponding calibration curve for the specific test in order to obtain a quantitative result.

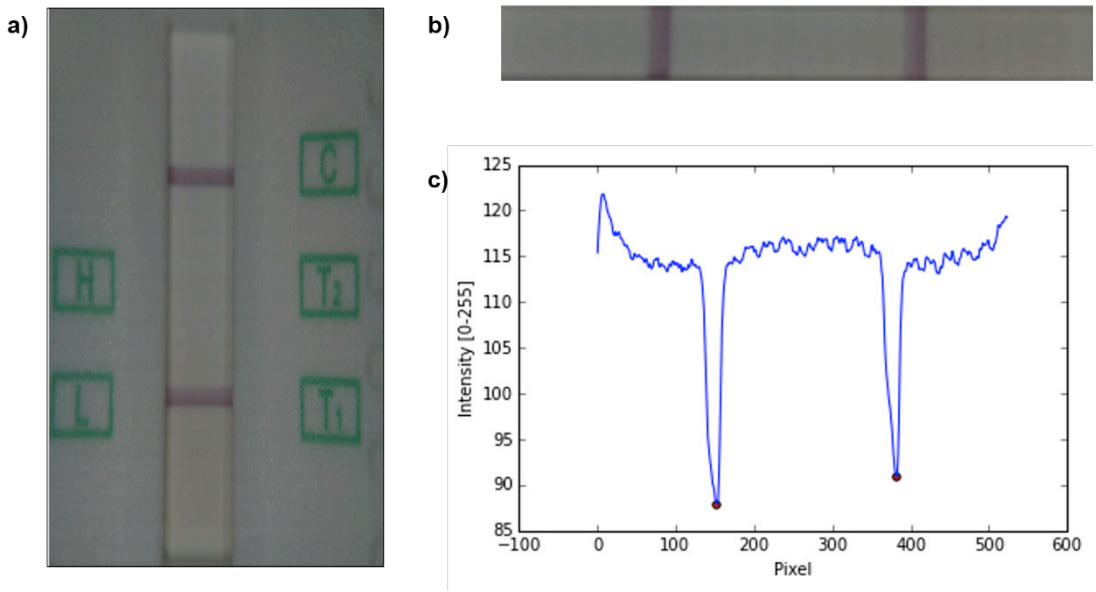

Figure 25: Image processing of LFA. (a) An image of a LFA from the prototype reader device. (b) After the image is sent to the smart device, the image is cropped to the region of interest. (c) The intensity of the lines is detected algorithmically and converted via a calibration curve to analyte concentration.

After the analyte concentration is determined using calibration data stored in the app, the test result is displayed on the smart device screen. The results are also time stamped and stored in a local database. If desired, the results database and/or raw images can be encrypted and securely sent to a medical practitioner. The results are not stored on the Tidbit, so it does not contain any sensitive patient data. This allows one reader to be used by many smart devices without sharing individual results.

Quality control

One of the main advantages of quantitation using image analysis over visual inspection of LFA is that the testing protocol can be carefully controlled to minimize user error. The reaction signal is only valid over a certain time interval, as evaporation

and backflow can alter the results with time[20], so it is important to capture the results during the optimal time window. Using the Tidbit, every measurement is taken at the same controlled time after the LFA is inserted, which removes the possibility of accidentally reading the results outside of the specified time window.

The image processing performed by the app also ensures quality measurements by checking for test strip errors and misalignment. For a valid test, the control line must be visible. If it is not, the strip did not develop properly or is misaligned in the reader. In either case, the software will reject this measurement and instruct the user to repeat the process, reducing the possibility of a false negative result. Similarly, a resulting T/C ratio that is too low to be statistically significant can be repeated to minimize the possibility of false positive results.

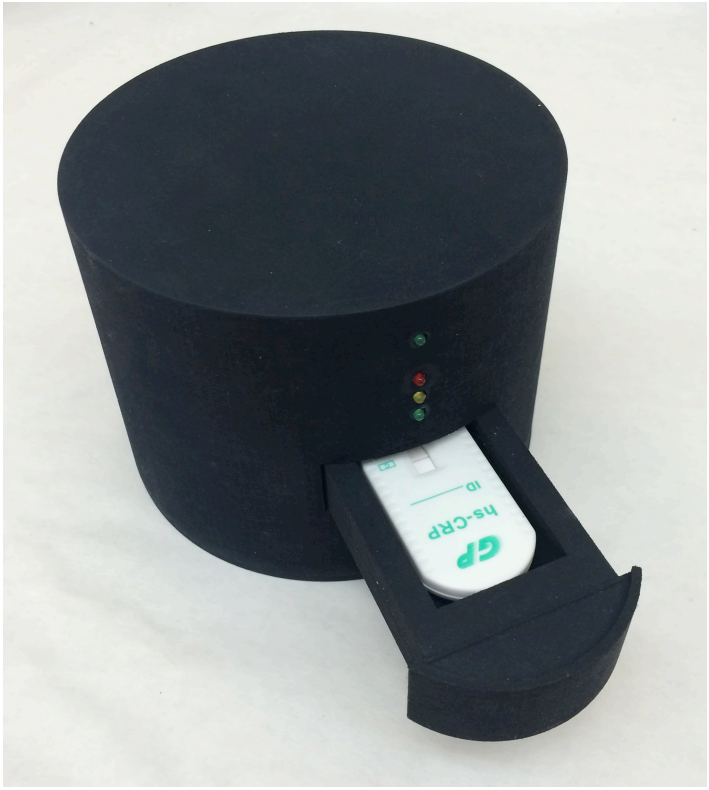

Figure 26: Tidbit prototype with CRP test strip cassette inserted in pull out tray.

USABILITY STUDY

A small-scale usability study was recently performed to gain feedback from users on the new Tidbit reader device. The actual assembled prototype is shown in Figure 26. The findings have been used to motivate future design iterations, and usability studies should continue with each new version of the reader. A procedure for these studies is in place and has been approved by the Cornell University Institutional Review Board for Human Participants (Protocol ID #1602006140).

Procedure

The study examines how participants feel about the Tidbit device and gains insights about potential use cases. Participants are given information about the general purpose of the device and the function of the test strips. They are not given any background on how the device works or how to use it. The current studied use case is with a computer, but future studies could incorporate smart phones or other internet-enabled devices. Before beginning the test, the reader is already wirelessly connected to the computer with the corresponding app open and ready. Even though a real test run would require a blood draw and placing a sample on a LFA, this usability study simply aims to see how participants feel about the Tidbit and the app, so a blood draw is not incorporated. Without instruction, the participant runs through the steps of the app and eventually places a pre-run test strip into the device, which generates a pre-determined result. Participants are informed and understand that this result is a random example and does not actually reflect their personal medical data. After getting the "result", participants are asked to take a SUS test and are then asked various questions to provide feedback about the Tidbit and process.

System usability scale

Thoroughly evaluating usability of systems is often not practical or cost-effective, so certain metrics have been created to get a quick and general indication of the overall level of usability of a system[42]. The SUS is one of these metrics. It is a simple, ten-item Likert scale that gives a global view of subjective assessments of usability.

To create a Likert scale, a large pool of potential questions is assembled, and the questions that result in extreme agreement or disagreement among respondents are chosen. These questions are the best for discriminating attitudes and make up the final scale.

For the SUS, a pool of 50 potential questions was assembled, and two software systems, one being “really easy to use” and one being almost impossible to use, were chosen. 20 people rated both systems against all questions on a 5-point scale ranging from “strongly agree” to “strongly disagree”, and the questions resulting in the most extreme responses were selected[42]. The resulting SUS, along with an example score, can be seen in Appendix A.

The SUS correlates well with other subjective measures of utility and is freely available for use in usability assessment[42].

Use and scoring

The SUS is usually given after the participant has used the system being evaluated, but before any discussion or debriefing[42]. This way, the responses reflect immediate reactions, and the participant has not had time to think too much about how they want to answer.

The result of the SUS is a single number that represents the overall usability of the system being evaluated, with the scores for individual questions not being meaningful on their own. To score each SUS, the score contribution from each item, ranging from 0 to 4, must be summed. For items 1, 3, 5, 7, and 9, the score contribution is the scale position minus 1. For items 2, 4, 6, 8, and 10, the score contribution is 5 minus the scale position. The sum of all score contributions is then multiplied by 2.5 to obtain an overall SU value. An example of the scoring process can be seen on the SUS in Appendix A. SU values range from 0 to 100, with 68 being considered an average score[58].

Participants and results

The first small scale usability study of the Tidbit reader device yielded interesting results to aid in further design refinements and future ideas. Six participants took part in the trial. The ages of these participants ranged from 22-72, with a mean age of 46 (σ=23.0). All participants were native English speakers residing in the northeastern United States. Participants had varying levels of education, ranging from some high school to graduate degrees.

The resulting mean SU score was 87.1 (σ=9.8), which is significantly above the average SU score of 65. Although the sample size was small and may not generalize to wider population groups, this gives a good initial indication that the current design is usable among different age groups and education levels.

The feedback given by participants was particularly interesting and provided concrete ideas for new design motivations. One of the most noteworthy occurrences was the fact that the older participants expressed much more excitement about the new reader and process than the younger participants. One participant specifically mentioned how he often needs to give himself Vitamin B12 shots and go to a doctor to

get his levels tested and that this point-of-care system would be especially beneficial. Multiple participants also acclaimed the battery power and wireless aspects of the Tidbit. A few users expressed confusion about which way the test strip should be inserted into the device, and one participant took a while to figure out that the drawer needed to be pulled out. Many users commented that more instructive pictures in the app would be very helpful.

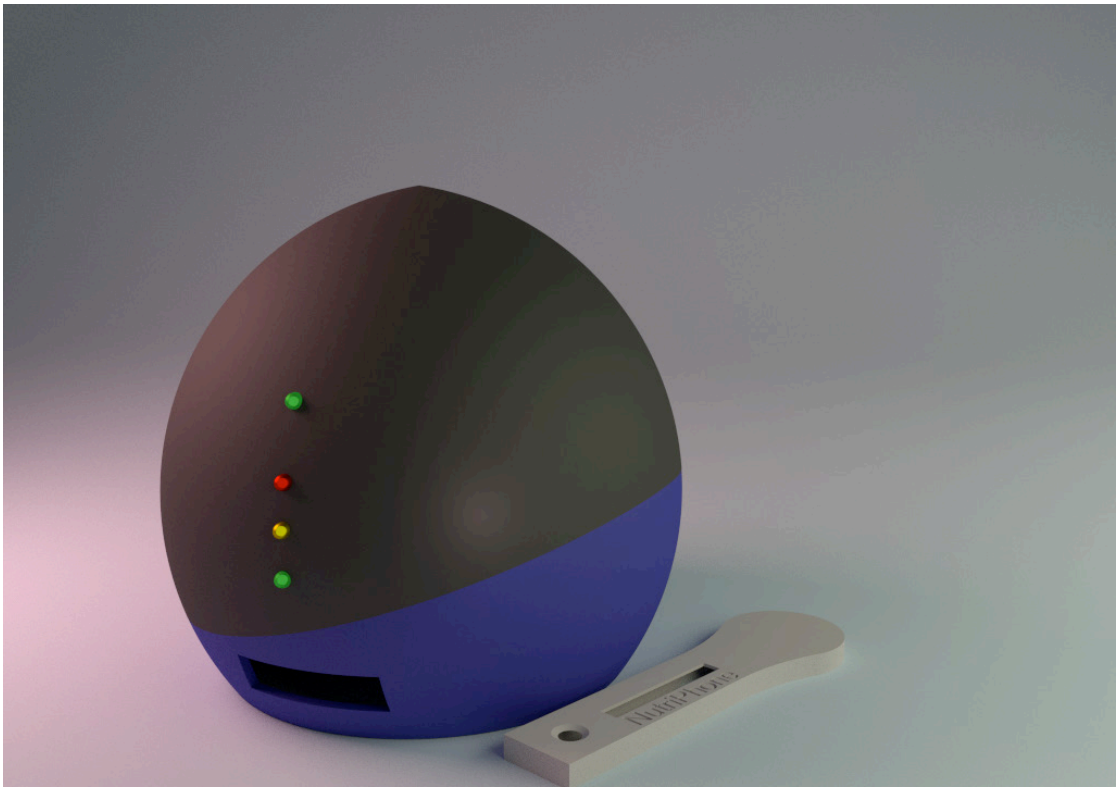

Figure 27: Rendering of new optical reader device being created currently to meet customer requests and improve upon first device.

IN PROGRESS DESIGN

Based on feedback gained in the usability study and customer requests, a new design is currently being created, pictured in Figure 27. The internal components and functionality of the device are the same as the previous device, but the battery has

been removed to cut down the size constraints. This means that the new device will require external power.

The new reader features a novel egg shape, which was viewed positively by many users and potential customers. It is smaller than the old reader, with a diameter of approximately 3.5” and a height of approximately 4”. The design features two colors that can be changed to suit the customers needs or tastes. The two separate pieces also make assembly of the device easier, as there is no longer an awkward bottom piece that needs to be attached. The pull-out tray has been eliminated, and the test strip cassette is now inserted directly into the reader. The design is also no longer symmetrical, so users cannot inadvertently place the device upside down. A custom test strip cassette has also been designed for use with the new reader.

The new test strip cassette is also shown in Figure 27. It features “NutriPhone” text indented into the top surface and will be printed in unique colors to match the egg device. The cassette features a large, curved end so that users will inherently know which side to grab. The curved end also prevents users from inserting the cassette too far into the device, as the slot in the reader only allows the cassette to be inserted to a certain width before it is too large to be inserted further. The blood insertion point is on the opposite end of the cassette so that users do not need to grab the cassette at the point where blood has been applied.

After this design has been completed and assembled, usability testing should be performed to ensure that users understand and like the new device, and further improvements should be made based on feedback received.

FUTURE IMPROVEMENTS AND ALTERNATE VERSIONS

From the feedback gained in the usability study, some further modifications could be made to the app and device. A new design with an even smaller size and

footprint was ideated to meet these needs, but assembly of such a device cannot yet be completed with the currently available internal components. Besides the use cases previously presented, alternative functions and procedures are also possible.

Modifications for future designs

Based on the feedback from this usability study, a few things need to be implemented into future designs. Firstly, more instructive pictures need to be added to the app to decrease user confusion. This modification alone might solve other possibly confusing steps, such as pulling out the tray and correctly inserting the strip. It may also be useful to eliminate the pullout tray and instead allow test strip cassettes to be inserted directly into the device.

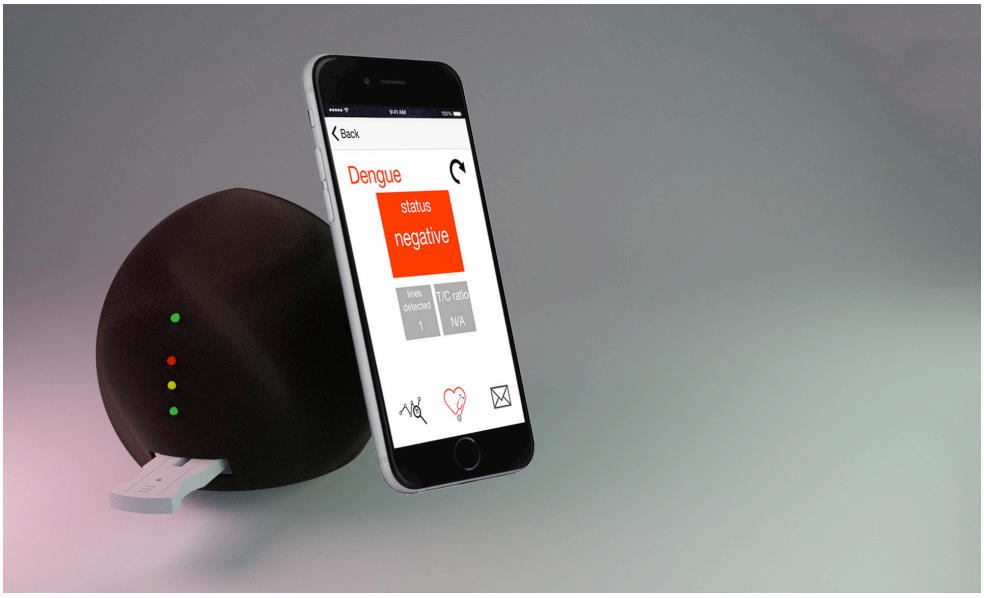

Figure 28: Render of smaller device form factor. The test strip cassette inserts partially with the region of interest inside of the reader. The reader cannot be placed upside down because of the absence of top-bottom symmetry.

Small form factor design

Eventually, an alternate version of the device with a smaller form factor could be created. A smaller reader would likely decrease costs, increase the mobility of the reader, and potentially lead to an even simpler operating procedure. The current reader size is limited by the necessary internal components and the desire to house the entire test strip cassette inside the device. The internal components, shown in Table 2, that specifically limit the size include the SBC and breadboard. In order to eliminate this constraint, VLSI could be used to create a small-scale integrated circuit to replace the current large components. To eliminate the test strip cassette size constraint, a new design would need to function with the cassette being partially inserted into the reader. This would also help to eliminate any confusion involved with the current pullout tray design. To accommodate multiple cassette sizes and also block external light, a flexible, opaque material, such as rubber, could be used to create flaps at the test strip insertion point. An internal stopper will also need to be incorporated so that the user cannot accidentally insert a small strip completely into the reader. It is very important to maintain incorporation of battery power to ensure complete portability of the reader.

A potential design that meets these needs is shown in Figure 28. Test strip cassettes are inserted directly into the reader. There is also no top-bottom symmetry, so the reader cannot be inadvertently placed upside down.

Alternative control devices and functionality

The current reader was discussed for integration with a smartphone or tablet. However, it can interface with any device that can transmit data over a wireless network or Bluetooth, including but not limited to a computer running any operating system, an iPod, a smart watch, smart glasses, a smart headset, or other wearable smart

technologies. The software is hardware agnostic and can be implemented as a native app (e.g. Android, iOS, or OSX app) or as a web-based interface.

The current design is a portable tabletop model that is large enough to allow more complicated optics, including fluorescence, bright field, or dark field filters. Presently, an internal ring of white LEDs is used for broadband absorbance microscopy. In the future, tunable LEDs controlled via the software will be used to perform fluorescence microscopy at multiple wavelengths. With the applicable filters and/or polarizers inserted, a range of microscopy techniques, including bright field, dark field, reflectance, fluorescence, and differential interference contrast microscopy are possible. These systems can also be used to quantify signals from a wide variety of detection labels including fluorescent proteins, colloidal gold, silver, or carbon nanoparticles, latex beads, and quantum dots.

In addition to more optical components, there is also sufficient space inside of the reader to image larger, multiplexed test cassettes for multiple analytes. The software is capable of containing an indefinite number of calibration curves, so the reader can be used for a wide range of LFA tests.

CONCLUSION

A new reader device for quantification of LFA signal has been developed. The design thinking process was applied in order to foster creative, novel solutions. Based on customer requests (Table 1) and the original problem statement, the Tidbit was designed and prototyped. The features of this new reader are compared with the most frequent customer requests for such a device in Table 3.

Table 3: Most common customer-requested features available on the Tidbit

Customer request	Feature of Tidbit
Ease and convenience of operation	• user can use their own familiar smartphone or device interface • test strip is automatically analyzed at optimal time • results displayed in easy-to-read visual • can be powered by internal rechargeable battery or AC adapter
Quantitative read out	• high accuracy imaging algorithm • result output in numbers with units and/or qualitative
Automatic electronic documentation of results	• records and saves data on user's smart device • results can be sent to physician
Higher sensitivity	• highly optimized sensor • ability to incorporate fluorescence and other detection labels
Objective interpretation of results	• raw image data stored
Use of reader in QC for test strip manufacturing	• rapid testing time allows high throughput • standalone reader • portable • automatic checks for test strip validity
Handheld reader format or mobility	• small format reader • portable
Operational robustness	• place test strip into reader and image • protected data • automatic checks for result validity • data assigned a time, date, and patient ID automatically
Physical robustness	• can be used in any lighting conditions
Audio/visual display of results	• visible display of analysis progress and results
Connectivity to PC, printer, barcode reader, or other data management system	• wireless connectivity to any internet or Bluetooth-enabled device
Hard copy of test results	• results stored on smart device and can be shared and printed
Compatibility to clinical workflow and systems	• easy incorporation into clinical systems
Standalone reader without use of computer	• rechargeable battery power • works with any smart device
Batch and calibration data management system	• automatically builds secure database of results on smart device being used
Low price	• integrated flexibility: - any cassette format can be used - any detection label can be read

Appealing design	• convenient shape and feel • trendy professional design
Fast read out	• imaging and analysis takes only a few seconds
Wireless data transfer	• standard feature
Compatibility to customer's unique cassette format	• standard feature
Compatibility to customer's unique label	• standard feature
Availability of professional software	• standard feature
Compatibility to different tests and test formats	• standard feature
Multiplexing	• can analyze multiplex signals

A small-scale usability test was performed on the cylindrical Tidbit prototype, and ideas for future refinements and designs were garnered. As the design process continues, usability tests should be consistently performed as new versions of the reader are created.

The Tidbit has many potential applications in rapid, point-of-care immunoassay-based diagnostics. Because it is portable, low-cost, and simple to use, it has many conceivable uses, including in-home personal health monitoring, use in a clinic, doctor’s office, or pharmacy, and diagnostics in resource-limited settings.

CHAPTER 8

FUTURE DIRECTIONS AND OTHER WORK

Conclusions

Dengue Lateral Flow Assay

Addition of NS1 detection

Multiplexing

Tidbit: Continuing the Design Thinking Cycle

Abstracts of Other Published Works

CONCLUSIONS

This thesis describes the groundwork for a highly sensitive fluorescence LFA for dengue detection and a novel optical reader for LFA signal quantification. Together, these advances can allow the diagnosis of dengue and potentially other febrile illnesses in resource-limited settings.

A lack of available resources in many dengue endemic settings often means that the tests with the highest sensitivity and specificity are inaccessible[9]. LFA technology has been used to lay the groundwork for a dengue diagnostic test that permits early and rapid diagnosis while being affordable and easy to perform. After experimentation with other detection labels, fluorescent particles were chosen for their relatively high sensitivity. The fluorescent particle conjugation chemistry is more complicated than with other detection labels, so further optimization of this process is needed.

Many devices currently exist for LFA signal quantification. However, these devices have inherent problems in providing accurate results, and they are often expensive, complicated, or bulky[20]. The design thinking process was applied to create an inexpensive, easy-to-use, portable optical reader. Customer requests and usability testing were both incorporated to ensure a robust design. Potential uses for the new device include diagnostics in resource-limited settings, use in a clinic, doctor's office, or pharmacy, or in-home personal health monitoring.

DENGUE LATERAL FLOW ASSAY

Besides the immediate work discussed in Chapter 4 that should be completed to create a dengue LFA, a few other potential improvements could be made in the future.

Addition of NS1 detection

One of the goals in creating a robust dengue LFA is detecting the disease in the earliest possible stage. As shown in Figure 2, the NS1 antigen appears earliest in the onset of dengue. This means that a LFA to detect dengue IgG and IgM has the potential of missing some cases that are in the earliest stage of the illness. To solve this problem, another LFA could be developed to detect dengue NS1 and run simultaneously with the dengue IgG and IgM LFA. A custom cassette to hold multiple strips could be easily created, and the Tidbit software could easily be modified to read multiple signals.

Multiplexing

A promising idea for a multiplexed test for various IgGs involves the use of Protein G conjugated to a detection label. Protein G has a high affinity towards IgG and could therefore be used to detect IgG from multiple diseases on a single LFA. Because Protein G is purchasable pre-conjugated to labels such as AuNP and fluorescent labels, the process of creating LFA is simplified by eliminating the conjugation process.

Eventually, different fluorescent detection labels could be used to label anti-human IgG and anti-human IgM so that one LFA could test for IgG and IgM for multiple diseases. This concept has been demonstrated using red and blue latex beads[69].

TIDBIT: CONTINUING THE DESIGN THINKING CYCLE

Because design thinking is a cycle without an end, it is important to constantly continue empathizing with different users, redefining the problem, coming up with new ideas and solutions, prototyping, and usability testing each new design iteration.

Important next steps will be incorporating new features into the current device as previously discussed, including filters and LEDs.

With the applications of the reader, especially in resource-limited settings, further usability concerns will have to be considered. The usability test that was performed may not generalize to other regions or cultures, so the device should be tested for usability through field trials in possible regions of interest. One specific concern is the language of the instructions. It may be useful to have a native multilingual speaker of the target language(s) translate the English instructions and integrate a language choice option into the software.

ABSTRACTS OF OTHER PUBLISHED WORKS

The following are abstracts detailing my other published work completed at Cornell University.

Shorter Wait Times: The Effects of Various Loading Screens on Perceived Performance

Hohenstein, J., Khan, H., Canfield, K., Tung, S., Perez Cano, R. *Chi '16 Extended Abstracts*, May 07-12, 2016, San Jose, CA, USA. ACM 978-1-4503-4082-3/16/05.

Loading screens are unavoidable in modern software applications, and providing graphical user feedback during wait times is a well-established way to increase perceived performance. Previous research has indicated that perceived performance is essential to the success of an application, and progress bars have been specifically shown to decrease perceived wait time. This study is the first to examine the effect of animated loading screens on perceived wait time as compared to the popular progress bar. Study participants compared a progress bar with both a passive and interactive animation. Results suggest that with an interactive animation,

perceived wait time is shorter and user satisfaction is higher than with a progress bar or passive animation.

NutriPhone: a mobile platform for personalized diagnosis of vitamin B12 deficiency

Lee, S., O'Dell, D., **Hohenstein, J.**, Colt, S., Mehta, S., Erickson, D. 2016. Under review.

Background Vitamin B12 is necessary for formation of red blood cells, DNA synthesis, neural myelination, brain development, and growth. Vitamin B12 deficiency is often asymptomatic early in its course; however, once it manifests, particularly with neurological symptoms, reversal by dietary changes or supplementation is difficult. Access to easy and low cost nutritional diagnostics will help with early detection and enable individuals to better understand their own status as well as track the effects of dietary changes.

Methods We developed the NutriPhone, a mobile platform, for the analysis of blood vitamin B12 levels in 15min and report the results from the first generation prototype in this manuscript. This prototype comprises of a smartphone accessory, an app, and a competitive-binding lateral flow test strip. We developed the NutriPhone system to quantify physiologically relevant levels of vitamin B12 and performed human trials where it was used to evaluate serum B12 status of 12 participants from just a drop (~40μl) of finger prick blood, comparing results to those obtained from a chemiluminescence immunoassay.

Findings The results obtained from the NutriPhone system had a correlation of 0.93 ($p<0.0001$) with those obtained from the conventional measurement and the median

bias was -0.3%. For a cut-off of 221pmol/L (vitamin B12 insufficiency), the current NutriPhone system shows 75% sensitivity and 83% specificity.

Interpretation The next generation of the NutriPhone will focus on improving sensitivity and specificity for vitamin B12 deficiency. Meanwhile, it can immediately play a key role in point-of-need screening of vitamin B12 insufficiency and B12 quantification.

"I mean, I'm not a doctor": Designing Interfaces for Interpreting Patient-Administered Medical Test Results

Baumer, E., Reynolds, L., O'Dell, D., **Hohenstein, J.**, Lee, S., Rieger, E., Adams, P., Qi, Y., Guha, S., Gay, G. 2016. Under review.

Technological advances allow people to track their own health in a variety of ways, potentially dramatically changing the patient-clinician relationship. However, research regarding patient interpretation of traditional medical tests highlights the challenges in making complex medical data comprehensible for a general audience. While medical and policy researchers debate about direct patient access to medical results, the imminent future of personal medical testing signals the need to explore direct patient access to medical data from an HCI perspective. To that end, we conducted two studies, an online survey and an in-lab prototype with debrief interviews, to explore whether and how people accurately interpret blood test results. Overall, participants often correctly interpreted the test result. However, many also expressed confusion about the result and uncertainty about their interpretation. These findings illustrate both promises and perils of direct access to medical data, and they suggest an alternative approach to personal medical testing.

APPENDIX A

System Usability Scale[42]

	Strongly disagree				Strongly agree	
1. I think that I would like to use this system frequently						4
	1	2	3	4	5	
2. I found the system unnecessarily complex						1
	1	2	3	4	5	
3. I thought the system was easy to use						1
	1	2	3	4	5	
4. I think that I would need the support of a technical person to be able to use this system						4
	1	2	3	4	5	
5. I found the various functions in this system were well integrated						1
	1	2	3	4	5	
6. I thought there was too much inconsistency in this system						2
	1	2	3	4	5	
7. I would imagine that most people would learn to use this system very quickly						1
	1	2	3	4	5	
8. I found the system very cumbersome to use						1
	1	2	3	4	5	
9. I felt very confident using the system						4
	1	2	3	4	5	
10. I needed to learn a lot of things before I could get going with this system						3
	1	2	3	4	5	

Total score = 22

SUS Score = 22 *22.5 = 55

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