Development and evaluation of point-of-care diagnostic technologies for providers and consumers

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ABSTRACT

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Point-of-care (POC) diagnostic technologies aim to expand access to traditional laboratory-based testing to near-patient settings. These settings can range from emergency or intensive care-units (ICUs) in the United States, to remote health posts in sub Saharan Africa. Differences in budget and infrastructure play a role in characterizing the wide array of possible "near patient" settings and must be taken into consideration in the engineering design process. In this dissertation we use translational engineering to develop practical and accessible microfluidic POC immunoassays for diverse settings, that include both provider and consumer facing applications.

First, we examined Lyme Disease in the U.S., where existing diagnostic technologies face the challenge of rapid and accurate serodiagnosis in the face of largely non-specific clinical symptoms. We developed a multiplexed rapid test that could replicate enzyme-linked immunosorbent assay (ELISA) performance for Lyme Disease diagnosis. After screening candidate biomarkers, we evaluated performance of the multiplexed microfluidic test against ELISA using clinical serum samples and illustrated the potential to streamline current clinical algorithms requiring two immunoassays (ELISA and Western Blot) into one standalone test suitable for physician's offices or urgent care clinics in the U.S. We also showed exploratory work towards a similar multiplexed test design for another bacterial spirochete infection, Leptospirosis.

Next, we built on previous work towards a POC HIV-syphilis antenatal screening tool, to develop a smartphone-integrated, microfluidic assay for healthcare workers to use in low resource settings. The low-cost ($34), re-usable device ("smartphone dongle") costs $34 to produce and provides results in 15 minutes. In this work, we focus on assay development efforts undertaken towards development of a fully integrated POC product suitable for deployment in the field, with practical considerations for the use of fingerstick blood, stability, scale-up and transport. We also streamlined the number of manual steps for end-user operation, through the use of lyophilized secondary antibodies, preloaded reagents on cassette, and an automatic result readout. While laboratory demonstration with clinical samples is important for initial characterization of POC devices, field evaluation reveals diagnostic performance under real-world conditions. We tested the device in the hands of minimally trained healthcare workers in Rwanda and saw comparable performance to other immunoassays run under field conditions. We also performed a follow-up pilot field study in Rwanda to evaluate the feasibility of the smartphone dongle platform for self-testing by patients/consumers in a low-resource setting, one of the most challenging use-cases for POC devices.

Finally, we sought to integrate intellectual frameworks from behavioral research and user-experience (UX) design in creating a new framework for evaluation of consumer-facing microfluidic devices, specifically towards HIV home-testing in the U.S. While overall rates of HIV are decreasing in the U.S., the population of gay, bisexual and other men who have sex with men (MSM) are disproportionately affected. Self-testing products for sexually transmitted infection (STI) testing could address unmet needs for these target populations in both increasing access and frequency of testing, as well as integrating use with sexual partners for early diagnosis or even prevention. We worked with a cohort of MSMs at high risk for HIV/STI

transmission in New York City, and performed for the first time, a structured assessment of completely naïve users interacting with a smartphone interfaced microfluidic diagnostic device ("SMARTtest"). We integrated UX design value model of device usability, credibility, accessibility and acceptability into our evaluation framework, which influence user's information, knowledge, motivation and behavioral skills towards engaging with a prevention method ("IMB" model). Thus far, such frameworks have rarely been applied to other consumer health monitoring devices, including microfluidic POC devices. As the microfluidic field moves towards more field demonstrations of devices, more untrained and minimally trained users will have access to such tools. It is important to understand how they use devices, what the device failure points are and what the most relevant design features are to spur user adoption and meaningful usage.

Underlying our work in creating accessible and practical POC immunoassay tools for infectious disease detection, is the illustration of the translational development roadmap from proof-of-concept assay development to field studies and user-based evaluations for intended end-use settings that range from U.S. based primary care clinics, rural health centers in low-resource settings as well as self-testing environments in both. Incorporating an understanding of the target use-case setting is critical in translating technologies for clinical use, whether in the infrastructure and services that are available, or end-user needs and constraints such as clinical workflow patterns, level of technical expertise and perceptions of usefulness and value. We show how user/use-case focused application of downstream translational engineering and testing informs upstream design choices and accelerates development of POC devices for real-world use. The sum of this work aims to illustrate tenets of translational engineering design and testing

to advance insight into building POC products that are poised for greater adoption by target end users, whether they are health providers or consumers.

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Chapter 1

Introduction

The goal of this thesis is to illustrate the translational "pipeline" of point-of-care (POC) immunoassay diagnostic development, from proof-of-concept assay development efforts in the lab, to testing with clinical specimens and integration of hardware and software elements for evaluation in the field. We illustrate practical considerations in POC development towards end-user operation, scale up, manufacturing, and product shelf life for health settings both in the U.S. and in developing countries. Finally, we explore structured evaluations with users themselves to evaluate potential for consumer-facing device deployment. The overall objective is to develop microfluidic diagnostic devices that are better poised for adoption by target end-users in diverse settings.

The field of POC diagnostics offers the exciting possibility of providing rapid diagnostic results in non-laboratory settings. The goal of POC diagnostics is to provide results with a fast turnaround time to enable decision making and potentially start of treatment within one clinical visit. With the rise of connected consumer devices, entire sectors of the economy (including retail, transportation, housing, and freelancing services) have been unmistakably transformed. The potential reach of POC diagnostics into all sectors of healthcare – and increasingly into daily

routines of individual patients and consumers – demand that technical advances take into context this broader transformation.

For healthcare providers, the landscape for medicine is poised for a dramatic shift. In any decentralized setting, a connected POC diagnostic device could be available to aid the diagnosis of disease and selection of treatments. With therapeutics becoming increasingly tailored to individuals' precise genetics and biomarkers, the value chain of healthcare delivery could move up to accurate and accessible POC diagnostics as opposed to therapeutics.

For consumers and patients, the impact could be even more dramatic. With the rise of connected consumer electronic devices, patients and consumers are adjusting in how they interact with POC medical technologies alongside mobile devices, and more broadly, in how they manage and seize control of their own health. Opportunities for home or self-testing can shift the burden of lost work hours and productivity in the appointment-based clinical care model. While patients with greater socio-economic agency can offset these burdens, daily-wage earners, particularly those in lower and middle-income countries could feel the benefits of increased access to care through POC tools. In due course, POC diagnostic and monitoring devices are expected to become ubiquitous, whether in an at-home setting, in a doctor's office or hospital, or in low- and middle-income countries.

In today's connected age, this progress is being made within the context of a broader and more diverse POC ecosystem than before (**Fig. 1**). Some of these components, such as assay chemistry and microfluidics, cover traditional ground for researchers in POC diagnostics, and are shown in

this work in developing new assays for diverse disease applications. Other components, ranging from data analytics to regulation, reflect broader forces which are influencing how POC technologies are being designed and developed.

For POC diagnostic devices to be accessible to end users, the disparate steps of a multi-step diagnostic assay – including fluid handling, sample processing, signal amplification, washings, and detection – must be seamlessly integrated. In this regard, microfluidics serves as an

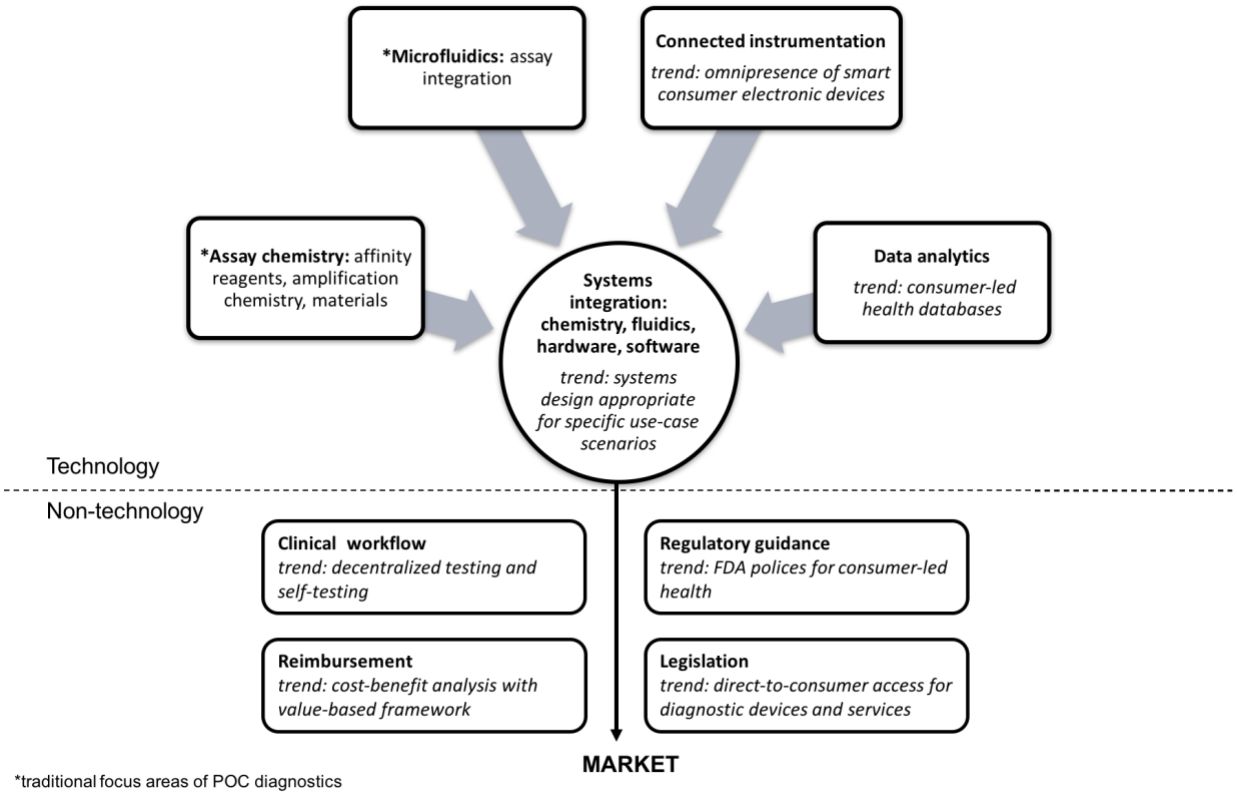

Figure 1. The POC ecosystem in a connected age.

integrating force. In this work, we have used microfluidic and microscale engineering techniques as part of a larger trend in producing integrated devices which are self-contained, automated, easy-to-use, and rapid [1-4].

1.1 Current landscape

1.1.1 Upstream Development

Traditionally, microfluidic POC developers have focused on individual upstream components such as assay chemistry, fluidic handling methods and instrumentation or sensor development (**Fig. 2**). Recent assay chemistry developments have been made in engineering antibodies with high affinity and specificity[5], antibody-mimetic proteins such as designed ankyrin repeat proteins (DARPins) which are thermally stable[6], non-immunoglobulin (non-Ig) scaffolds[7], and antigen-specific, single-domain antibodies[8-11]. Other assay chemistry developments include the use and modification of aptamers (short, single-stranded oligonucleotides with high specificity) as affinity reagents[12], enhancements to labeling molecules/nanoparticles for signal detection (e.g. multicolored silver nanoparticles for tunable absorbance spectra and multiplexed detection[13], europium (Eu) (III) chelated nanoparticles for bacterial detection in a lateral flow format[14]). With fluid handling methods, researchers have developed novel strategies in fabrication and fluidic actuation mechanisms, such as micromilling Lego blocks for highy reproducible, modular microfluidic components[15], optically-driven photodeformable polymers that could be manipulated to exert control over a variety of fluids[16], which could represent a new tool in microfluidic design (feasibility of actuating one component without affecting others in a high density chip still needs to be demonstrated), no-power valves based on magnetic adhesives for mixing reagents[17], electrostatic mixing method of reagent droplets on a chip[18] etc. Researchers have also made efforts in developing label-free sensing platforms for detecting multiple targets from clinical specimens (e.g. microcantilever-based detection based on their

potential for label-free and sensitive detection[19]). Work has been done to integrate microfluidics with plasmonic sensors, which are a label-free detection method by monitoring interactions among electromagnetic waves and free electrons in metals (including surface plasmon resonance and surface-enhanced Raman scattering [20])[21]). Other instrumentation efforts have been made for lateral flow assays such as electronic readers [22, 23] compact smartphone-based fluorescence detectors[24] etc.

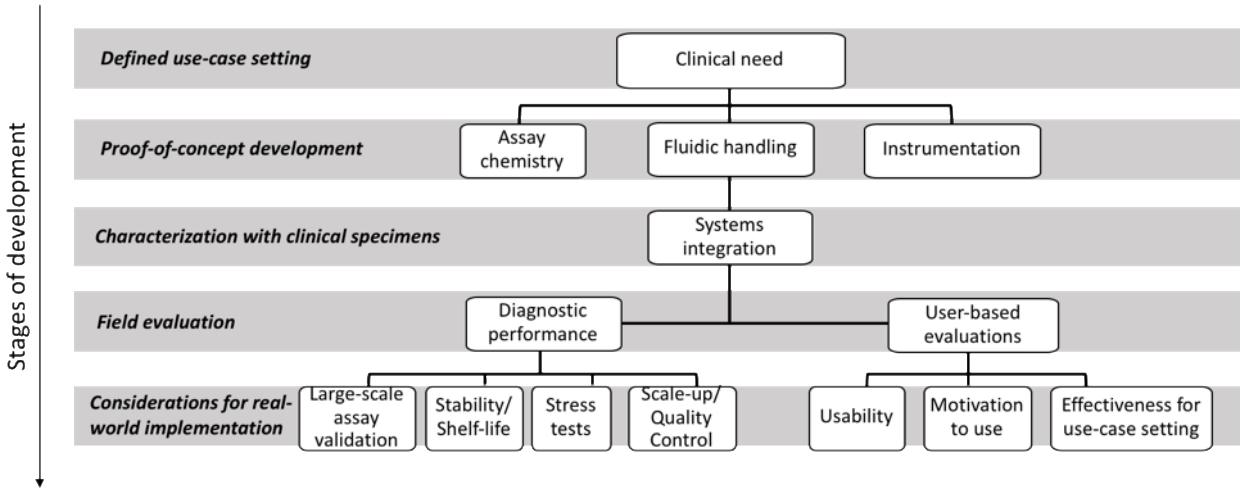

Figure 2. Translational framework for microfluidic POC diagnostic development

1.1.2 Downstream Development

A few groups have examined downstream considerations towards stability, an important aspect of producing POC assays suitable for field deployment. Some notable examples include the demonstration of long term dry storage of ELISA reagents horseradish peroxidase (HRP) conjugated antibody label and colorimetric substrate diaminobenzidine (DAB) at 45°C for over 3 months. This was achieved by leveraging formulations commonly used in the pharmaceutical industry for preservation of biomolecules, through the use of sugars like trehalose, bovine serum albumin (BSA) and ferrous irons[25]. Reconstituted HRP-antibody and DAB substrates were

used in 2D paper network format to detect malarial biomarker *Plasmodium falciparum* histidine rich protein 2 (*Pf*HRP2) in a sandwich immunoassay. While this format of colorimetric ELISA could be detected by eye, all quantification was still performed with flatbed scanner and image anlsysis software. Additionally, the test was demonstrated with antigen-spiked simulated samples (vs. whole blood or on chip plasma separation), and did not contain fluidic delivery and metering integration. Other efforts include the incorporation of freeze-dried reagents in a paper-based sensor for the detection of the Zika virus RNA genome, paired with a battery-powered electronic reader (manufacturing cost of $250)[26]. Sample preparation steps are not yet included, and the overall assay time is several hours. Integration of disparate assay steps for a complete sample-to-result process remains a barrier to translational efforts by many POC microfluidic developers

In one of the few studies highlighting integration, developers created a smartphone based video microscope and showed quantification of blood-borne filarial parasites could be performed under 2 min, and required only a glass capillary and lancet as peripheral equipment[27]. A custom algorithm tracked the motion of the microfilaria by quantifying the displacement of red blood cells, and the results were tested in the field in Cameroon, showing good correlation gold standard thick smear microscopy[27]. Key components of this technology that differentiate from other mobile-phone microscopy efforts is the automated quantitative algorithm and software interface allowing for one-touch completion of the assay. While diagnostic performance was not explicitly evaluated in a structured format for local doctors and technicians handling the assay compared to the study team, including local healthcare workers in testing efforts illustrates the potential of this platform for use by minimally trained health care workers.

1.1.3 Commercial Efforts

Table 1 illustrates examples of commercial efforts with fingerprick blood based technologies[28]. Abbott's i-STAT device has been one of the more successful POC products, with deployment in almost a third of U.S. hospitals after two decades of service[28, 29]. Most of these products however, are still under development and evaluation.

Recent epidemics of infectious diseases such as Ebola and Zika have highlighted the importance of rapid testing that can be field-deployable or used in emergency field-laboratories. A number of commercial efforts were undertaken to develop cartridge-based, sample-to-answer real-time RT-PCR systems for detection of the EBOVL gene of Ebola. The Xpert Ebola system (from the company Cepheid) adapted the company's previous work on tuberculosis to integrate sample preparation, virus inactivation, nucleic acid amplification, and detection[30]. In Sierra Leone, the Xpert Ebola assay showed high sensitivity and specificity for both venipuncture whole blood and oral swab clinical samples compared to laboratory RT-PCR[30]. In another system, the FilmArray Ebola system (from the company Biofire Defense) used a pouch with lyophilized reagents that were rehydrated, followed by sample dilution and injection into the reconstituted reagent pouch[31]; the pouch was inserted into the FilmArray instrument with results available after 1 hour[31, 32].

Table 1. Selected commercial efforts with finger prick blood testing technologies (Taken from [28])

Company/location	Technology	Sample size/tests	Status
Abbott	Electrochemical detection	26 tests: chemistries, electrolytes, hematology, blood gasses, coagulation, cardiac markers, and a pregnancy test; 2–3 drops from a capillary, venous, or plasma sample, depending on the test; results in minutes	i-STAT on the market
Abionic/Lausanne, Switzerland	Nanofluidic biosensors, fluorescent anti-IgE antibodies, read with miniaturized fluorescent microscope	2 drops of blood: 10 allergens in 20 min	abioSCOPE (reader) and abioGuide (apps) has CE marking from EU
Archimej Technology/Évry, France	High sensitivity spectroscopy where the emission spectrum is dynamically controlled	Microvolumes of blood: multiple biomarkers for liver, kidney, heart status in real time	Beta-Bioled in development
Cepheid (now part of Danaher)	PCR on a cartridge	3–4 drops of capillary blood: quantitative and qualitative assays for HIV, hepatitis C and Ebola	Quantitative assays for HIV viral load and hepatitis C under development; qualitative assay for HIV has CE marking and WHO pre-qualification; qualitative assay for Ebola has CE marking and US and WHO emergency authorization
Cor	Vibrational spectroscopy	Drop of blood taken from the arm: HDL, LDL, total cholesterol, fasting glucose, triglycerides, fibrinogen	Cor Wellness System in development
DMI	Fluorescence and light scattering from multi-plexed nanostrips	5–10 ml of blood: hundreds of tests	rHealth under development
Eva Diagnostics/London	Multi-wavelength optical absorption	10 ml of capillary or venous blood: hemoglobin and hematocrit in 30 s	AnemiPoint: Undergoing evaluation
Genalyte	Photonic detectors lithographically printed on silicon chips	One drop of venous or capillary blood: 128 tests, starting with rheumatology	Maverick in development
Magarray/Milpitas, California	Magneto-nanosensing technology adapted from computer disc drive technology	Single drop of blood: modular with biomarkers in cancer, autoimmunity, heart diseases in minutes	In development for laboratory use or point of care
Pixcell Medical/Yokneam Illit, Israel	Viscoelastic focusing	Finger prick (capillary) or venous blood: 20 standard CBC parameters	Hemoscreen, CE mark in EU, going through 510K approval
Philips	Antibody-coated magnetic nanoparticles and biosensor	Finger prick: troponin in 5 min	Minicare I-20: CE marking
Roche	Reflectance photometry	30 ml of blood: 17 tests in 2–3 min	Reflotron Plus (marketed in the EU)
Zepto Life Technology/Minneapolis	Giant magneto-resistance sensing with magnetic nanoparticles	Small volumes of serum: multiple biomarkers in minutes	Under development

HDL, high-density lipoprotein; LDL, low-density lipoprotein; WHO, World Health Organization; CBC, complete blood count.

Comparison of FilmArray with RT-PCR were also performed for whole blood and urine samples[31]. Finally, the Idylla Ebola Virus Triage Test (from the company Biocartis NV) used a RT-PCR instrument and console, and provided results in 100 min from EDTA venipuncture whole blood samples[33]. The reagent cartridges did not require cold chain storage[33], and clinical results from the field are pending. All three systems were given Emergency Use Authorization status by the regulatory authorities, and use was still confined to facilities with moderate to high complexity[34]. There exist challenges to implementation in more decentralized field settings, where even greater public health impact could be achieved. Field use requires uninterrupted electricity, temperature control, and moderately trained personnel to conduct sample preparation[34]. In addition, without subsidies, cost of instruments and cartridges are also prohibitive for lower-resource settings (for example, the manufacturer list price for Filmarray reader is $39,500, with $129 per test kit)[35].

1.1.4 Use-Cases

While it is widely acknowledged that POC testing is completed at or near the patient, this definition spans a large range of possible POC settings, each of which imposes a different set of specific design constraints on POC devices. Previously, different levels of healthcare delivery have been described. For example, one popular public-health model describes different “levels” (Level 1 being community outreach, Level 2 being primary health, and Level 3 being district level lab)[36]. Another model describing POC diagnostics for infectious diseases in low- and middle-income countries presents five different scenarios, from home to hospital[37]. Although these distinctions are useful in certain areas of POC diagnostics (i.e. global health), in our view, another description is needed to distinguish the key features across all POC settings.

Specifically, a distinction should be made to uncouple infrastructure and budget. For example, a "clinic" which is a non-profit entity in developing countries could be as geographically remote as a "clinic" in a military setting, but their cost constraints for the POC device are very different. In another example, a device in the "field" for global health is as similarly removed from lab testing facilities as a monitoring device used at home in the U.S., but their cost constraints are again very different. For developers of POC diagnostic devices, infrastructure and cost are two important and independent sets of constraints.

Hence, we propose a general description of POC use cases in a 2 x 2 matrix, which separates budget (low and moderate) from infrastructure (clinic and home) (**Fig. 3**). Design of POC devices can be targeted towards four distinct use cases.

Use Case 1 (Clinic level, moderate budget) is the least-constrained category, allowing for higher material costs and greater access to accessory equipment and trained personnel. Examples include hospitals, emergency rooms, urgent care clinics, operating rooms, intensive care units, private pharmacies, and military base clinics. At the other end of the spectrum, Use Case 4 (In the field, constrained budget) is the most constrained for a developer of POC device, operating at a low budget and minimal to no infrastructure. Examples include global health applications in low-resource settings, remote clinics, and self-testing at low cost and with minimal accessory resources. Use Cases 2 and 3 are intermediate scenarios. Use Case 2 (In the field, moderate budget) is constrained by its portability in the field but maintains a moderate budget, encompassing consumer devices for self-testing and healthcare monitoring, military personnel in the field, space travel, ambulance, and industrial and agricultural field testing. Use Case 3

(Clinic level, constrained budget) has the amenities of a basic clinical setting but is constrained by its budget. Examples include non-profit health centers and some primary care clinics.

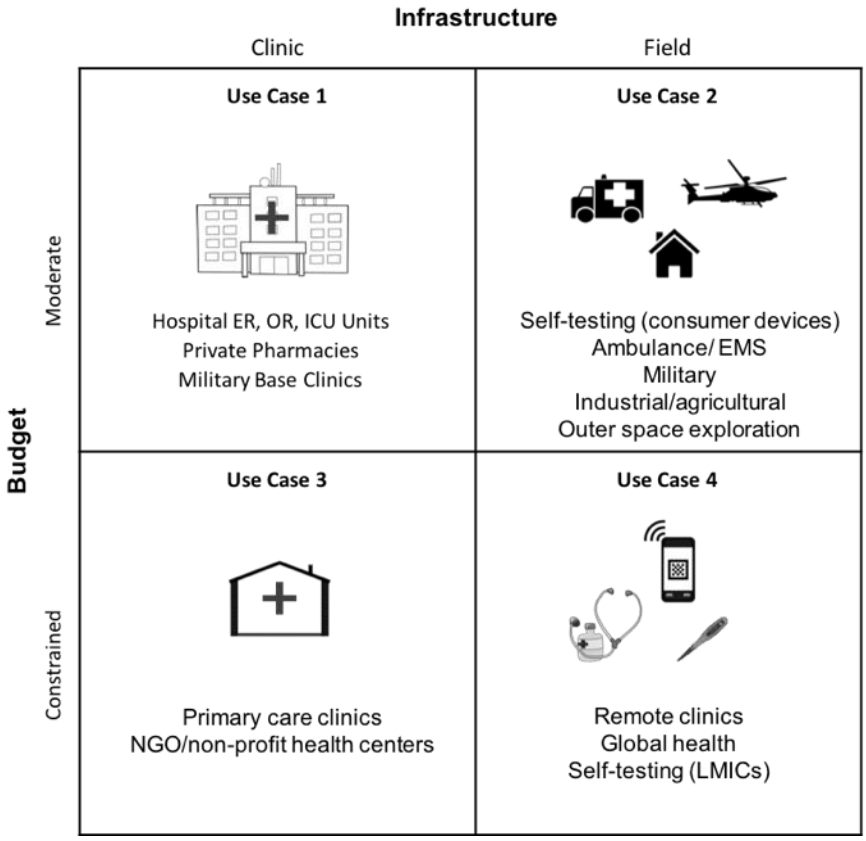

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Figure 3. POC use cases, uncoupling cost from infrastructure. Differences in budget and infrastructure level play a role in categorizing four distinct use cases for POC devices.

1.2 Objectives

In recent years, there have been a number of advances in individual microfluidic components and academic demonstrations of POC technologies[38, 39], but less progress on integration and translation to "real-world" use[40].

Paper based, lateral flow assays (sometimes called "dipstick" assays) on the other hand, have been very successful in deployment and adoption due to features like low cost, low to no-power usage, long-term stability, interpretation by minimally trained users (usually discrimination of visual bands or dots by eye), usability with a wide range of specimens, and minimal cold chain requirements for shipping/storage[41]- in sum, a consideration for usage in many POC settings (particularly for Use Case 4 resource-constrained, field usage). The World Health Organization lists 28 lateral flow rapid tests as "prequalified" in vitro diagnostic products for HIV and malaria (from companies such as SD Bioline, Chembio, Alere, Trinity Biotech, bioMerieux, OraSure, ABON Biopharm, ARKRAY Healthcare, Access Bio, Premier Medical etc[42]. Drawbacks of lateral flow assays however, are challenges with multiplexing and complex fluid handling integration as well as subjective interpretation of test lines (which can be affected by lighting conditions, amount of time passed and faintness of lines with biologically weak positive samples).

Novel microfluidic technologies can address these challenges as well as provide enhanced clinical solutions for other diagnostic use-cases. A lot of recent work in the microfluidic POC field have shown proof-concept demonstrations towards achievement of clinically relevant

sensitivity and specificity using simulated samples (and in some cases, clinical samples evaluated in laboratory conditions). Few however, have connected systems integration and consideration of downstream design and evaluation elements to push these advances closer to clinical or field use.

For systems integration for POC diagnostic devices, it will be critical to recognize that different POC settings (which include emergency room, ambulance, physician's office, pharmacy, home, or in the field) hold vastly different constraints and requirements (see Use Cases in **1.1.4**). By incorporating these design and user-experience criteria towards microfluidic POC development and evaluation, we aim to create a framework for tools that are poised for greater adoption and subsequently greater impact.

Our objective is to consider downstream translational engineering to iterate on upstream design choices and accelerate development of POC devices for real-world use. **Fig. 4** illustrates stages of development, including traditional focus areas for microfluidic researchers. We highlight areas of focus in this thesis that are guided by a holistic consideration for the use-case setting and target end-user in design and testing. For example, a key guidance parameter of assay development was to achieve clinically relevant diagnostic performance but also to minimize end-user operation for minimally trained or untrained workers, which had implications for sample type/processing, reagent delivery and fluid handling. We also undertook efforts to consider stability and preliminary methods to scale up production in a controlled manner.

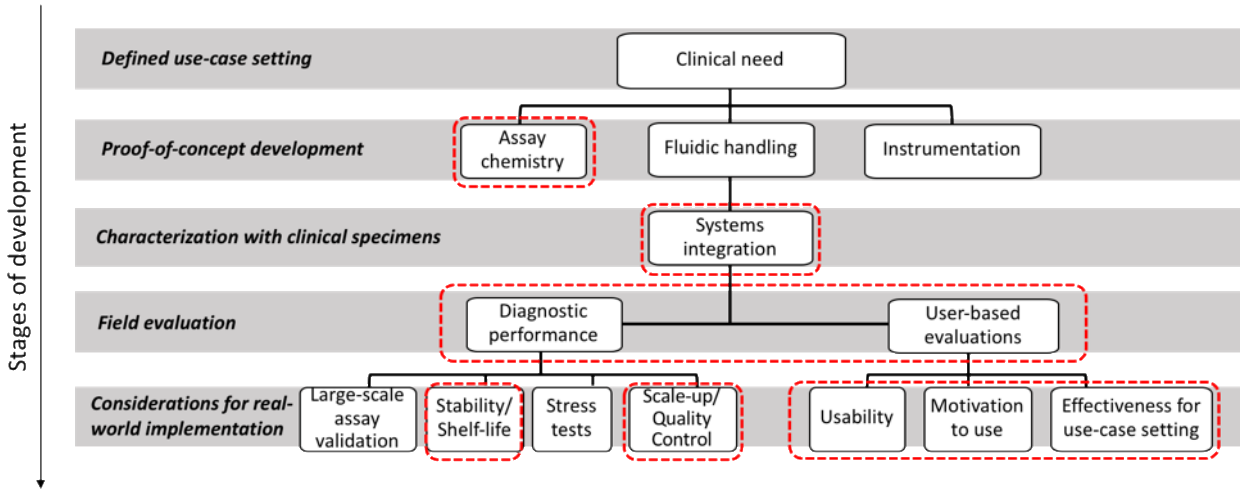

Figure 4. Translational design and evaluation focus areas of this thesis

Additionally, microfluidic devices must be integrated with hardware and software to ensure an appropriate user experience and highlight value for adoption. In this thesis, we considered user experience for systems integration with hardware and software to minimize the need for peripheral equipment and allow users to run a complex assay in a simplified format with low training burden. An understanding of the use-case setting also provides additional design considerations for hardware such as portability, ruggedness, cost, reliance on electricity etc. Implementing downstream evaluations in the field provided us with information on the diagnostic performance our devices have under real-world conditions, as well as feedback from users on feasibility of device use and acceptability for adoption. These evaluations are necessary to iterate on upstream design choices that can improve diagnostic performance, address friction points in usability and extend effectiveness for use-case implementation.

1.3 Overview of thesis

Our goal was to design and evaluate microfluidic POC diagnostic assays and platforms for specific use-cases. In doing so, we form a greater understanding of the practical engineering design criteria and considerations that could allow for new POC technologies to have greater clinical or consumer impact. We demonstrated these considerations within the full development spectrum, starting from a proof-of-concept demonstration of new assays (**Chapter 2**) to a more translational integration of components, leveraging the power of smartphones as platform devices (**Chapter 3**). We also explore the impact of these devices in the hands of different target end-users in the field, both from a provider and patient context (**Chapter 3 and 4**). Finally, we sought to perform for the first time a structured assessment of completely naïve users interacting with a smartphone interfaced microfluidic diagnostic device towards self- or home-testing (**Chapter 4**).

The contents of this dissertation are organized in the following manner:

Chapter 1: Introduction

Chapter 2: Creating rapid, accurate and multiplexed serodiagnostic tests for bacterial spirochete infections. We sought to develop rapid tests with increased specificity and sensitivity for bacterial illnesses that largely present with non-specific symptoms in humans. We focused primarily on the development of a rapid multiplexed test for Lyme Disease (**2.1-2.4**), a tick-borne illness with incidence rates that have doubled in the last decade in the United States. We aimed to replace the current two-tier serodiagnostic test format (ELISA followed by Western

blot) with a single rapid test suitable for a doctor's office or urgent care setting (Use Case 1). We sought to replicate ELISA performance in a microfluidic rapid test format as well as screen candidate antigens for potential disease staging. We also explored development of a rapid test for Leptospirosis **(2.5-2.7**), another bacterial spirochete infection with diverse and non-specific symptoms where current standard microscopic-agglutination tests (MAT) are underused due to laborious and expensive procedures.

Chapter 3: Developing a microfluidic, smartphone integrated HIV-syphilis immunoassay and demonstrating use at the point-of-care. We sought to build on previous work in introducing a dual rapid test for HIV and syphilis for low resource settings by creating an integrated diagnostic system that can be used in the field (Use Case 4). Our aims were to use whole blood as a sample input, consider scale-up in fabrication, characterize stability and optimize pre-packaging of reagents to develop a "plug and play" system. Designing for this use-case also entailed significant consideration towards cost, portability and automation. We sought to leverage the power and ubiquity of smartphones as a platform device to build a robust, integrated system with minimal user-operation steps to conduct a triplex immunoassay (HIV, treponemal syphilis, and non-treponemal syphilis). We characterized test performance of the device in the hands of minimally trained healthcare workers in Kigali, Rwanda (**3.1-3.4**). From this, we expanded assay and device development efforts in a followup pilot study with naïve users in Kigali, Rwanda to assess feasibility of smartphone-integrated diagnostic platform for home-testing in a developing country (**3.4-3.8**).

Chapter 4: Assessing the potential of a smartphone accessory for HIV home-testing. Self-testing has been proposed as the next step in HIV prevention, particularly with high risk groups, where uptake of traditional testing and counseling services remains low. We sought to expand access to HIV and other STI testing outside of the clinic by exploring the use of our smartphone accessory and accompanying application (app) for home or self-testing in the U.S. (Use Case 2). We applied the Fisher & Fisher Information-Motivation-Behavioral Skills (IMB) model as well as elements of user-experience (UX) design towards a structured evaluation of microfluidic POC technology. We sought to assess value of our product beyond an assessment of clinical sensitivity and specificity, by understanding how untrained users use our device, what the device failure points are and the most relevant design features to spur user adoption and meaningful usage. We also sought to explore user motivations and behaviors through the smartphone app with the goal of gathering large-scale testing data to improve linkage to care and health outcomes. We conducted an initial study with completely naïve users: a sample of the men who have sex with men (MSM) population in New York City who qualified as a "high risk" cohort based on self-reported sexual behaviors.

Chapter 5: Conclusions

1.4 My role in each chapter and relevant publications

Developing integrated diagnostic devices requires significant interdisciplinary and collaborative effort from team members with diverse backgrounds and expertise. The contributions from team members, collaborators and partners in the public and private sector were critical to the studies

described in this thesis. Here, I would like to clarify my role in each study. For Chapter 2, I led the multiplexed assay design, characterization and testing with clinical samples. For Chapter 3, my role was leading assay development efforts to improve the HIV/syphilis assay for field usage and helping conduct evaluation of the device in the hands of local healthcare workers in Rwanda for the first field trial. For the subsequent pilot study in Rwanda evaluating feasibility of the device for self-testing in a resource limited setting, I led study/field trial design and implementation and also guided assay and device development. For Chapter 4, I guided app and device development, designed evaluation tools and helped conduct user-testing visits in New York City.

Manuscripts related to these sections are listed below:

Chapter 2: **Nayak, S.***, Sridhara, A.*, Melo, R., Richer, L., Chee, N. H., Kim, J., ... & Gomes-Solecki, M. (2016). Microfluidics-based point-of-care test for serodiagnosis of Lyme Disease. Scientific reports (*denotes equal contribution)

Chapter 3: Laksanasopin, T.*, Guo, T. W.*, **Nayak, S.**, Sridhara, A. A., Xie, S., Olowookere, O. O., ... & Chin, C. D. (2015). A smartphone dongle for diagnosis of infectious diseases at the point of care. Science translational medicine (* denotes equal contribution)

Chapter 4: **Nayak, S.**, Guo T.W., Lopez-Rios, J., Lentz, C., Dolezal, C., Hughes, J., Frasca, T., Carballo-Diéguez, A., Balán, I., & Sia, SK. Structured evaluation of user experience with microfluidic-based point-of-care diagnostic device towards HIV home-testing (Manuscript in preparation).

CHAPTER 1. INTRODUCTION

Note: This Overview (Chapter 1) and Conclusions (Chapter 5) contain elements of a previously written review:

Nayak, S.*, Blumenfeld, N. R.*, Laksanasopin, T., & Sia, S. K. (2016). Point-of-care diagnostics: Recent developments in a connected age. Analytical chemistry (* denotes equal contribution)

Chapter 2

Creating rapid, multiplexed tests for bacterial spirochete infections

In this chapter, we examine two bacterial spirochete infections of public health interest, Lyme and Leptospirosis, where rapid multiplexed tests could provide significant clinical value. This work was performed in collaboration with Dr. Maria Gomes-Solecki's group at University of Tennessee.

Lyme Disease

2.1 Background

Lyme disease (LD) is widely distributed throughout temperate zones of the Northern Hemisphere [43] but lacks a reliable point-of-care (POC) diagnostic test. Its prevalence is high and increasing. Newly diagnosed cases have doubled in the United States over the last decade [44]. The number of probable cases of Lyme disease in the U. S. has been revised upwards by 10 fold

by the Centers for Disease Control and Prevention (CDC) to account for widespread under-reporting [45]: this number is now estimated at ˜300 000 cases per year [46].

Lyme disease is a progressive disease with a wide array of largely non-specific clinical manifestations gradually developing from early to late stage. Late disseminated infection is associated with permanent damage to the nervous and musculoskeletal systems [47]. *Erythema migrans* (EM) is the clinical marker of early infection (stage 1) in up to 80% of patients with classic Lyme disease [48]. Of the patients presenting with stage 1 LD, ˜35% present with atypical rashes that are often misdiagnosed [49], thereby putting a large group of patients at risk for developing late Lyme, antibiotic-refractory arthritis and/or post-treatment Lyme disease syndrome. In addition to *Borrelia burgdorferi* sensu stricto, the CDC recently reported the discovery of a new spirochete species (*Borrelia mayonii*) that causes LD in people in the upper Midwest [50]. The newly discovered Lyme causing *B. mayonii* is associated with additional symptoms not previously described for LD (nausea and vomiting) and with diffuse rashes rather than the classic Bull's Eye of EM which further complicates clinical diagnosis of the disease [50]. About 15% of patients treated with the recommended 2-4 week course of antibiotics will have lingering symptoms of fatigue, pain or joint and muscle aches that can last more than 6 months. Between the population of patients that present at the clinic with atypical rashes [49] and patients that are correctly diagnosed but go on to develop symptoms of late LD, a physician in an endemic area can be faced up with ~50% of patients at risk of developing late disease.

CHAPTER 2. CREATING RAPID, MULTIPLEXED TESTS FOR BACTERIAL SPIROCHETE INFECTIONS

Prompt diagnosis and treatment is critical to prevent disease progression. Unlike most bacterial diseases that can be defined microbiologically by direct observation, LD is currently defined indirectly through serologic assays given that Lyme-causing *Borrelia* grows slowly (up to 6 weeks) in culture [51]. Current laboratory based serologic assays employ the C6 ELISA or a two-tier test comprised of whole-cell or recombinant antigen ELISA followed by IgG Western blot containing a number of *B. burgdorferi* antigens such as VlsE, p100, p66, p58, p45, p41, p39, p30/31, p28 and p18. The sensitivity of these assays varies between 35-56% for Early Stage I, 73-77% for Early Stage II and 96-100% for Late Stage III LD [52-56]. However, only 10 to 50% of patients with culture confirmed very early localized Lyme disease (EM rash < 7 days) presented a detectable antibody response using the sero-analysis technology tested [57, 58].

A recent study found that the C6 ELISA can substitute for immunoblots in the two-tiered testing protocol for LD without a loss of sensitivity or specificity [59]. Thus, a rapid serodiagnostic assay which can reproduce the performance of the C6 ELISA would fill a significant void. Here, we describe how we used microfluidics technology to develop a quantitative multiplexed rapid lab-on-a-chip point of care (POC) assay for the serodiagnosis of human Lyme disease towards U.S. based urgent care or physicians' office settings (Use Case 1). We evaluate the diagnostic potential of various candidate antigens for a multiplexed serodiagnostic test (**Table 2**). Development of an assay or biomarkers that allow physicians to diagnose LD at the point of care enables prompt and proper treatment of patients.

2.2 Methods

2.2.1 Lyme Disease characterized human serum panel

Blinded de-identified surplus serum samples from patients with signs and symptoms of Lyme disease enrolled in previous studies conducted by the Lyme Disease Center at Stony Brook University, reference Lyme disease panels from CDC, and reference healthy individuals from a commercial source were used. Informed consent was obtained from all patients enrolled in the studies that originated the samples. Use of these samples was approved under FWA00021769 by IntegReview, Inc. Ethical Review Board IRB #2. The involvement of human subjects in the proposed studies falls under Exemption 4 as outlined under HHS regulations (45 CFR Part 46) and is not considered "clinical research" as defined by NIH. The methods were carried out in accordance with the relevant guidelines.

Three different archived serum panels were used for testing and evaluation:

1) *Stony Brook Lyme Disease panel, n=20 samples.* Twenty samples from patients presenting at the clinic with signs and symptoms of Lyme disease, some of which were culture confirmed. These samples were used to do preliminary studies and improvement of testing parameters.
2) *CDC LD panel, n=40 samples.* Thirty-five samples in this panel were collected from patients diagnosed with Lyme disease by experienced physicians in endemic areas (Northeast and upper-Midwest). Five of the samples included in the panel were obtained from healthy individuals from the same areas. An extensive set of information, ranging from detailed clinical symptoms at presentation to serologic data from ELISA and Western Blot for these patients has been characterized. In addition, *B. burgdorferi* was cultured from 88% of the early Lyme cases and the remaining patients met a rigorous case definition for early

disseminated or late Lyme disease and had a seropositive ELISA result. This panel as well as all information on its clinical characterization was kindly provided by Dr. Martin Schriefer from the NCID/CDC.

3) *Cross-Reactive Human Sera Panel, n=25 samples.* Sera from healthy patients from a non-endemic area (Golden West Bio, Tennessee) was used to do specificity studies.

2.2.2 Peptides (Lyme)

The following peptides were used: pepBBK07 (kindly provided by Dr. Utpal Pal, U. of Maryland) and PepVF (synthesized at GenScript, Piscataway, NJ). PepVF design was modified from a peptide based in the core sequence from the full length 25-residue IR6 from *B. burgdorferi* B31 [60] by adding a 13 amino-acid sequence from FlaB. Crude extract of PepVF was synthetized by GenScript.

2.2.3 Protein Purification

OspA (outer surface lipoprotein A), OspB (outer surface lipoprotein B), OspC-K (outer surface lipoprotein C type K) and OspC-B (outer surface lipoprotein C type B) were purified in Dr. Gomes-Solecki's laboratory as follows. Recombinant *E. coli* clones were grown in Tryptone Broth Yeast (TBY) medium supplemented with 50 μg/ml Kanamycin (Kn) at 37°C, shaking at 225 rpm, until it reached an OD_{600} of 0.8. The expression of 6xHis tagged recombinant proteins was induced by adding 1 mM IPTG (isopropyl-β-d-thiogalactopyranoside) to the cells followed by incubation at 37ºC for 3h. The cells were harvested by centrifugation at 4000 x g for 10 min at 4ºC. The proteins were purified by affinity chromatography using the Ni-NTA Purification

System (Invitrogen) following the manufacturer instructions. Protein concentration was determined by the Bradford protein assay (Bio-Rad, Hercules, CA, USA), and was stored at -80^{o}C. Pure recombinant proteins (crude extract) were analyzed on a 10% denaturing polyacrylamide gel and electrotransferred to a polyvinyldene difluoride membrane (PVDF, Millipore, Billerica, MA) for analysis with antigen-specific- polyclonal mouse antibody. Purified recombinant proteins from *B. burgdorferi* such as flaB (flagellin B, p41), p100 (membrane lipoprotein p100/p93), BmpA (laminin binding protein A, p39), DbpA (decorin binding protein A), DbpB (decorin binding protein B) were purchased from ProSpec (Rehovot, Israel); VlsE (Variable Major Protein like sequence E, surface exposed lipoprotein) was purchased from My BioSource (San Diego, CA).

2.2.4 Serological immune responses using ELISA

Serum was tested for the presence of IgG against purified recombinant proteins and peptides by indirect ELISA, performed at Dr. Maria Gomes-Solecki's laboratory. Antigens were coated on flat-bottom ELISA plates at 2µg/ml (Nunc MaxiSorp™, ThermoFisher) and indirect ELISA was performed using human serum (1:100). Goat anti-human (Lyme) or goat-anti-mouse (Leptospirosis) IgG (1:50,000) horseradish peroxidase-conjugated antibody (Jackson Immunoresearch, USA) was used as secondary antibody. Four healthy samples from the CDC panel were used (3 standard deviation above the mean) to determine the cutoff of the assay in Method 1; one healthy sample was positive against all recombinant *B. burgdorferi* proteins and was excluded from this study. Method 2 cutoff was determined by ROC curve analysis and choosing a point that maximized sensitivity and specificity.

2.2.5 Point-of-care immunoassay

Injection molded plastic microfluidic cassettes were functionalized by direct adsorption of antigen candidates (purified recombinant proteins and peptides of *B. burgdorferi*) at the following concentrations: 100 μg mL^{-1} pepVF, 20 μg mL^{-1} rOspC-K, 30 μg mL^{-1} rOspC-B, 1 μg mL^{-1} rVlsE, 200 μg mL^{-1} rP41, 60 μg mL^{-1} rP100, 30 μg mL^{-1} rDbpB, 100 μg mL^{-1} rBmpA, and 20 μg mL^{-1} rDbpA. Avidin-biotin conjugation was used for pepBBK07 functionalization: 50 μg mL^{-1} streptavidin followed by 60 μg mL^{-1} pepBBK07. Functionalized cassettes included an internal negative control zone, spotted with no antigen, an internal positive control zone, spotted with 20 μg mL^{-1} rabbit anti-goat IgG antibody (Life Technologies) and multiplexed combination of three target antigen zones. All zones were treated for 1 hour with 1% BSA-0.05% Tween-20 in PBS for blocking and stabilizing. Further details on cassette preparation can be found in previous studies[61-64]. To run the assay, polyethylene tubing (inner diameter: 0.86 mm, Zeus) was pre-loaded with all reagents and connected to the cassette inlet for delivery, in a method previously described [61, 62, 65]; initial wash of 2 μL of 0.05% Tween-20 in PBS, 30 μL of serum/plasma sample (10X dilution in 1% BSA), four 2 μL 0.05% Tween-PBS washes, 14.5 μL secondary gold-conjugated anti-hIgG (1.06ug/mL) and anti-hIgM (0.54 ug/ml) antibodies (OPKO Diagnostics) in 3% BSA-0.2% Tween-20 in PBS, followed by two 2 μL 0.05% Tween-PBS washes and four 2 μL water washes, each separated by air spaces (**Fig. 3A**). As in previous work, these steps take about 10 minutes to flow (which includes all the binding steps of the immunoassay)[63]. Silver nitrate and reducing agents (OPKO Diagnostics) were subsequently drawn through the cassette. An initial intensity reading (I_0) was taken immediately after silver

entered the channel, and another intensity reading (*I*) was taken after 4.5 minutes of silver development. All experiments were read on a bench-top analyzer (OPKO Diagnostics) (**Fig. 3A)**. Optical density was calculated as:

$$OD = - log [I/ I_0]$$

2.2.6 Statistical analysis

Assay performance of test was reported in term of sensitivity and specificity compared to reference test. Given the number of true positives (TP), true negatives (TN), false positives (FP), and false negatives (FN), the performance metrics are:

$$sensitivity = \frac{TP}{FN+TP}$$

$$specificity = \frac{TN}{FP+TN}$$

For Lyme panel analysis, reference tests were culture confirmation and clinical diagnosis. In one method, receiver operating characteristic (ROC) analysis was performed to determine cutoff values and assess sensitivity and specificity. In another method, cutoff values were established at 3STDEV above the average of four negative control samples. 95% Confidence Intervals (95% CI) were calculated. Calculations were performed using Graphpad Prism. For markers containing multiple antigens (e.g. the three antigens multiplexed Lyme tests), we used a 1:1:1 ratio of weighting constants to add up the signals, and then used ROC analysis to compare area-under-the-curve (AUCs) with a new cutoff, similar to evaluation of different permutations of biomarkers as demonstrated in other works evaluating pooled set of markers [66, 67].

Table 2. Descriptions of Lyme antigens screened

Family	Antigen	Name	Description
Membrane Proteins	OspA	Outer surface lipoprotein A	Protein in borrelial outer surface membrane; expressed mainly by B.burgdorferi in ticks [68]
	OspB	Outer surface lipoprotein B	Protein in borrelial outer surface membrane; expressed mainly by B.burgdorferi in ticks [68]
	OspC-K	Outer surface lipoprotein C, type K	Protein in borrelial outer surface membrane; expressed by B.burgdorferi during transmission of spirochetes from ticks to mammals as well as in the vertebrate host [68]
	OspC-B	Outer surface lipoprotein C, type B	Protein in borrelial outer surface membrane; produced during early infection [69]
	BmpA	Basic membrane protein A (p39)	Borrelial outer membrane protein that binds to laminin in host's extracellular matrix; implicated as playing a role in some symptoms of Lyme disease [70]
	DpbA	Decorin binding protein A	Adhesin protein of B. burgdorferi that binds to decorin (a proteoglycan on surface of human cells); implicated in mediating tissue adherence of B. burgdorferi [71]
	DbpB	Decorin binding protein B	Adhesin protein of B. burgdorferi that binds to decorin (a proteoglycan on surface of human cells); implicated in mediating tissue adherence of B. burgdorferi [71]
	VlsE	Variable major protein-like sequence E	Surface exposed lipoprotein; belongs to a family of immunodominant variable major surface lipoproteins or VMPs that were involved in multiphasic antigenic variation in related Borrelia species [72]
Peptides	PepVF	Peptide VF	Synthetic peptide isolated from a conserved region of VlsE
	p100	Membrane lipoprotein p100 (p93)	Immunodominant polypeptide
	BBK07	Lipoprotein BBK07	Peptide isolated from a surface exposed lipoprotein
Flagellar protein	flaB (p41)	Flagellin B, p41	Protein found in borrelial flagella

2.3 Results

2.3.1 Lyme Disease point-of-care device

The POC device, which we call mChip-Ld, consists of a signal detection device and a disposable, injection molded plastic cassette on which all the biochemical steps of the immunoassay are carried out (**Fig. 5**). Lyme-specific antibodies in a patient blood sample bind to antigens immobilized on the surface of the microfluidic cassette. Subsequent automated reagent delivery of all washes, secondary antibodies and silver amplification reagents results in silver ion reduction on gold nanoparticles attached to cassette surface. The detection mechanism, as previously described[61], consists of paired light-emitting diodes (LEDs) and photodetectors aligned directly with the microfluidic test zones (**Fig. 5A**). When inserted into the analyzer, each microfluidic test zone of the cassette is sandwiched between a red LED aligned above with a 1mm pinhole and red-sensitive photodiode aligned directly below each test zone. Silver development on the cassette results in a proportional decrease in the light sensed by the photodiode and can be quantified by optical density values (**Fig. 5B**). Assay time to result is approximately 15 minutes.

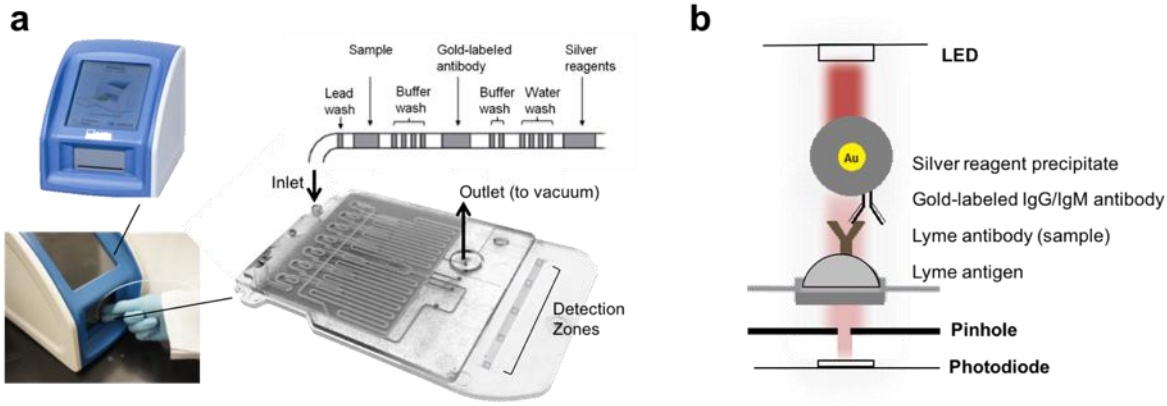

Figure 5. Overview of the mChip-Ld Device. (A) The plastic microfluidic cassette is inserted into a benchtop analyzer (Claros1 Analyzer, OPKO) which is used to power fluid flow, control temperature setpoints and detect signals. Sera/plasma samples as well as all washes, gold-labeled secondary antibodies and silver amplification reagents are pre-loaded and delivered automatically in sequence, passing over the five detection zones of the microfluidic channel. Pressure driven flow is achieved through attachment of a vacuum (simple syringe or benchtop analyzer) to the microfluidic cassette outlet. (**B)** Schematic of the biochemical and optical set up. Lyme antigens are adsorbed to the surface of the plastic microfluidic cassette. Sequential binding of the sample antibodies, gold-labeled detection antibodies and silver amplification reagents results in a visible signal that can be quantified as the optical density of the detection zone. Light emitted from a LED above the detection zone is collected by a photodiode. The presence of silver development, which absorbs incident light, reduces light sensed by the photodiode.

2.3.2 Testing candidate antigens on ELISA

In order to develop multiplexed panel designs for the POC test, we first screened candidate antigens using conventional ELISA. We examined 12 candidate recombinant antigens: rP100, rBmpA, rP41 (FlaB), rDbpA, rDbpB, rOspA, rOspB, rOspC-K, rOspC-B, rVlsE, pepBBK07, and PepVF (**Fig. 6, Table 3**). We used a panel of Lyme positive samples (n=35) characterized by the CDC and healthy samples taken from non-endemic areas (n=25). Two cutoff methods were evaluated in the screening of candidate markers on ELISA. In Method 1, four healthy samples from the same area of collection as the Lyme positive samples were tested. Cutoff for each antigen was determined as 3 standard deviations above the mean signal of these four samples, thus prioritizing a high specificity test. In Method 2, receiver-operator curve (ROC) analysis was used to select a cutoff maximizing sensitivity and specificity looking at area under the curve

(AUC), prioritizing a high sensitivity test. The full table of results is shown in **Table 3**. For the intended application of designing a high sensitivity test for screening decisions, Method 2 was chosen for subsequent analysis.

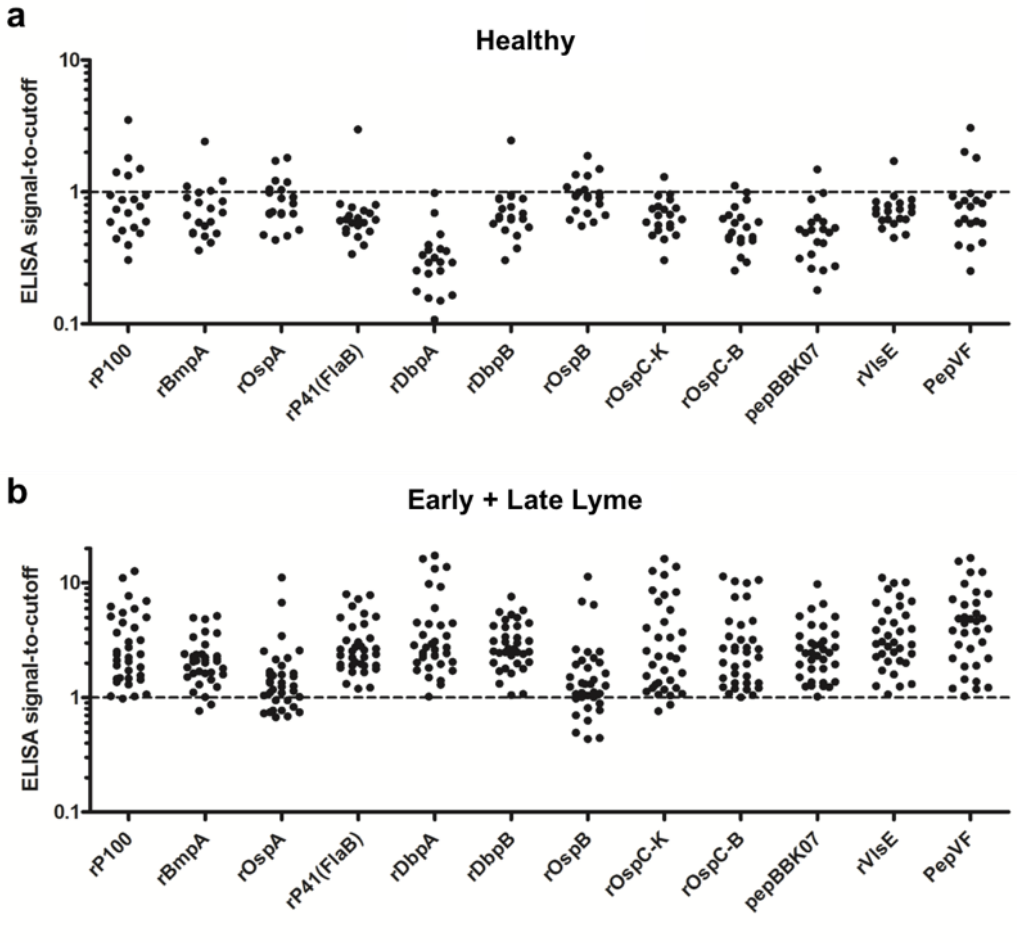

Figure 6. ELISA screening of candidate antigens for Lyme diagnosis. Signal to cutoff plots showing specificity and sensitivity of candidate antigens using IgG ELISA compared to reference testing with clinical evaluation and cultures. **(A)** 25 sera samples from healthy individuals in a non-endemic area and **(B)** 35 sera samples clinically characterized as positive by the CDC for Lyme Disease and were used to test specificity and sensitivity of candidate antigens of rP100, rBmpA, rOspA, rP41 (FlaB), rDbpA, rDbpB, rOspB, rOspC-K, rOspC-B, pepBBK07, rVlsE and PepVF.

Table 3. Performance of two ELISA cutoffs for diagnosing Lyme disease (at any stage)

ELISA Performance with Cutoff Method 1 (High Specificity): Early + Late Lyme						
	Sensitivity (%)	**CI (95%)**	**Specificity (%)**	**CI (95%)**	**Cutoff**	**AUC**
rP100	42.9	26.32 - 60.65	100.0	88.78 - 100.0	0.250	0.971
rBmpA	31.4	16.85 - 49.29	95.0	75.13 - 99.87	0.296	0.936
rOspA	22.9	10.42 - 40.14	95.0	75.13 - 99.87	0.128	0.784
rP41	68.6	50.71 - 83.15	95.0	75.13 - 99.87	0.310	0.967
rDpbA	42.9	26.32 - 60.65	100.0	83.16 - 100.0	0.190	1.000
rDbpB	34.3	19.13 - 52.21	100.0	83.16 - 100.0	0.194	0.981
rOspB	20.0	8.44 - 36.94	100.0	83.16 - 100.0	0.154	0.714
rOspC-K	34.3	19.13 - 52.21	93.6	78.58 - 99.21	0.408	0.976
rOspC-B	54.3	36.65 - 71.17	100.0	88.43 - 100.0	0.444	0.996
pepBBK07	28.6	14.64 - 46.30	100.0	83.16 - 100.0	0.319	0.989
rVlsE	82.9	66.35 - 93.44	100.0	83.16 - 100.0	0.303	0.993
PepVF	77.1	59.86 - 89.58	95.0	75.13 - 99.87	0.124	0.961
ELISA Performance with Cutoff Method 2 (High Sensitivity): Early + Late Lyme						
	Sensitivity (%)	**CI (95%)**	**Specificity (%)**	**CI (95%)**	**Cutoff**	**AUC**
rP100	97.1	85.08 - 99.93	75.0	50.90 - 91.34	0.090	0.971
rBmpA	94.3	80.84 - 99.30	80.0	56.34 - 94.27	0.128	0.936
rOspA	71.4	53.70 - 85.36	70.0	45.72 - 88.11	0.070	0.784
rP41	100.0	90.00 - 100.0	95.0	75.13 - 99.87	0.157	0.967
rDpbA	100.0	90.00 - 100.0	100.0	83.16 - 100.0	0.064	1.000
rDbpB	100.0	90.00 - 100.0	95.0	75.13 - 99.87	0.060	0.981
rOspB	74.3	56.74 - 87.51	70.0	45.72 - 88.11	0.071	0.714
rOspC-K	94.3	80.84 - 99.30	95.0	75.13 - 99.87	0.106	0.976
rOspC-B	100.0	90.00 - 100.0	95.0	75.13 - 99.87	0.188	0.996
pepBBK07	100.0	90.00 - 100.0	95.0	75.13 - 99.87	0.103	0.989
rVlsE	100.0	90.00 - 100.0	95.0	75.13 - 99.87	0.158	0.993
PepVF	100.0	90.00 - 100.0	85.0	62.11 - 96.79	0.057	0.961

Abbreviations: CI, confidence interval; AUC, area under the curve.

Table 4. Results of ELISA screening using 12 antigens, segmented by Early-Lyme and Late-Lyme samples.

Table 4A

ELISA: Early Lyme					
	Sensitivity (%)	**CI (95%)**	**Specificity (%)**	**CI (95%)**	**AUC**
rP100	96	79.65 - 99.90	75	50.90 - 91.34	0.904
rBmpA	92	73.97 - 99.02	80	56.34 - 94.27	0.928
rOspA	68	46.50 - 85.05	70	45.72 - 88.11	0.755
rP41	100	86.28 - 100.0	95	75.13 - 99.87	0.964
rDpbA	100	86.28 - 100.0	100	83.16 - 100.0	1.000
rDbpB	100	86.28 - 100.0	95	75.13 - 99.87	0.984
rOspB	68	46.50 - 85.05	70	45.72 - 88.11	0.685
rOspC-K	96	79.65 - 99.90	95	75.13 - 99.87	0.983
rOspC-B	100	86.28 - 100.0	90	68.30 - 98.77	0.998
pepBBK07	100	86.28 - 100.0	95	75.13 - 99.87	0.988
rVlsE	100	86.28 - 100.0	95	75.13 - 99.87	0.990
PepVF	100	86.28 - 100.0	85	62.11 - 96.79	0.960

Table 4B

ELISA: Late Lyme					
	Sensitivity (%)	**CI (95%)**	**Specificity (%)**	**CI (95%)**	**AUC**
rP100	100	69.15 - 100.0	65	40.78 - 84.61	0.960
rBmpA	100	69.15 - 100.0	75	50.90 - 91.34	0.955
rOspA	80	44.39 - 97.48	65	40.78 - 84.61	0.855
rP41	100	71.51 - 100.0	90	68.30 - 98.77	0.973
rDpbA	100	69.15 - 100.0	95	75.13 - 99.87	1.000
rDbpB	100	69.15 - 100.0	90	68.30 - 98.77	0.975
rOspB	90	55.50 - 99.75	65	40.78 - 84.61	0.785
rOspC-K	90	55.50 - 99.75	90	68.30 - 98.77	0.960
rOspC-B	100	69.15 - 100.0	95	75.13 - 99.87	0.990
pepBBK07	100	69.15 - 100.0	90	68.30 - 98.77	0.990
rVlsE	100	69.15 - 100.0	90	68.30 - 98.77	1.000
PepVF	100	69.15 - 100.0	80	56.34 - 94.27	0.965

Abbreviations: CI, confidence interval; AUC, area under the curve

When testing the full panel of Lyme samples by ELISA, covering both Early and Late Stage Lyme, rP41, rDbpA, rDbpB, rOspC-B, rVlsE, pepBBK07 and PepVF showed the highest sensitivities (100% sensitivity) and specificities (> 90%) (**Fig. 6, Table 3**). The following proteins were highly sensitive as diagnostic candidates using Early Lyme test samples (>95% sensitivity): rP100, rP41, rDbpA, rDbpB, rOspC-K, rOspC-B, rVlsE, pepBBK07 and PepVF (**Table 4A**). Using Late Lyme samples we identified the following antigens (100% sensitivity): rP100, rBmpA, rP41, rDbpA, rDbpB, rOspC-B, rVlsE, pepBBK07 and PepVF (**Table 4B**). The least cross-reactive antigens (> 90% specificity) tested against serum samples from healthy individuals were: rP41, rDbpA, rDbpB, rOspC-K, rOspC-B, rVlsE and pepBBK07 (Fig. 2A). rOspA with sensitivity of 71.4% (95% CI: 54-85%), specificity of 70.0% (95% CI: 46-88%) and AUC of 0.784, as well as rOspB with sensitivity of 74.3% (95% CI: 57-87%) specificity of 70% (95% CI: 46-88%) and AUC of 0.714 showed the poorest performance compared to other antigens and were eliminated as candidates for POC testing (**Table 3**). By deconstructing diagnostic performance with the full sample panel into Early Lyme (**Table 4A**) and Late Lyme (**Table 4B**), we also illustrate the potential of candidate antigens for disease staging as well as diagnosis.

2.3.3 Screening candidate antigens on the microfluidic platform

To characterize sensitivity and specificity of these antigens for application in Lyme diagnosis using the rapid microfluidics format, we performed a preliminary screening test. Due to the large number of antigens being screened (ten), we did not optimize this screening test as much as some previous studies on the mChip system[61-64]. For example, we did not optimize the

conjugation chemistry beyond physiosorption, concentration of coating protein, and blocking conditions. Instead, we looked for the markers that produced the best relative performance within the mChip-Ld data set. Twenty patient samples were used to evaluate various permutations of multiplexed markers with surface conditions suitable for a panel (i.e. balancing conditions for optimal signal-to-noise ratios for most markers on a panel, not necessarily the highest signal-to-noise ratios that can be recorded on the mChip-Ld platform for an individual marker).

Next we screened the immunoassay potential of individual candidate antigens (rP100, rP41/FlaB, rDbpA, rOspC-K, rOspC-B, rVlsE, pepBBK07 and PepVF) on the mChip-Ld POC platform against the same Lyme positive and healthy sample panels used in ELISA screening experiments (**Fig. 7, Table 5**). rOspC-K and PepVF proteins were highly sensitive (>80% sensitivity) as diagnostic candidates using Early Lyme test samples (**Table 6A**). Using Late Lyme samples, we identified the following antigens (>70% sensitivity): rP100, pepBBK07, rVlsE, rOspC-B and PepVF, with the latter two markers having >90% sensitivity (**Table 6B**). The least cross-reactive antigens (> 80% specificity) tested against serum samples from healthy individuals were: rVlsE, rOspC-K and PepVF (**Fig. 7A**). A breakdown of Early and Late Lyme classification by candidate antigen on the POC platform shows that OspC-K skews towards ‘positive” disease classification for Early Lyme and ‘negative’ for Late Lyme, suggesting potential for discrimination between disease stages (**Table 6**).

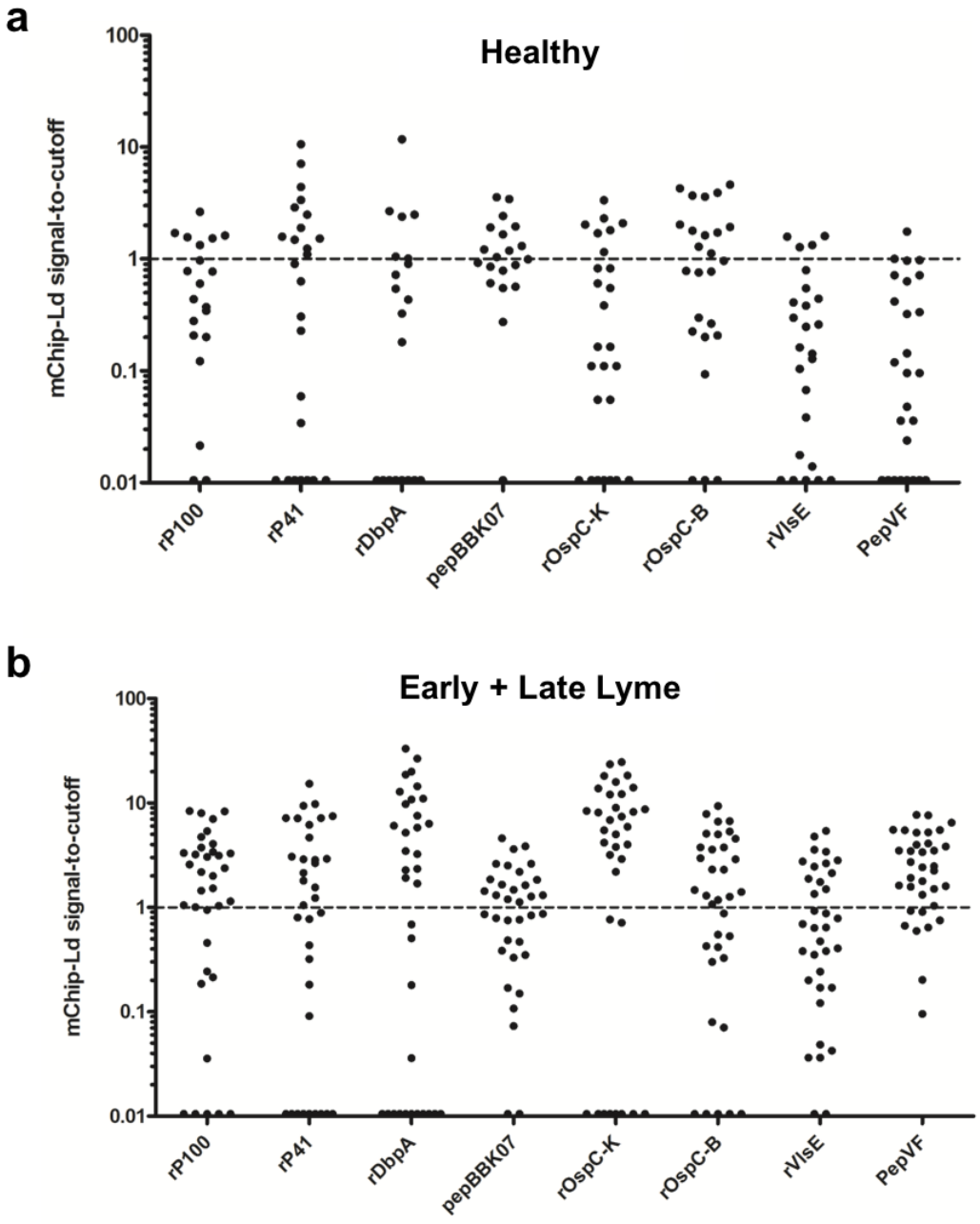

Figure 7. Preliminary screening of candidate antigens with mChip-Ld. Signal to cutoff plots showing specificity and sensitivity of candidate antigens on the mChip-Ld platform. Recombinant antigens rP100, rP41 (FlaB), rDbpA, pepBBK07, rOspC-K, rOspC-B, rVlsE and PepVF were chosen for testing from the ELISA screening study. **(A)** 25 sera samples from healthy individuals in a non-endemic area and **(B)** 35 sera samples clinically characterized as positive by the CDC for Lyme Disease were used to test specificity and sensitivity of candidate antigens.

Table 5. Performance of mChip-Ld screening using 8 antigens, for diagnosing Lyme disease at any stage.

	POC: Early + Late Lyme				
	Sensitivity (%)	**CI (95%)**	**Specificity (%)**	**CI (95%)**	**AUC**
rP100	68.6	50.71 - 83.15	70.0	45.72 - 88.11	0.699
rP41	62.9	44.92 - 78.53	44.0	24.40 - 65.07	0.579
rDbpA	51.4	33.99 - 68.62	80.0	56.34 - 94.27	0.650
pepBBK07	51.4	33.99 - 68.62	50.0	27.20 - 72.80	0.515
rOspC-K	68.6	50.71 - 83.15	92.3	74.87 - 99.05	0.778
rOspC-B	62.9	44.92 - 78.53	48.0	27.80 - 68.69	0.581
rVlsE	40.0	23.87 - 57.89	84.0	63.92 - 95.46	0.714
PepVF	94.3	80.84 - 99.30	73.1	44.33 - 82.79	0.934

Table 6. Results of mChip-Ld screening using 8 antigens, segmented by Early-Lyme and Late-Lyme samples

Table 6A

POC: Early Lyme					
	Sensitivity (%	**CI (95%)**	**Specificity (%)**	**CI (95%)**	**AUC**
rP100	68.0	46.50 - 85.05	70.0	45.72 - 88.11	0.680
rP41	60.0	38.67 - 78.87	56.0	34.93 - 75.60	0.643
rDbpA	56.0	34.93 - 75.60	80.0	56.34 - 94.27	0.621
pepBBK07	56.0	34.93 - 75.60	65.0	40.78 - 84.61	0.560
rOspC-K	84.0	63.92 - 95.46	92.3	74.87 - 99.05	0.877
rOspC-B	76.0	54.87 - 90.64	56.0	34.93 - 75.60	0.717
rVlsE	68.0	46.50 - 85.05	64.0	42.52 - 82.03	0.682
PepVF	92.0	73.97 - 99.02	73.1	52.21 - 88.43	0.914

Table 6B

POC: Late Lyme					
	Sensitivity (%)	**CI (95%)**	**Specificity (%)**	**CI (95%)**	**AUC**
rP100	70.0	34.75 - 93.33	70.0	45.72 - 88.11	0.745
rP41	60.0	26.24 - 87.84	64.0	42.52 - 82.03	0.582
rDbpA	60.0	26.24 - 87.84	95.0	75.13 - 99.87	0.723
pepBBK07	70.0	34.75 - 93.33	70.0	45.72 - 88.11	0.703
rOspC-K	40.0	12.16 - 73.76	96.2	80.36 - 99.90	0.529
rOspC-B	90.0	55.50 - 99.75	64.0	42.52 - 82.03	0.758
rVlsE	70.0	34.75 - 93.33	100.0	86.28 - 100.0	0.794
PepVF	100.0	69.15 - 100.0	96.2	80.36 - 99.90	0.985

Abbreviations: CI, confidence interval; AUC, area under the curve

2.3.4 Comparison of ELISA with the multiplexed microfluidic chip test (mChip-Ld)

Next, we analyzed four potential multiplex panel designs: (1) rP100+ PepVF + rOspC-K; (2) rVlsE+ PepVF+ rOspC-K; (3) rVlsE+ rP41+ rOspC-K, and (4) rVlsE+ rDbpA+ rOspC-K on both ELISA and POC platforms compared to gold standard reference tests of clinical classification and culture tests (**Fig. 8, Table 7)**. A simple multivariate model was used to combine the signals for each marker and develop a new cutoff in order to classify samples as positive or negative in these multiplexed tests. rP100+ PepVF + rOspC-K, had 94.3% sensitivity 90% specificity on ELISA compared to 88.5% sensitivity, 90% specificity (AUC: 0.844) on the mChip-Ld system (**Fig. 8A, Table 7**). The remaining combinations of: (2) rVlsE+ PepVF+ rOspC-K, (3) rVlsE+ rP41+ rOspC-K and (4) rVlsE+ rDbpA+ rOspC-K all had 100% sensitivity, and >95% specificity on ELISA. On the mChip-Ld system, combination (2) of rVlsE+ PepVF+ rOspC-K yielded the highest sensitivity of 94.3%. with 75% specificity (AUC: 0.932) (**Fig. 8B, Table 7**). The triplexed panel rP100+ PepVF + OspC-K (**Fig. 6A**) showed comparable performance on both the ELISA and POC plaforms when compared to clinical classification and culture tests. VlsE+ PepVF+ OspC-K (**Fig. 8B**) had higher sensitivity than p100+ PepVF + OspC-K on the POC platform, however with a tradeoff of lower specificity with the cutoff chosen here.

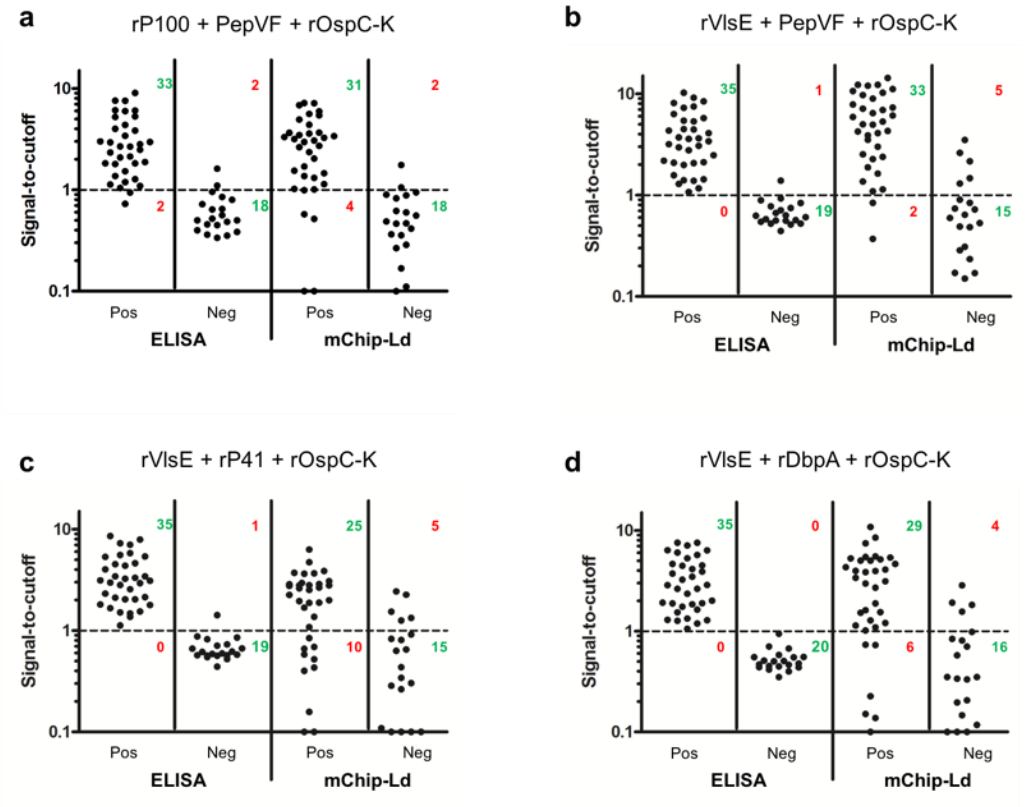

Figure 8. Comparison of ELISA and an initial version of multiplexed microfluidic chip platform (mChip-Ld). A vertical scatterplot showing signal-to-cutoff ratios of samples positive (Pos) or negative (Neg) for Lyme Disease, as determined by gold standard tests of clinical confirmation and culture tests, on ELISA and mChip platforms using a multiplexed combination of **(A)** rP100, PepVF and rOspC-K; **(B)** rVlsE, PepVF and rOspC-K; **(C)** rVlsE, rP41 and rOspC-K, and **(D)** rVlsE, rDbpA and rOspC-K.

Table 7. Comparison of ELISA and point-of-care (POC) multiplexed tests

ELISA: Early + Late Lyme					
	Sensitivity (%)	**CI (95%)**	**Specificity (%)**	**CI (95%)**	**AUC**
rP100+PepVF+rOspC-K	94.3	80.84 - 99.30	90.0	68.30 - 98.77	0.976
rVlsE+PepVF+rOspC-K	100	90.00 - 100.0	95.0	75.13 - 99.87	0.994
rVlsE+rP41+rOspC-K	100	90.00 - 100.0	95.0	75.13 - 99.87	0.997
rVlsE+rDbpA+rOspC-K	100	90.00 - 100.0	100	83.16 - 100.0	1.000
POC: Early + Late Lyme					
	Sensitivity (%)	**CI (95%)**	**Specificity (%)**	**CI (95%)**	**AUC**
rP100+PepVF+rOspC-K	88.5	69.74- 95.19	90.0	68.30 - 98.77	0.844
rVlsE+PepVF+rOspC-K	94.3	80.84 - 99.30	75.0	50.90 - 91.34	0.932
rVlsE+rP41+rOspC-K	71.4	53.70 - 85.36	75.0	50.90 - 91.34	0.807
rVlsE+rDbpA+rOspC-K	82.9	66.35 - 93.44	80.0	56.34 - 94.27	0.844

Abbreviations: CI, confidence interval; AUC, area under the curve

We compared the sensitivity of the current standard C6 ELISA to a multiplexed POC test consisting of p100+ PepVF + OspC-K (**Fig. 9**) . The multiplexed POC test achieves comparable results to the C6 ELISA in identifying Lyme positive samples (88.5% mCHIP v 85.7% C6 sensitivity).

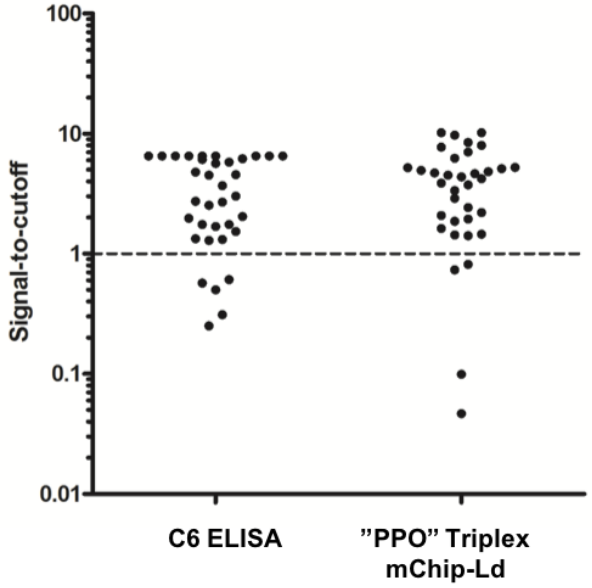

Figure 9. Comparison of C6 ELISA Lyme test with "PPO" triplexed mChip-Ld test. Vertical scatter plots showing samples previously validated to be positive for Lyme disease (Early and Late Stage) as tested on the C6 ELISA test (left) and multiplexed mChip device (right). The multiplexed mChip test used a combined signal of three proteins (rP100, PepVF and rOspC-K), and produced results comparable to the single-antigen C6 ELISA.

2.4 Discussion

Here, we report the development of a microfluidics based rapid assay for the serodiagnosis of Lyme disease. The platform is designed for intended use in primary or urgent care settings in the U.S., with an assay that can be done by moderately trained personnel in 15 minutes.

Previously we have demonstrated a dual HIV and syphilis immunoassay using low-cost hardware for lower resource settings (Use-Cases 3 & 4)[61, 64]. In designing a POC assay primarily for a doctors's office or an urgent care setting in the U.S. (Use Case 1), some design criteria such as portability and cost are less constrained. Here, we leveraged the simple and low cost optics required for signal detection with a proprietary analyzer developed by OPKO Diagnostics, that can be packaged as a benchtop instrument or battery-operated hand held unit, with wireless, wired or printer output capabilities. Fluid handling, temperature control and signal detection modules are integrated and automated into a single device that is suitable for primary or urgent care settings. The detection mechanism, as previously described [61], consists of paired light-emitting diodes (LEDs) and photodetectors aligned directly with the microfluidic test zones (**Fig. 5**). The material cost of each disposable cassette is about $1.50[62] [63].

Our prior work showed an application for our POC system in a Sub-Saharan setting where temperature and humidity can be significant factors; here, we anticipate primary usage in a Use Case 1 setting where refrigeration is available for shipping and storage, and where environmental conditions are typically controlled. Thus, assay reagents such as secondary antibodies were left in liquid form under refrigeration. Future steps include optimization of assay parameters (such as anti-coagulation reagents and washing conditions) to work with whole-blood samples, as performed previously[62, 63].

All the biomarkers tested have been shown to detect antibodies in blood from Lyme disease patients. Most antigens (rVlsE, rP93/100, rBmpA/P39, rFlaB/P41, rDbpA/P18, rDbpB/P18,

rOspC/P23) are currently included in Western blot or line blot assays [73, 74]. We found that two types of OspC (type K and type B) might be ideal to identify early Lyme disease samples [75]. OspA and OspB were identified as potential good candidates to discriminate late Lyme [76]. BBK07 immunodominant peptides were defined as good serodiagnostic markers for Lyme disease [77, 78]. In addition, the C6 peptide ELISA was widely adopted for diagnosis of Lyme disease [52, 79-81]. We modified a peptide based in the core sequence from C6 from *B. burgdorferi* B31 [60] and added a 13-aminoacid sequence from FlaB (pepVF). In the studies reported here, ELISA screening (**Fig. 6**) showed six recombinant proteins, p100, p41, DbpA, OspC-K, OspC-B, VlsE and two peptides pepBBK07 and pepVF, were promising antigens for diagnosis of Lyme disease. rOspA and rOspB showed the poorest sensitivity and specificity performance compared to other antigens and were eliminated as antigens for point of care.

The mChip system has comparable performance to ELISA with respect to sensitivity and specificity but is faster and can be implemented at the point of care. Further, we can separate antigens per zone of detection, which allows the physician to make a comprehensive diagnostic decision. Our ELISA results identified the eight antigen candidates for the microfluidics screen (**Fig. 7**). These studies led to the further elimination of two antigenic candidates, OspC-B and pepBBK07 due to high cross-reactivity with healthy samples. At this point we compared 4 combinations of antigens in multiplexed microfluidics and ELISA formats, rP100-pepVF-rOspCK, rVlsE-pepVF-rOspC-K, rVlsE-rP41-rOspCK and rVlsE-rDbpA-rOspC-K (**Fig. 8**). On ELISA our best combination was rVlsE-rDbpA-rOspC-K which detected accurately 35 LD positive samples and 20 negative healthy samples (100% sensitivity and specificity). On the

microfluidics format, the best combination of antigens was the only one that did not contain rVlsE (rP100-pepVF-rOspC-K), which detected 31/35 positive LD samples and 18/20 negative healthy samples (88.57% sensitivity, 90% specificity, AUC of 0.844). Here, VlsE underperformed in the microfluidics format as it produced higher cross-reactivity with healthy samples. In these studies, we did not optimize the conjugation chemistry beyond physiosorption for most antigens, and also did not optimize concentration of coating protein and blocking conditions. Specificity in the microfluidics format would have to be optimized further before clinical use, though we note that all screening work performed here was done with an older, sera sample set. Validation of findings may have to be repeated with a newer, well characterized Lyme sera panel.

With additional clinical data sets, a more detailed, powered analysis for multiplexing can be undertaken, to refine coefficients in this model [66, 82]. Further, we did not perform advanced assay development testing to identify positive samples from a blinded mixture of healthy and LD samples. Future optimization of this assay would include those studies. Another limitation of the POC assay, which can be generalized to all other serologic assays, is that it does not detect antibodies which may not be present in serum in the first two weeks post infection.

We compared our best mChip-Ld candidate (P100-pepVF-OspC-K) to the C6 ELISA which is generally recognized as the best first tier assay for serodiagnosis of Lyme disease (**Fig. 9**) and we found that the sensitivity for overall diagnosis of Lyme is just as high for microfluidic test as the C6 ELISA test. More than one-third of the Lyme-positive samples, covering both Early and Late

Stage Lyme, were classified as "Negative" by Western Blot, though classified as "Positive" on C6 ELISA. In addition, the OspC-K antigen in a microfluidic format shows promising results as detection of early-stage LD.

One of the antigens used in our lead microfluidics assay, PepVF, is based on the 26-mer invariable region (IR(6)) of the variable surface antigen of *B. burgdorferi* (VlsE). This antigen is conserved among European pathogenic genospecies [83] and it was reported recently that *B. mayonii* infection was identified by C6 ELISA [50]. Thus, we speculate that a rapid detection assay based on PepVF, as described in this study, should identify *B. bugdorferi* sensu lato infections.

Diagnostic testing for Lyme disease is traditionally achieved by determination of the serologic responses to *B. burgdoferi* sensu lato, with the exception of the very early localized phase of disease (EM<10 days), in which the diagnosis must be done clinically due to the recognized lack of antibody availabe for detection by serologic assays [84]. In this study, we show that we can detect culture confirmed clinically characterized LD using an assay suitable for use at the point of care. The versatile nature of the microfluidcs platform allows us to explore development of a single tiered assay for the future rapid diagnosis of Lyme disease that provides the physician with information on reaction to individual antigens.

Leptospirosis

2.5 Background

Like Lyme, Leptospirosis is a bacterial spirochete infection with largely non-specific clinical manifestation. Symptoms can range from those of general febrile illness (fever, chills, muscle aches) to severe kidney or liver failure, meningitis, and even death[85]. Leptospirosis occurs in urban environments of both industrialized and developing countries as well as rural regions around the world [86]. Human infection largely results from direct or indirect exposure to reservoir hosts and to contaminated water or soil with infected urine from carrier mammals[86]. Although incidence in the U.S. is low (~ 100-150 identified cases per year), the CDC estimates more than 1 million cases occur worldwide each year, including 59,000 deaths[85]. Urban epidemics are reported in cities in developing countries and will likely increase as the world's slum population doubles to 2 billion by 2030 [87]. An unexpected outbreak was reported in New York City recently and climate change may account for its re-emerging status [88]. Incidence rates are currently considered to be underestimated due to lack of awareness of the disease and lack of accessible, rapid diagnostics[86].

Recovery of leptospires from blood or cerebrospinal fluid (CSF) through culture remains the definitive diagnostic test, though the drawbacks of this method are discussed above. Molecular methods for direct detection using PCR are currently being explored, however serology remains the most frequently used diagnostic approach for leptospirosis [86]. The microscopic agglutination test (MAT), which detects agglutinating antibodies in serum, is the most widely

used reference standard serological test for Leptospirosis[89]. This involves incubating patient serum with various Leptospirosis serovars (serological strains of bacteria) to determine the infecting serovar before testing various serum dilutions for antibody titer determination[89]. As MAT involves maintenance of live serovars and human interpretation, it is laborious, difficult to standardize and limited to specialized, reference laboratories. Alternate serologic tests like conventional ELISAs are also not suitable for near-patient or seroepidemiologic settings.

Here we describe exploratory work towards a rapid, quantitative assay for Leptospirosis. We investigate the potential of several candidate antigens comprised of recombinant Leptospira proteins with a high degree of homology between the many pathogenic serovars. In an initial study, we show that successful translation from an ELISA format to a quantitative microfluidic format is possible, laying the groundwork for subsequent development efforts towards clinical usage.

2.6 Methods

2.6.1 Leptospirosis mouse serum panel

Antigen-specific polyclonal serum (i.e. serum containing antibodies for one specific antigen) was generated in mice for initial reactivity testing experiments. Serum from mice infected with 10^7 cells of *L. interrogans* serovar Copenhageni Fiocruz (n=12) as well as serum from non-infected control mice (n=6) were used for testing and evaluation of candidate biomarkers on both ELISA and mChip platforms.

2.6.2 Candidate antigen biomarkers

Five recombinant, purified *L. interrogans* (serovar Copenhageni) proteins were chosen for preliminary analysis: rLigA$_{7\text{-}13}$, rLigB$_{1\text{-}6}$, rLoa22, rTolC and rLipL32 (**Table 8**).

Table 8. Recombinant biomarkers from *L. interrogans* serovar Copenhageni

Immunoglobulin-like proteins	rLigA$_{7\text{-}13}$, rLigB$_{1\text{-}6}$
Outer membrane lipoproteins	rLipL32, rLipL41, rLipL42, rLipL53, rLemA
Virulence factors	rLoa22, rColA
Transporters	rTolC, rPhnL
Flagellar proteins	rFliE, rFlgB, rFlaB, rFlgC, rFlgJ, rFlgH, rFlbC

2.6.3 Point-of-care immunoassay

Microfluidic cassettes were functionalized by direct adsorption of antigen candidates (purified recombinant proteins of *L. interrogans*) at concentrations ranging from 5-50 μg mL^{-1} for initial optimization testing reactivity between each purified protein and its respective polyclonal serum. Multiplexed panels were functionalized with the following concentrations: 5 μg mL^{-1} rLigA, 20 μg mL^{-1} rLigB, 5 μg mL^{-1} rLoa22, 20 μg mL^{-1} rLipL32 and 20 μg mL^{-1} TolC. All other protocol and assay conditions are identical to those of the POC Lyme assay (**2.2.5**), with the exception of gold-conjugated secondary antibody consisting of goat anti-mouse IgG molecules.

2.6.4 Statistical Analysis

ROC analysis was performed as described previously (**2.2.6**) to report sensitivity and specificity values for each antigen. Gold-standard reference tests were culture confirmation.

2.7 Results & Discussion

We aim to develop an antibody capture assay (**Fig. 10**). for rapid, quantitative detection of Leptospirosis at the POC. By investigating the diagnostic potential of candidate antigens and evaluating performance on both ELISA and the mChip microfluidic platform, we aim to follow a similar development process to that of the rapid Lyme Disease detection platform (mChip-Ld system).

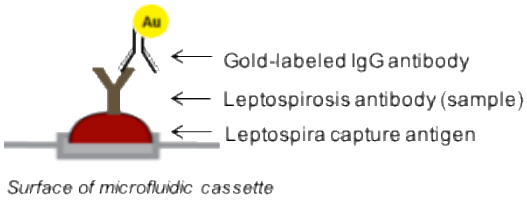

Figure 10. Schematic of Leptospirosis sandwich immunoassay design. Serum samples that contain disease positive antibodies will bind to the surface of microfluidic cassettes functionalized with Leptospira capture antigens. A sequence of washes is followed by gold-labeled IgG antibodies, which bind to the primary antibodies immobilized on the surface. Subsequent automated reagent delivery of remaining washes and silver amplification reagents results in silver ion reduction of gold nanoparticles, as described previously [90].

The first step in trying to recapitulate an ELISA assay on our microfluidic platform requires choosing an appropriate capture concentration on the surface of the microfluidic cassettes. Preliminary ELISA testing indicated that each candidate antigen showed high reactivity when tested against its respective polyclonal serum. We proposed using the same matched antigens and polyclonal serum samples to help select capture concentrations based on reactivity. Thus, in microfluidic cassettes functionalized with rLigA antigens for example, polyclonal serum containing only LigA antibodies was used as the "sample". Just as in ELISA, we observed generally high reactivity between all the candidate antigens and their respective polyclonal serum

across all concentrations that were tested (**Fig. 11**). In choosing a preliminary capture antigen concentration, we aim to pick the lowest concentration that shows the best signal (high magnitude of signal and low variability). Choosing a lower concentration allows us to maximize the most expensive components of an assay (the biological capture molecules) which could play a greater role in downstream scale-up efforts.

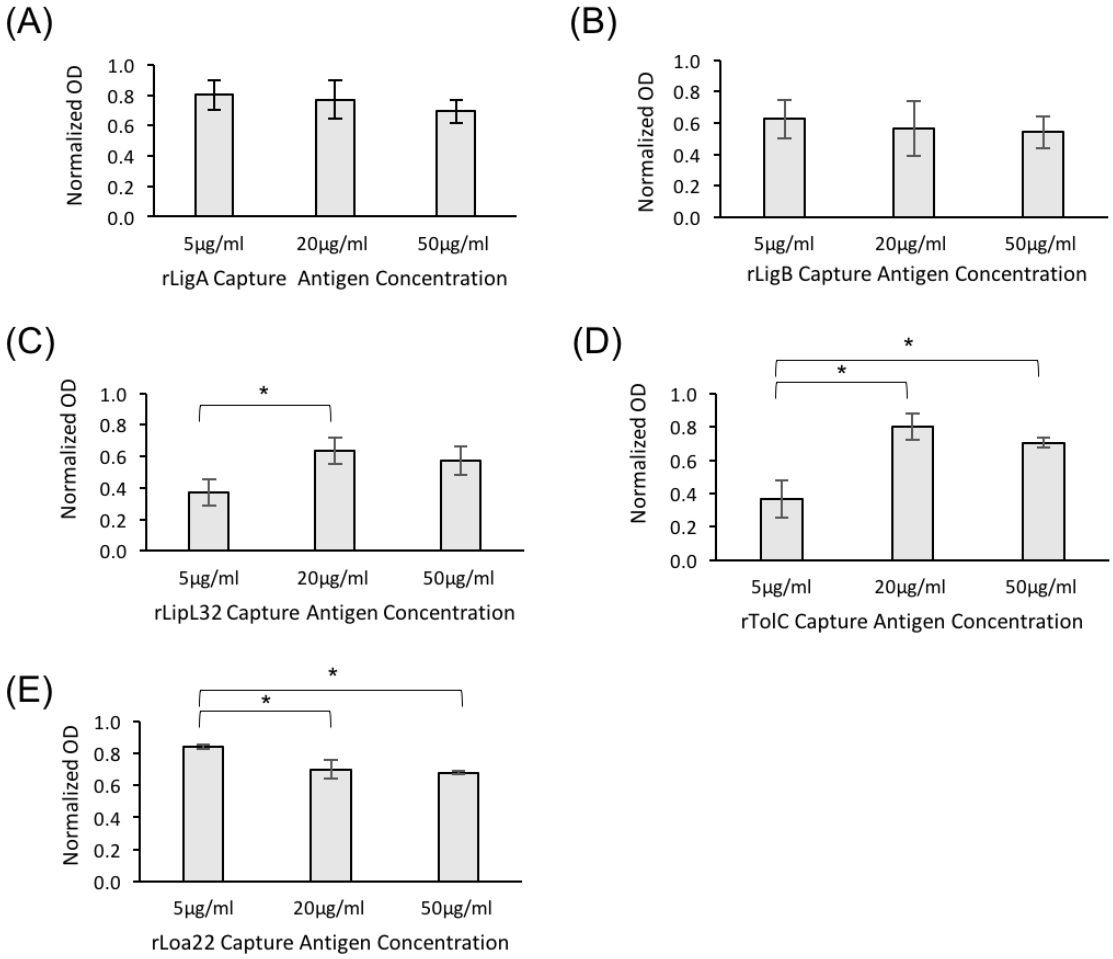

Figure 11. Reactivity of candidate Leptospira antigens at various capture concentrations. Each candidate antigen of **(A)** rLigA, **(B)** rLigB, **(C)** rTolC**, (D)** rLipL32 or **(E)** rLoa22 was spotted on microfluidic cassettes at capture concentrations ranging from 5-50 µg-mL^{-1}. Serum containing polyclonal antibodies specific to each antigen were tested on corresponding cassettes to assess reactivity, shown in plotted bars. Data are averages ±1 SD (*n*=3). Asterisk (*) indicates statistical significance ($p<0.05$) using Student's t-test.

After choosing initial capture antigen concentrations for each candidate marker, we worked to develop preliminary multiplexed assay panels (**Fig. 12**). Given that each microfluidic cassette could hold three target detection zones (in addition to internal positive and negative control zones), we functionalized microfluidic cassettes with 2 designs: (1) rLoa22–rTolC-rLipL32 and (2) rLigA-rLigB-rLipL32 in Target Positions 1 to 3 respectively. A second, more unexplored factor in developing multiplexed assays on our platform is the role of zone order (**Fig. 12**) on resulting signals. For highly specific matched antigen-antibody combinations, zone order should have minimal impact on reactivity. In cases where cross-reactivity effects are stronger, optimization of zone order position could be required. Preliminary multiplexing optimization indicated that rLigB performed slightly better with a higher capture antigen concentration (20 μg mL^{-1} instead of 5 μg mL^{-1}) when placed in Target Zone Position 2 (behind rLigA in Position 1). While there was no statistically significant difference between the two concentrations when tested against the LigB polyclonal serum, the 20 μg mL^{-1} concentration produced a slightly lower standard deviation in repeat runs (**Fig. 13**).

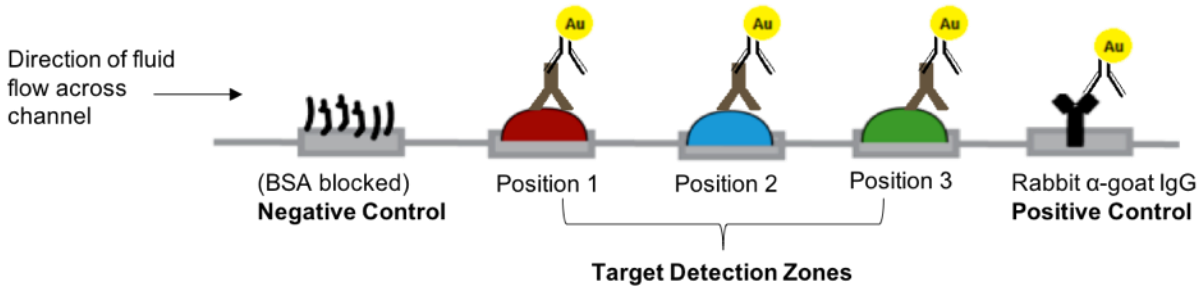

Figure 12. Schematic of multiplexed microfluidic channel design. In the current microfluidic cassette format, there are five zones available for functionalization; addition of internal positive and negative controls reduces the number of target zones to three. The direction of fluid flow across the channel is such that Target Zone Position 1 is the first to encounter the sample (or subsequent reagents), followed by Position 2 and then 3.

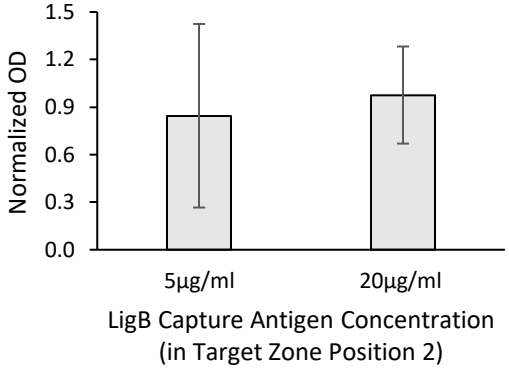

Figure 13. Reactivity of rLigB in Target Zone Position 2 on multiplexed cassettes. Zone order of multiplexed cassettes was negative control, rLigA in Target Position 1, rLigB at either 5 µg mL^{-1} or 20 µg mL^{-1} in Target Position 2, blank Target Position 3 and a positive control. LigB polyclonal serum was tested on both cassette designs and resulting signals are plotted. Data are averages ±1 SD (*n*=3). No significant differences using a Student's t-test.

The next step was to screen each candidate antigen on ELISA using a mouse serum panel consisting of 18 samples (12 infected and 6 controls. ROC analysis of the ELISA screening results showed that rLigA, rLigB, rLoa 22 and rLipL 32 all had 100% sensitivity and specificity compared to culture reference test (**Fig. 14, Table 9)**. rTolC had the lowest performance with 83.3% sensitivity and 66.7% specificity (AUC of 0.694) on ELISA (**Table 9**).

Next, we did a preliminary assessment of performance using the same give antigens on the microfluidic platform. ROC analysis showed that rLigB, rLoa22 and rLipL32 performed the best out of the candidate antigens, with sensitivities > 90% and specificities of 100% (**Fig.15, Table 10**). As with the ELISA results, rTolC had the lowest diagnostic performance (**Table 10**).

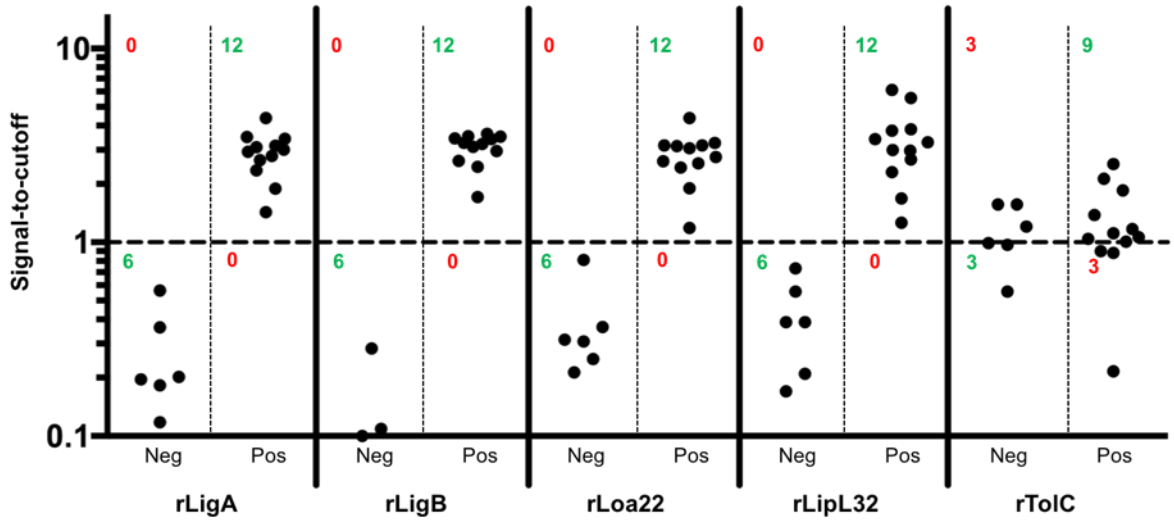

Figure 14. ELISA screening of candidate antigens for Leptospirosis diagnosis. Signal to cutoff plots showing specificity and sensitivity of candidate antigens using IgG ELISA compared to reference testing with culture. 6 sera samples from healthy mice and 12 sera samples from mice infected with 10^7 cells of L. interrogans (serovar Copenhageni Fiocruz) were used to test specificity and sensitivity of candidate antigens of rLigA, rLigB, rLoa22, rLipL32 and rTolC.

Table 9. Performance of ELISA screening using 5 antigens to diagnose Leptospirosis

	Cutoff	Sensitivity	95% CI	Specificity	95% CI	AUC
LigA	> 0.339	100	73.54% to 100%	100	54.07% to 100%	1
LigB	> 0.763	100	73.54% to 100%	100	54.07% to 100%	1
Loa22	> 0.302	100	73.54% to 100%	100	54.07% to 100%	1
LipL32	> 0.076	100	73.54% to 100%	100	54.07% to 100%	1
TolC	> 0.028	83.3	51.59% to 97.91%	66.7	22.28% to 95.67%	0.694

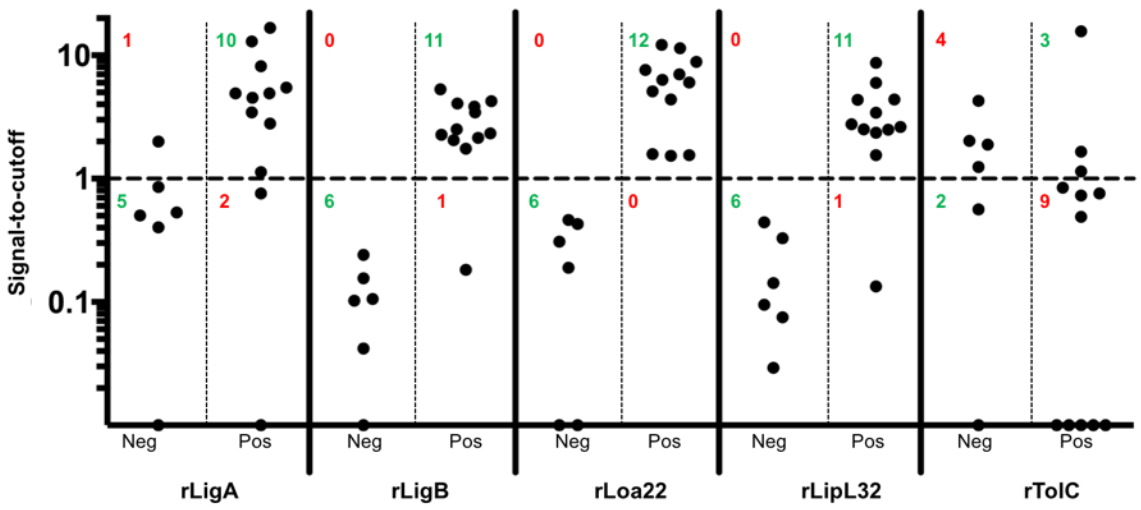

Figure 15. Preliminary screening of recombinant Leptospira candidate antigens with mChip. Signal to cutoff plots showing specificity and sensitivity of candidate antigens using the mChip platform compared to reference testing with culture. 6 sera samples from healthy mice and 12 sera samples from mice infected with 10^7 cells of L. interrogans (serovar Copenhageni Fiocruz) were used to test specificity and sensitivity of candidate antigens of rLigA, rLigB, rLoa22, rLipL32 and rTolC.

Table 10. Performance of mChip screening using 5 antigens to diagnose Leptospirosis

	Cutoff	Sensitivity	95% CI	Specificity	95% CI	AUC
LigA	> 0.522	91.7	61.5% to 99.8%	83.3	35.9% to 99.6%	0.931
LigB	> 0.1324	100	73.5% to 100%	100	54.1% to 100%	1
Loa22	> 0.0424	100	73.5% to 100%	100	54.1% to 100%	1
LipL32	> 0.0873	91.7	61.5% to 99.8%	100	54.1% to 100%	0.958
TolC	> 0.0116	83.3	51.6% to 97.9%	66.7	22.3% to 95.7%	0.750

rLoa22 was a better diagnostic candidate than rLigA when placed in Target Zone Position 1 on the microfluidic platform. We also observed that TolC was not a good marker with the mouse sera panel, which could potentially be the same for human sera panels as well. From the preliminary assessment of candidate antigens in multiplexed microfluidic panels, the next round of multiplexed testing could include rLoa22 in Position 1, rLigB in Position 2 and rLipL32 in Position 3.

In this exploratory work, we have shown that a quantitative POC test is possible for discriminating between positive and negative Leptospirosis diseased sera samples. We also illustrate the steps that are needed in creating a new multiplexed assay on the mChip microfluidic platform. Next steps would entail potential addition of IgM antibodies for early infection detection, testing with human sera samples and validation with a larger sample size. Additional cross-reactivity studies may be necessary to determine if further optimization of zone order placement is necessary. The assay development work presented lays the groundwork for future development efforts towards a fully integrated POC system that can be implemented in clinical settings. For Use-Case 3 implementation (e.g. primary care settings in developing countries), additional work towards low cost, usability for minimally trained personnel as well as considerations for limited ground electricity for equipment and refrigerated reagents should be considered. Understanding clinical workflow for Leptospirosis screening in local settings would also enhance design and development process particularity if higher clinical utility lies in Use Case 4 implementation (in the field).

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Chapter 3

Developing a smartphone-integrated HIV-syphilis assay and demonstrating use in the field

Antenatal Screening Tool for Healthcare Workers in the Field

3.1 Background

Previous work in our lab focused on miniaturizing of the complex ELISA into a simpler, microfluidic assay that could expand access to diagnostic testing. Significant efforts were also made in building custom hardware to be used with the microfluidic assay cassette platform. From a proof-of-concept device using low-cost optical components to a fully integrated form factor with fluidic control and data transmission modules, we demonstrated the ability to design

technology that could be used outside of a laboratory setting[61, 64]. Our goal for the next phase of POC device evolution, was to create diagnostic tools that could be used in the most remote regions of the world, particularly in lower-infrastructure settings of the developing world (Use Case 4). This work also represents the next stage of POC device development, by integrating assay chemistry, microfluidics and connected instrumentation towards a fully integrated system that can be deployed in the field (**Fig. 4**).

3.1.1 Connected instrumentation

The uptake of smart consumer electronic devices has moved forward with breathtaking speed. Their omnipresence has transformed how services are delivered for entire sectors of the economy, with several consequences for POC diagnostics, namely the use of smartphones as platform devices. Towards health and monitoring, smartphones are increasingly adapted for imaging, sensing, and diagnostics[91]. Miniaturized components used in consumer electronic devices can be adapted for use in POC diagnostic devices. The camera lens in smartphones can be used for microscopy, cytometry, and optical readouts for different detection methods[1], low-energy Bluetooth modules for data connectivity are becoming standardized and prevalent, and batteries are becoming longer lasting. The use of these components for POC devices can be extended from prototyping to large-scale manufacturing by leveraging the established supply chains for smartphones and smart devices. For design of POC tools for resource constrained use-case settings, smartphones provide a platform that leverages powerful processing, communication tools and an extremely portable interface, which can replace peripheral equipment needed to run conventional diagnostic tests.

3.1.2 Prevention of mother-to-child transmission of sexually-transmitted infections (STIs)

Developing countries experience a higher burden of death and disability due to infectious diseases compared to those in developed settings. The World Health Organization estimated around 170,000 infants are born with HIV every year, predominantly in sub-Saharan Africa[92]. Congenital syphilis accounts for 500,000 stillbirths every year (CDC, 2014). While there has been progress made towards reducing new infections, efforts are still needed in reducing congenital syphilis and new HIV infections among children.

Most transmissions could be eliminated by routine diagnosis and simple treatment of infected mothers. High-burden countries thus require antenatal care testing to screen for both HIV and syphilis. By enabling early detection of STIs, both mothers and children can avoid significant adverse health outcomes. The Fast Track Targets towards the UN Sustainable Development Goals of ending the AIDS epidemic by 2030 include the "90-90-90" target: 90% of people living with HIV know their HIV status, 90% of people who know their HIV-positive status are getting treated and 90% of people receiving treatment have suppressed viral loads[93]. Our aim is to tackle the first component of expanding access to HIV diagnostic tools.

Currently, lateral flow rapid diagnostic tests (RDTs) are amongst the most widely used class of diagnostic tools for screening, particularly in resource-constrained settings. This is mainly due to the use of paper, which has long been held as an inexpensive and resourceful material choice for a resource-limited, field use case (Use Case 4). Typically, these RDTs rely on lateral flow or

immunofiltration technologies, require minimal cold-chain requirements for shipping/storage and forego instrumentation for human interpretation of the result (e.g. visual presence of bands)[41]. The World Health Organization lists 28 lateral flow rapid tests as "prequalified" in vitro diagnostic products for HIV and malaria from companies such as SD Bioline, Chembio, Alere, Trinity Biotech, bioMerieux, OraSure, ABON Biopharm, ARKRAY Healthcare, Access Bio, Premier Medical etc[42]). While some dual-HIV-syphilis RDTs have been developed[94-96], the major drawbacks of lateral flow platforms include subjective user interpretation, and limited assay complexity capabilities for multiplexing and multi-step reactions. We aim to leverage the power of connected instrumentation such as smartphones to build an improved HIV-syphilis test suitable for healthcare workers to use at the POC.

3.1.3 Microfluidic, multiplexed STI test with a smartphone accessory

Previously our group has demonstrated reproduction of ELISA-like performance and objective readout capabilities with a microfluidic dual HIV-syphilis assay platform[61, 64]. We propose to build on this work to create a more integrated, triplex HIV-syphilis assay on disposable, plastic cassettes, pre-loaded with all reagents for field-deployment in Sub-Saharan Africa (**Fig. 17**). We also illustrate design and fabrication of a portable diagnostic device that attaches to the audio jack of a smartphone, which integrates fluid handling, signal detection and quantification. Finally, we demonstrate usage by healthcare workers in Kigali, Rwanda to evaluate field-performance as well as gather preliminary user feedback on acceptability of our device. The three main components of this project are: (1) assay development, (2) hardware design and (3) field trial design and testing.

3.2 Methods

3.2.1 Triplex HIV-syphilis assay

Surface modification of injection molded, plastic cassettes was done through direct physisorption of antigens onto plastic microfluidic target detection zones, as described previously[97]. We used recombinant multi-epitope chimeric antigens **(**gp41, gp36, and O-IDR) for an HIV 1/2 (Biolink International) marker, a 17-kDa recombinant outer membrane protein TpN17 (Lee Labs) [97, 98] for a treponemal syphilis marker and synthetic cardiolipin prepared from plant source [99] provided by CDC for a non-treponemal syphilis marker. Internal positive control zones were functionalized with an anti-goat IgG antibody (Life Technologies) as described previously (Chapter 2). Negative control zones were treated with a blocking agent. 36 µL each of 2 µg-mL^{-1} of HIV chimeric antigens, 15 µg-mL^{-1} of TpN17, and 10 µg-mL^{-1} of anti-goat IgG Ab in bicarbonate buffer solution (pH 9.6) were spotted on the detection zones using a robotic arm as an automated fluid dispenser (**Fig. 21**). Cardiolipin was covalently attached to the plastic surface using EDC-Sulfo-NHS reaction (which activates the carboxylate groups on cardiolipin for binding with amine-groups on poly-L-lysine coated plastic).

3.2.2 Blocking/stabilizing agent selection

To preserve the conformation and reactivity of the adsorbed antigens, we analyzed the performance of three blocking/stabilizing agents: casein (Thermo Scientific), SeaBlock (Thermo Scientific) and StabilCoat Immunoassay Stabilizer (Surmodics Inc.). Cassettes were functionalized with the various protein markers as described above. Fluid was dispensed by manual pipetting onto cassette surfaces. Each detection zone was spotted with 36 µl of blocking

agent and incubated for 1 hour at room temperature in a humid chamber. After incubation, the blocking agent solution was aspirated and plastic cassettes were placed in a vacuum desiccator for 20-30 minutes. The vacuum-sealed chamber containing the microfluidic cassettes was then placed in a 30°C oven for 4-6 hours of secondary drying. Cassettes were sealed with clear adhesive tape (OPKO Diagnostics) and stored at 4°C until use. An HIV/Syphilis co-infected sera sample as well as a disease negative sera sample both diluted 75X in 1% BSA – 0.05% Tween-20 in PBS were tested on cassettes prepared with each blocking agent.

3.2.3 Lyophilization of secondary antibodies

Lyophilization of secondary antibodies was performed by OPKO Diagnostics (Woburn, MA). 9.5 µL of gold-nanoparticle conjugated goat anti-human IgG (0.53 µg/mL) and goat anti-human IgM (0.27 µg/mL) antibodies were lyophilized into 2.5cm of plastic tubing. No detergent or anti-coagulant excipients were included in the lyophilization buffer. Lyophilized antibody holders were stored at 4ºC until use. Prior to running an assay, lyophilized antibody holders were filled with 9µL of 3% BSA-0.2% Tween in PBS solution and incubated for 8-10 minutes at ambient conditions.

3.2.4 Early dongle prototype

3.2.4.a Hardware

To investigate feasibility of scaling down the hardware footprint and power requirements, we built an early dongle prototype with two detection zones using the same low-cost LEDs and photodiode detectors as in previous protoypes ($0.50 and $6.00 per unit respectively) and power supplied by an iOS device (iPod Touch, 4th gen.) as shown in **Fig. 16**. The optical signal was converted to analog signal, and then converted to digital signal by the A/D converter of a

separately attached microcontroller. A laptop computer was used to obtain light transmission values through the TI Code Composer Studio with proper settings of the JTAG (used to test interconnects on PCBs) as shown in **Fig. 17.**

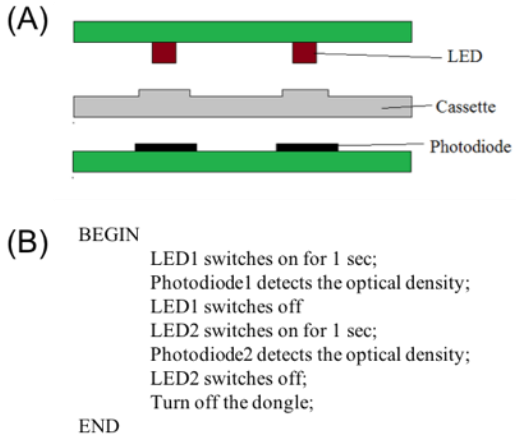

Figure 16. Schematic of early dongle prototype. (A) 2 zone detection system conserving previously developed hardware design of using low cost optical components. The light emitted from the LEDs transfers through the cassette zones that serve as filters, and then detected by the photodiodes. The optical signal is converted to analog signal, which is then converted to digital signal by the A/D converter of the microcontroller. **(B)** Control flow program for the dongle.

3.2.4.b Running a test

The PCBs had no casing, therefore manual adjustment was needed to correctly align the cassette with the dongle setup such that light emitted from the LEDS was transferred through the cassette zones and then detected by the photodiodes. Furthermore, no fluidic actuation module was integrated into this design; sample and reagent fluids were introduced through loading a PE tube to the inlet channel and drawn through the microfluidic cassette using syringe-actuated negative pressure (33kPa) at the outlet. Silver reduction of the gold nanoparticles was used, as in previous tests, for signal amplification. Readings were taken at the beginning and end of the 4 minute silver development sequence to calculate optical density (OD) values for each zone. Prism software was used to determine cutoffs and for ROC analysis.

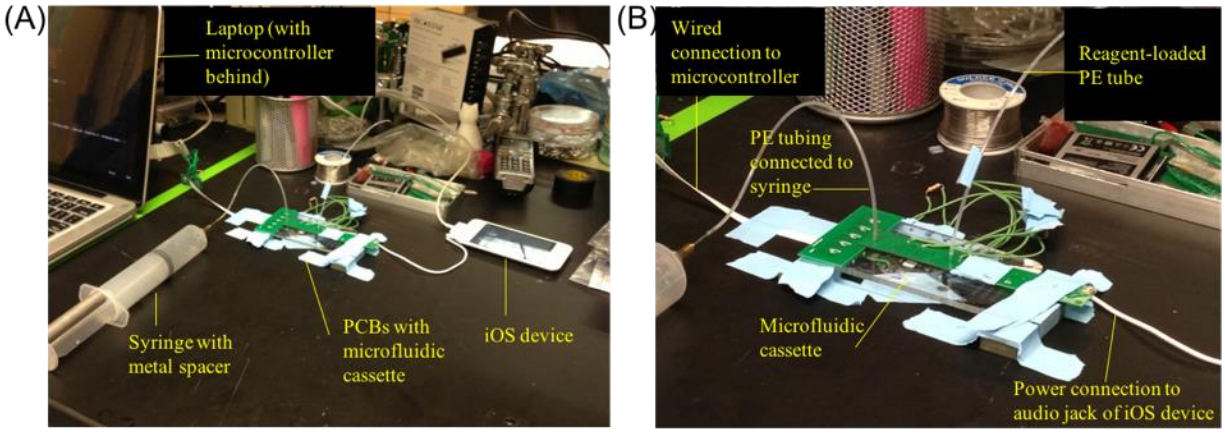

Figure 17. Components of early dongle prototype setup. (A) The microfluidic cassette is sandwiched between the PCBs holding the LED and photodiode arrays. With no casing to guide insertion or hold cassette in place, lab tape was used to secure alignment of the components such that the two detection zones of the cassette were aligned with the optical components of the dongle. An iOS device (iPod Touch, 4th gen.) and microcontroller (not pictured) were attached to the dongle, with the latter connected to a laptop computer for data visualization. A 60cc syringe with a metal spacer was used to induce constant negative pressure of 33kPa for fluid actuation. **(B)** Close-up of the cassette sandwiched between LEDs and photodiodes while a reagent loaded PE tube and syringe are attached to the inlet and outlet respectively. Wired connections to the iOS device and microcontroller are shown.

3.2.4.c Cassette functionalization

In the early dongle prototype, only 2 detection zones could be functionalized for the test. Thus, we designed cassettes to have a positive reference zone and HIV sample zone using coating methods similar to those previously developed for the mChip. Attachment of Protein G for the positive reference zone was done through EDC-SulfoNHS chemistry, which activates the carboxyl groups on the protein for binding with the amine-groups on the poly-lysine coated plastic of the cassettes. HIV plasma samples that were known to be positive (n=13) or negative (n=9) by rapid test and ELISA reference tests were selected for evaluation with the dongle prototype. Reagent tubes were loaded with a 100X sample dilution in 1%BSA-0.05%Tween-20 in PBS and 100X dilution of gold labeled goat-anti-human IgG (capture antibody) in 3% BSA-0.2% Tween-20 in PBS.

3.2.5 Smartphone Dongle "1.0"

3.2.5.a Design

The smartphone dongle features power-free fluid flow and objective signal read-out with powering and signal transmission solely via audio jack connection (**Fig. 18**).

Power-free fluid flow. We designed a power-free mechanically-activated vacuum source, using a simple mechanics of rubber bulb and one-way valve. With this mechanism, the dongle generates a reliable, repeatable vacuum at the time of the assay, while keeping the consumables low cost and simple to manufacture.

Audio jack powering and signal transmission. We use the audio jack for both power delivery to the device and signal transmission from the device, as has been shown by Kuo, *et. al.* [100]. We programmed a microcontroller that performed frequency-shift keying (FSK) by converting a decimal integer into binary and each bit was sent as a high-frequency (1632 Hz, or "1") or low-frequency (816 Hz, or "0") signal, and transmitted the photodiode readings through the audio jack and back to the phone.

User-friendly app interface. A custom smartphone application (app) was designed on an iPod Touch to assist in assay operation, power the device to take intensity readings, and demodulate the FSK signals sent by the device. The app provides step-by-step directions with pictures to assist the user through steps necessary to perform the assay. A results page provides objective readout of a positive or negative diagnosis for each of the three tests performed. The app also saves the raw data, diagnosis, time and date of testing, and patient ID number for review at a later time.

3.2.5.b Fabrication

Custom printed circuit boards (PCBs) were designed in Altium and printed from PCB Universe. LEDs and photodiodes were precisely aligned with the cassette slot so testing zones align without manual effort. 1-mm pinholes made of 1-mm thick black Delrin (McMaster-Carr) above each photodiode. The dongle casing was designed in SolidWorks and printed in-house (Objet 24 3D-Printer, Stratasys). Vacuum chamber was created with a one-way umbrella valve (Minivalve), a rubber bulb from a 140-mL syringe (Becton Dickinson), and a conical spring (Century Spring Corp) inside to aid re-expansion. Silicone rubber o-rings and sheets (McMaster-Carr) were used to connect to outlet and seal the venting port.

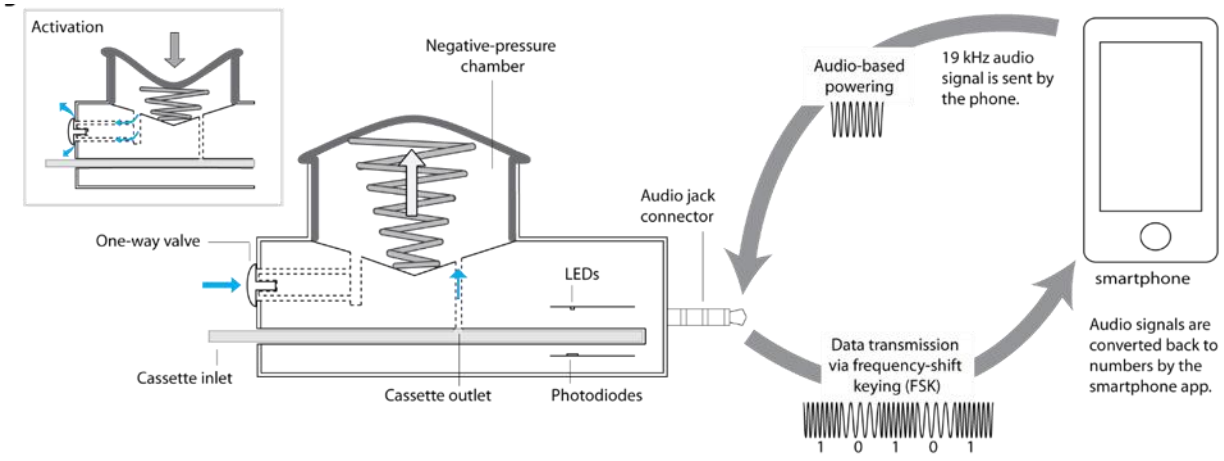

Figure 18. Mechanism of the smartphone dongle. Schematic diagram of dongle highlighting a power-free vacuum generator, with the sub-figure showing vacuum activation. The audio jack connector on the dongle is used for audio-based powering and FSK data transmission to a smartphone.

3.2.6 Running a test with the smartphone dongle

To perform a test (**Fig. 19**), the user (e.g. a health care worker) collects a fingerprick whole blood sample using conventional methods. 1 µL of whole blood is mixed with 9 µL of 1% BSA - 0.05% Tween-20 in PBS (10X dilution) and 2 µL of the mixed sample is pipetted into the inlet of

the disposable test cartridge. Step-by-step directions on the app guide the user to attach the antibody holder (pre-filled with 9 µL of 3% BSA – 0.05% Tween-PBS) to a microfluidic cassette, insert the cassette into the dongle, and press the rubber bulb fully to initiate a vacuum. The user is prompted to start "Phase 1" of the assay on the app, which consists of a 6 minute timer during which the sample and all wash buffers flow through the cassette. The user is then prompted to move a toggle to close a venting port on the reagent cassette in order to initiate silver development and signal measurement in "Phase 2" of the assay. OD readings were taken at the beginning and end of the 5 min silver development as described previously (Chapter 2). Afterwards, results for all markers were displayed on the screen, and raw absorbance values are recorded along with the study ID.

3.2.7 Field trial design

We wanted to evaluate the smartphone-integrated STI test performance and field-readiness by putting it in the hands of target end-users, in this case, local healthcare workers (HCWs) in Rwanda. We also wanted to get preliminary feedback from local patients, more than half of whom typically had to wait for results at the health centers without knowing how long it would take and over a third of whom typically had to wait for more than 2.5 hours to receive results from one clinic visit. Three community level health centers were selected as study sites in Kigali, Rwanda: 1) Kimironko (Remera) HC, serving 56,000 people, 2) Biryogo HC, serving 50,000 people, and 3) Gahanga HC, serving 28,000 people. This study was approved by the Columbia University IRB and Rwanda National Ethics Committee. All rules and regulations pertaining to human subject involvement and protection of confidentiality and

sensitive data were followed.

3.2.7.a Healthcare workers (HCWs)

Five HCWs across the three study sites were selected for study involvement based on willingness to participate. All five HCWs were lab technicians with no prior experience or training in ELISA, though all had experience with HIV rapid tests using plasma/sera and fingerprick sampling. Training consisted of a 30-minute session with the study team. HCWs were given background information related to the study and hands-on training on how to use the dongle to perform a test using fingerprick whole-blood.

3.2.7.b Patient recruitment and enrollment

A target sample size of 100 subjects was chosen for this study. No power analysis was performed as the study aims focused on a preliminary assessment of field-readiness of the device and appropriateness for local clinical work flow. Patient recruitment focused on individuals already enrolled in "Prevention of Mother to Child Transmission" (PMTCT) or "Voluntary Counseling & Testing" (VCT) programs in the local health centers. Patients in these programs were already scheduled to receive HIV and syphilis testing from venipuncture blood draws during their clinic visit. HCWs used a recruitment script provided by the study team during PMTCT and VCT information sessions, and emphasized that participation was completely voluntary and did not affect any services received at the health center. Interested patients were referred to the study team (Rwanda study facilitor) for more information and to undergo an informed consent process with a third party translator fluent in the local Kinyarwanda language.

3.2.7.c Study visit procedure

After patients received regular venipuncture blood draws as part of their regularly scheduled clinic visit, they were guided through a study consent process (**Fig. 19**). HCWs entered study participant ID numbers into the app before obtaining a fingerprick blood sample (< 20 uL) from the patient. Patients then completed a survey that consisted of a questionnaire regarding their medical history as well as their experience with the dongle assay. These questionnaires were administered by a third-party interviewer fluent in local Kinyarwanda as well as French and English. Responses to questionnaires were written in English by the third-party interviewer and returned to the study team for analysis. Participants were compensated 1000RWF (approximately $1.54 USD) for their time. Disease statuses were blinded to the Columbia study team and HCWs who conducted the test. HCWs ran the test as outlined in **3.2.6,** with the study team performing pre-filling of antibody holders and sample dilution steps (**Fig. 19**). Aliquots of corresponding venipuncture blood samples were tested on the dongle by the study team for comparison with tests run by healthcare workers using fingerprick blood samples.

3.2.7.d Confirmatory testing of samples

HIV RDTs (Colloidal Gold, Determine and Unigold) were completed at each site. HIV ELISA (Vironostika), syphilis TPHA (Spinreact), and RPR (Spinreact) as reference tests for HIV, treponemal syphilis and non-treponemal syphilis respectively were performed at the Rwandan National Reference Laboratory (NRL) using plasma (0.5mL) separated from

venipuncture blood. At the end of the trial, the results were unblinded to the study team, and reference tests were compared to the tests obtained by the dongle.

3.2.7.e Statistics

Averages, standard deviations, linear fit, and two-sided Student's t-tests ($\alpha = 0.05$) were calculated with Microsoft Excel. Student's t-test was chosen to compare two small sets of quantitative data when data in each sample set were related. Vertical scatter plots, sensitivity, specificity, 95% confidence intervals, ROC curves, and McNemar's test were created in GraphPad Prism.

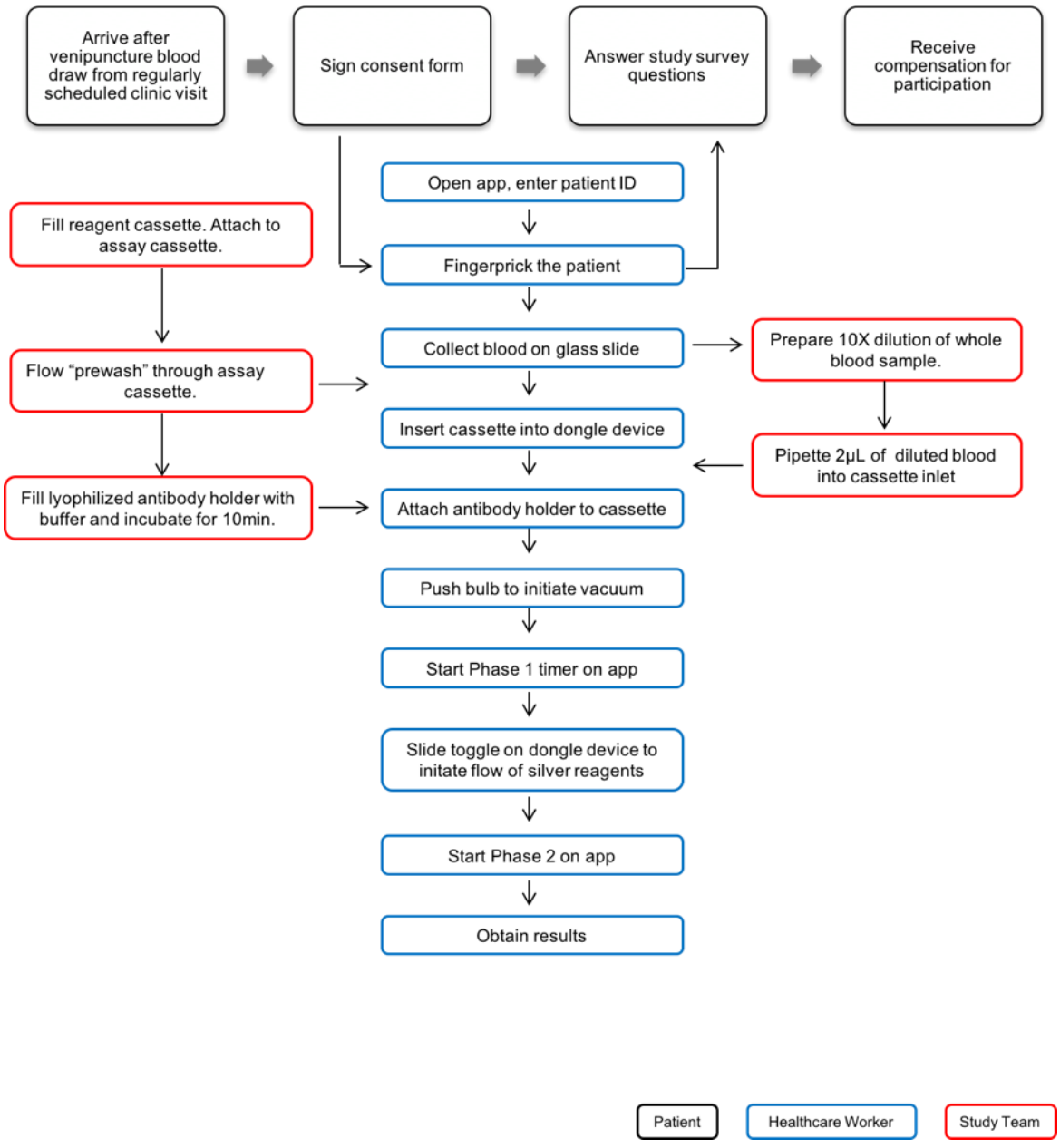

Figure 19. Process flow diagram for a study visit session in Kigali, Rwanda using the smartphone dongle STI test. Sequence of steps for patient (shown in black), healthcare worker (shown in blue) and study team (shown in red), are depicted for a typical study visit session.

3.3 Results

3.3.1 Triplex HIV-syphilis assay

Several changes were made with respect to the previously developed HIV-syphilis assay from our group[61]: (1) addition of IgM antibodies for early detection of infection, (2) addition of cardiolipin as a non-treponemal syphilis marker, (3) prepackaging of all wash buffers in to a reagent cassette, (4) lyophilization of gold-nanoparticule conjugated secondary antibodies as well as blocking agent optimization for increased assay stability and (5) scale up of fabrication using automated fluid dispensing robotic arms.

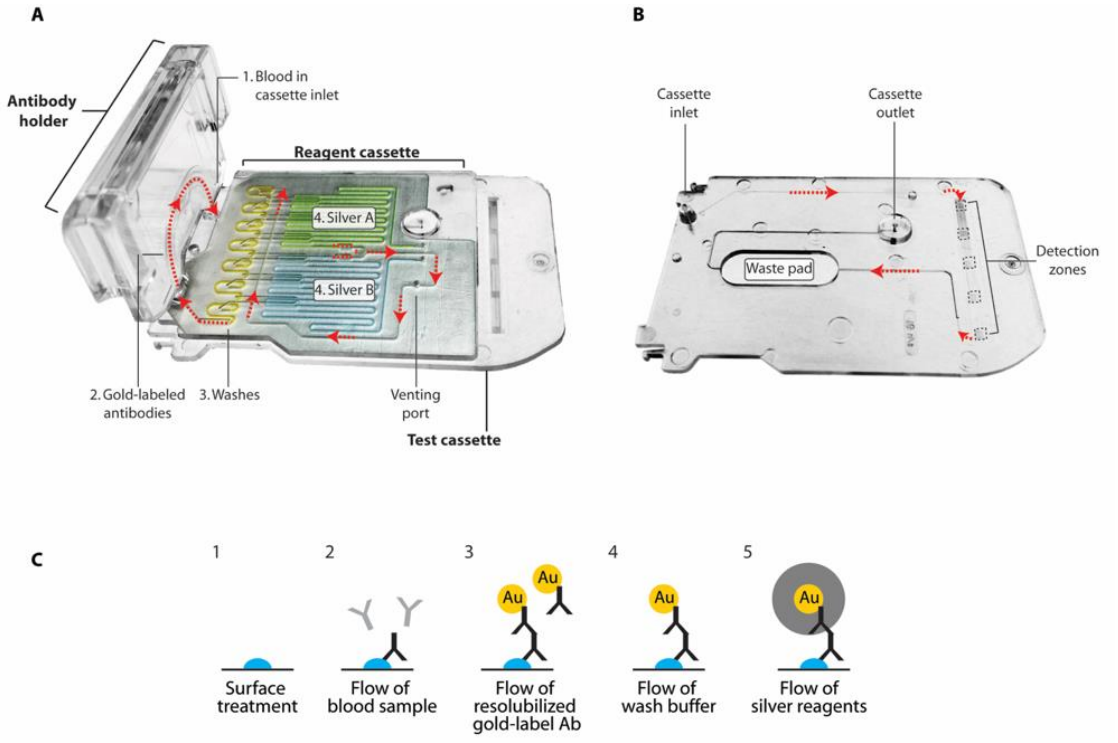

Figure 20. Two-layer microfluidic cassette and antibody holder for triplex HIV-syphilis immunoassay. (A) The reagent cassette (top layer) contains pre-loaded reagents (washes (yellow), silver nitrate (blue), and reducing agent (green)) and the assay test cassette (bottom layer) contains five detection zones. Reagents are numbered in the order they flow through the test cassette. (B) Sequence of reagent flow is shown from cassette inlet to the detection zones before exiting into the embedded waste-pad for containment of all sample and reagents. The power-free vacuum chamber connects to the cassette outlet, drawing fluids from the inlet towards the waste pad. (C) Schematic of immunoassay on the surface of target detection zones.

The addition of anti-human IgM antibodies as detection antibodies for early detection of infectious diseases was important for enhancing the sensitivity of the non-treponemal syphilis assay (**Fig. 21**). Antibodies produced against cardiolipin are used as a non-treponemal marker for syphilis and are commonly found in IgM antibodies. Cardiolipin is a lipoidal material released as a result of damage to the host cells because of the active infection and also from the cell surface of *Treponema pallidium*, the causative bacterial agent of syphilis [101]. It indicates active infection and is helpful in tracking the effectiveness of treatment, which is usually measured by an RPR, or rapid plasma reagin, test. In the RPR test, a nontreponemal serological test for syphilis, a reactive agglutination indicates a positive result. A subsequent quantification of RPR titer can be done to establish a baseline titer value and should go down at least 4-fold with successful treatment (e.g. 1:32 to 1:8)[102]. Non-treponemal antibodies may not always be specifically directed against the *Treponema pallidium* however, as other conditions such as pregnancy, Lyme Disease, certain types of pneumonia, malaria and tuberculosis could cause false-positive results [103]. Clinical algorithms for syphilis detection vary in first line test choice but typically include both a treponemal antibody test, which is highly specific for syphilis, as well as a non-treponemal antibody test, to confirm active infections.

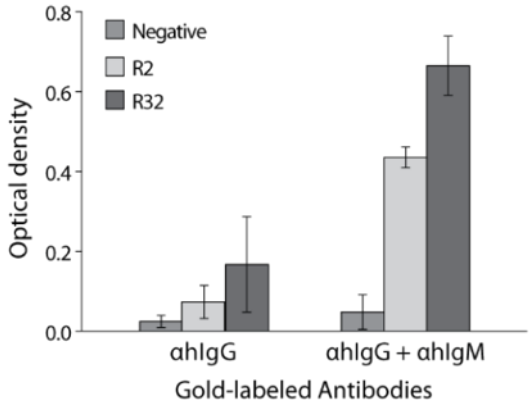

Figure 21. Addition of gold-labeled anti-human IgM for syphilis detection. Comparison of signal measurements obtained by additional of gold-labeled anti-hIgM to gold-labeled anti-hIgG and gold-labeled anti-hIgG alone as detection antibodies for negative, weak positive non-treponemal syphilis (RPR titer 1:2), and strong positive non-treponemal syphilis (RPR titer 1:32) plasma samples. Data are averages ±1 SD (*n*=4 for anti-hIgG and *n*=3 for anti-hIgG + anti-hIgM).

3.3.2 Blocking agent selection

In evaluating the blocking agents, the optimization goals for each detection zone were as follows: (1) minimize noise on the negative control zone; (2) minimize non-specific binding on target zones for tests with disease-negative samples; (3) maintain optimal conditions for antibody binding on target zones for tests with disease positive samples, and (4) maintain high positive control signals for all tests. StabilCoat satisfied these parameters better than Seablock or Casein blocking agents and was chosen for subsequent fabrication of the triplex HIV-syphilis microfluidic assay (**Fig. 22**).

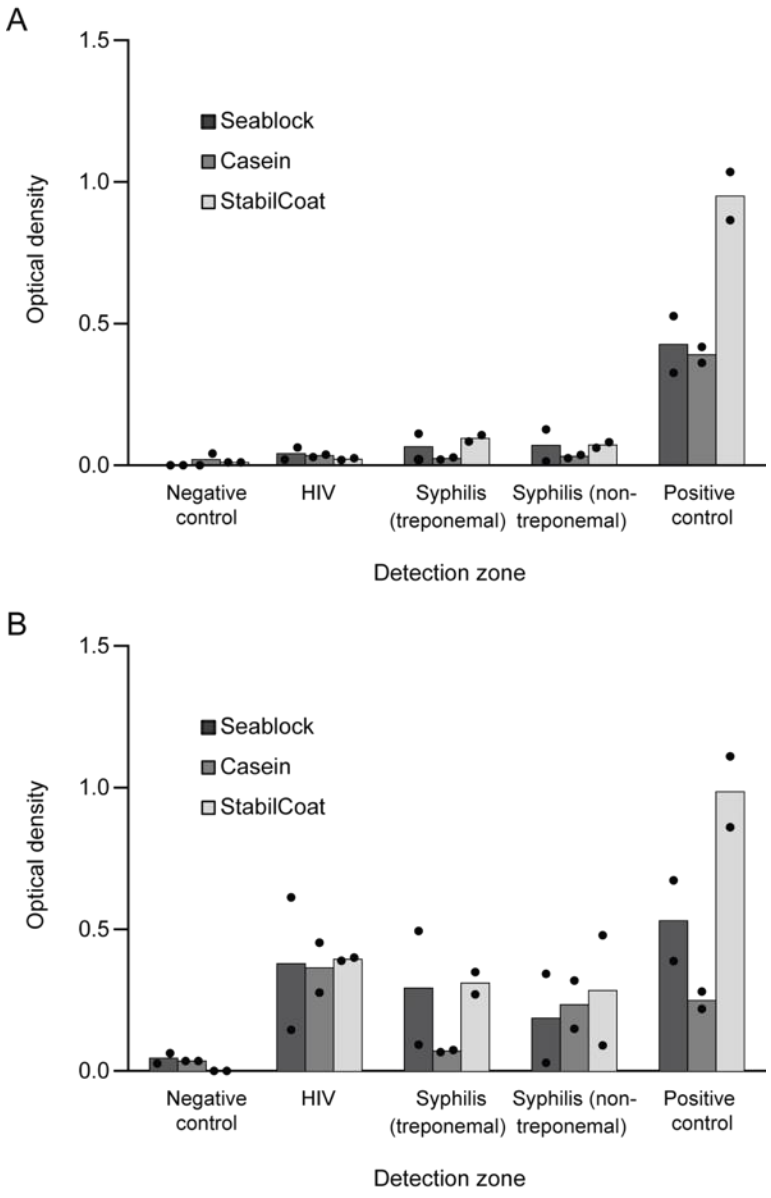

Figure 22. Comparison of blocking agents. SeaBlock, casein, and StabilCoat® were tested as blocking agents, using optical density measurements of microfluidic detection zones after testing. (**A**) Disease negative sample and (**B**) HIV and Syphilis disease positive sample. Data are averages (*n*=2), with individual data shown as dots (•).

3.3.3 Scale-up procedure for functionalization of microfluidic cassettes

An automated liquid dispensing robot (OPKO Diagnostics) was used for scale-up manufacturing efforts for reproducible and high-throughput cassette preparation (**Fig. 23**). The robotic

dispensing arm was used to prepare microfluidic cassettes at Columbia University prior to shipping to study sites in Rwanda.

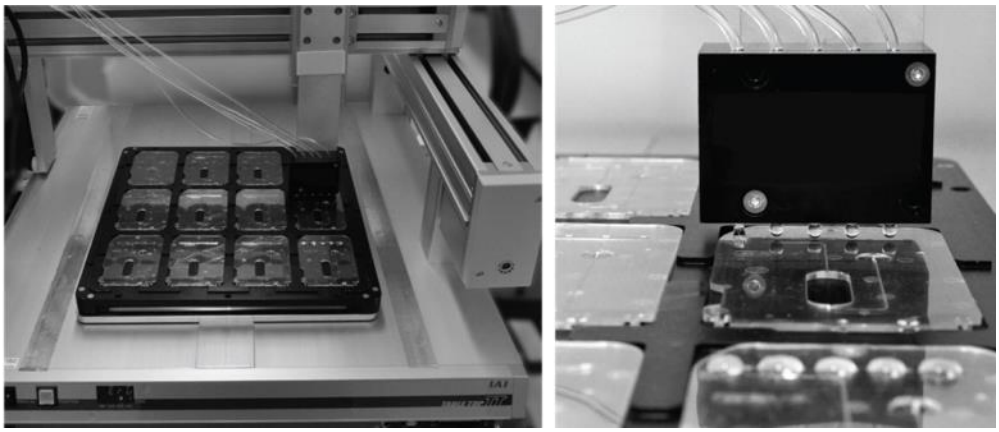

Figure 23. Robot-assisted manufacturing for cassette preparation. Spotting solutions of capture proteins are prepared in batches of 5mL and then connected to plastic inlet tube feeds to the robotic dispensing arm. Trays of microfluidic cassettes (12 cassettes per tray) are placed under the robot arm and a pre-programed dispensing path deposits 36uL of fluid per zone. For negative control zones, no solution was fed into the corresponding plastic inlet tubing line. Each tray requires ~1 minute for completion of spotting

3.3.4 Stability of microfluidic assay

3.3.4.a Reconstitution of lyophilized secondary antibodies for field work

To improve long term stability in shipping and storage of our device, we lyophilized gold-conjugated secondary antibodies inside the antibody holder, along with a stabilizer (but no detergent or anticoagulant) and packed the holder in an individual moisture barrier bag prior shipping them to Rwanda. We showed that lyophilized gold-conjugated antibodies had comparable performance to those freshly diluted in buffer from a refrigerated stock solution (**Fig. 24**). No significant differences were observed for IgG (as shown by the hIgG target zone and rabbit anti-goat IgG positive control zone signals) or IgM (as shown by the hIgM target zone) antibody yields.

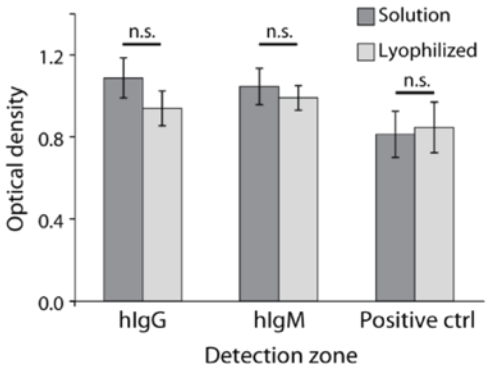

Figure 24. Comparison of signal from gold-labeled anti-hIgG/anti-hIgM antibodies lyophilized in a plastic antibody holder and freshly prepared in solution. Detection zones were functionalized with human IgG, human IgM and rabbit anti-goat antibodies (positive ctrl). Data are averages ±1 SD (*n*=3). n.s. not significant, Student's t-test. Comparison of signal from gold-labeled anti-hIgG/anti-hIgM antibodies lyophilized in a plastic antibody holder and freshly prepared in solution.

3.3.4.b Prepackaging of reagents

Two PBS-T and four water washes as well as Silver Reagents A and B (silver nitrate and hydroquinone respectively) were loaded into the reagent cassette (**Fig. 20**) manually by pipetting and sealed using an adhesive tape (OPKO Diagnostics). The two silver reagents are stable separately and thus stored separately on the reagent cassette; the venting port design allowed mixing of the silver reagents immediately prior to use, minimizing silver auto-catalysis. To prevent exposure of chemicals to the user, sample and reagents were contained in a membrane filter within the cassette, and the antibody holder was securely connected to the cassette. Industrial robotic techniques for loading reagents and applying adhesives can be employed for a high-throughput manufacturing.

3.3.4.c Shelf life and transportation of assay cassettes

To mimic real testing conditions, we prepared assay test cassettes ahead of time at Columbia University before transporting them to field testing sites in Rwanda. By using StabiliCoat, a blocking and stabilizing agent, during physisorption of capture proteins, we found the protein to

retain its original function over 3 weeks at 60°C (**Fig. 25**) (equivalent to roughly 28 weeks at 25°C according to Arrhenius-like approximations [104]). Assay cassettes were transported from New York City to Kigali, Rwanda through regular check-in baggage packaging and procedures, with cassettes stored in sealed plastic bags with desiccant, and some ice packs to counteract large spikes in temperature. Further stability experiments should be carried out at room temperature with humidity-controlled packaging for long-term stability of prepared cassettes. Additional stress tests for transportation and handling are also required for full characterization of shelf life.

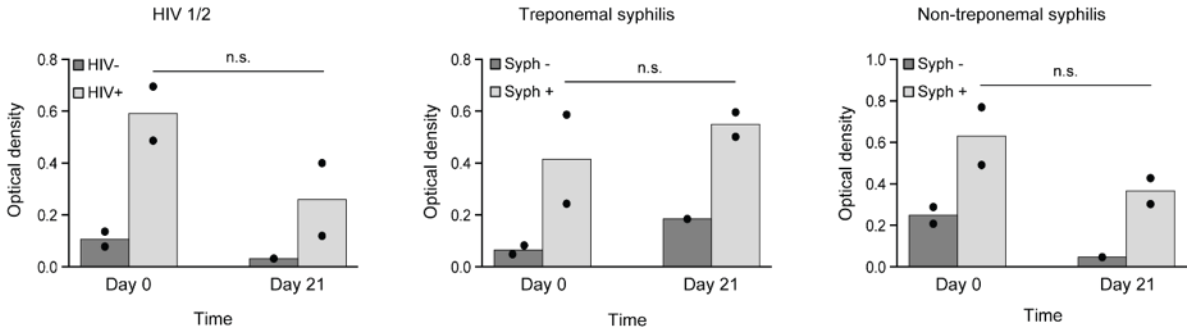

Figure 25. Stability of functionalized protein on microfluidic cassette. Cassettes were prepared using StabilCoat Immunoassay Stabilizer (Surmodics). Microfluidic surfaces were stored for 21 days at 60ºC, after which signals on HIV 1/2, treponemal syphilis, and cardiolipin zones were measured with stored plasma samples. Data are averages (*n*=2), with individual data shown as dots (•). n.s., not significant, two-tailed Student's t-test.

The silver amplification reagents remain the main component of our device that require cold-chain storage. The remaining other wash buffers (water, PBS-Tween solutions) are stable at ambient conditions and lyophilized antibodies have been shown to be stable for over 6 months at room temperature [97, 105].

3.3.5 Early smartphone dongle prototype

We demonstrated that a portable iOS device was capable of powering the dongle for HIV antibody detection. The sensitivity and specificity of the early dongle instrument in classifying

HIV plasma samples was 100% and 100%, respectively using an OD cutoff of 0.500 (**Fig. 26**). This could only be considered as a preliminary study however, as samples were pre-selected for strongly positive/negative status. Integration of electrical and optical components as well as a fluidic actuation module was also not completed. Significant manual adjustment was needed to place the microfluidic cassettes in correct alignment with the dongle optical components, obviating the need for a form factor with simpler insertion and stabilization of cassettes for run-to-run standardization and ease of use as a POC instrument.

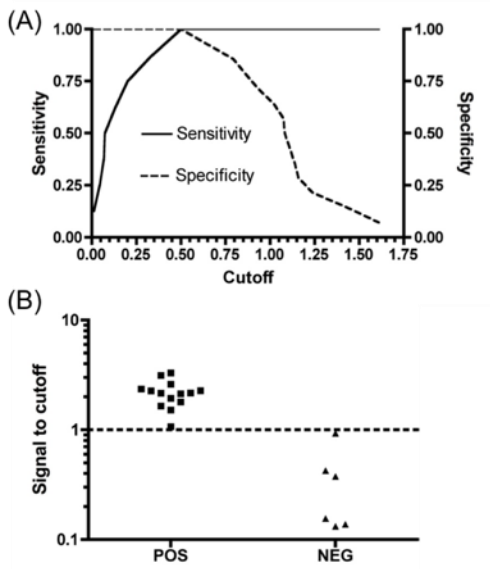

Figure 26. Diagnostic performance of early dongle prototype. (A) The receiver-operating characteristic (ROC) curve showing sensitivity and specificity as a function of OD cutoff values. **(B)** A vertical scatter plot showing signal-to-cutoff values for the HIV disease positive and negative specimens using cutoff normalization of 0.500.

3.3.6 Smartphone dongle for field-usage

In developing a device that could be deployed for field-usage in resource-constrained settings, our aims were to design a robust, portable and low-cost instrument that could expand access to ELISA-quality diagnostic tests and allow for "plug and play" operation. The smartphone dongle measures 7.5cm x 7cm x 5cm, weighs 130g and uses the audio jack of a phone for power and

data transmission (**Fig. 27**). Conventional ELISA assays require a plate-reader and computer instrumentation that can cost > $15000 to perform a quantitative test with objective readout; in contrast, the dongle cost is approximately $34 for a semi-quantitative test with objective readout. A full bill of materials is outlined in **Table 11**. This was achieved through the power-free vacuum design and the FSK implementation of power and data transfer between the dongle and smartphone.

The dongle measures the optical density (absorbance) of silver enhancement on each assay as described previously (Chapter 2). Power is only consumed during optical density readings (8.5 mW) and information transfer back to the smartphone (1.5 mW). Over the course of the 15-minute assay, the dongle has an average power consumption of only 1.6 mW as no power is consumed by the dongle while sample and washes are flowing.

Figure 27. Image of smartphone dongle with a microfluidic chip connected to an iPod Touch (4th Gen).

The dongle has no internal battery and uses only the power delivered by an audio signal sent from the smartphone. In our field work, a 19-kHz audio signal was sent from an iPod Touch (4th generation) through the audio jack to the dongle, and converted into a stable DC 3.0V. The

power harvested from the audio jack is stable and sufficient for reliable optical density (OD) measurements. The custom app converts the photodiode signals to OD (or absorbance) units and can be reported as "positive" or "negative" when compared to pre-set cutoff thresholds for each assay marker.

In a direct comparison of OD signal measurement of an HIV-positive sample on the smartphone dongle compared to a benchtop analyzer (OPKO Diagnostics, see Chapter 2), no significant differences were observed on both the R17 and HIV target zones (**Fig. 28A**). The negative control zone showed a slight difference ($p = 0.03$) between the two instruments, however the dongle also showed close linear correlation ($R^2 = 98.9\%$, **Fig. 28B**) between OD signals and RPR titers (1:128 titer) measurement.

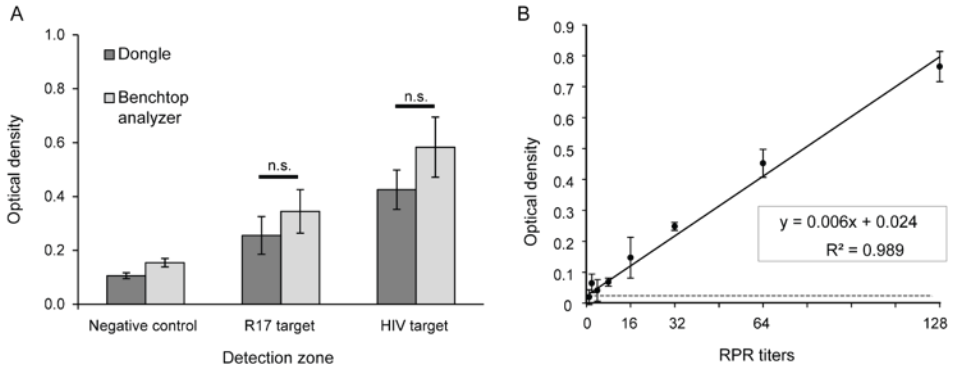

Figure 28. OD readings using smartphone dongle. (A) Optical density (OD) measurements on negative control, R17 (treponemal syphilis) and HIV target zones were recorded on both the dongle and a benchtop analyzer for an HIV-positive whole blood sample. Readings were done on the same cassette between both instruments. Data are averages ± 1 SD (*n* = 3). n.s., not significant, two-tailed Student's *t*-test. **(B)** OD measurements on the non-treponemal target zone were recorded for serial dilution of an RPR positive (1:128) serum. Data are averages ±1 SD (*n*=3) and plotted with a linear regression fit and correlation.

Table 11. Bill of components for dongle

Component	Vendor	Part no.	Qty	Cost per ($ USD)	Total ($ USD)
Audio connector	Amazon	n/a	1	1.000	1.00
Transformer	Coilcraft	LPR6235	1	0.770	0.77
MOSFET, N-Channel	Mouser	522-ZXM61N03FTA	2	0.210	0.42
MOSFET, P-Channel	Mouser	522-ZXM61P03FTA	2	0.210	0.42
Schottky diode	Digikey	DFLS120LDICT-ND	1	0.180	0.18
Voltage regulator	Digikey	576-1283-1-ND	1	1.150	1.15
Ultralow power microcontroller (MSP430F1611)	Digikey	296-22900-ND	1	10.310	10.31
Oscillator	Digikey	SE2413CT-ND	1	0.770	0.77
Operational amplifier	Digikey	ADA4505-1ARJZ-R2CT-ND	5	1.300	6.50
636-nm Red light-emitting diode (LED)	Digikey	67-2194-1-ND	5	0.190	0.95
Photodiode	Digikey	475-2659-1-ND	5	0.560	2.80
Capacitor, 0.1 µF	Digikey	1276-1443-1-ND	10	0.003	0.03
Capacitor, 1 µF	Digikey	1276-1010-1-ND	1	0.009	0.01
Capacitor, 2.2 µF	Digikey	1276-1183-1-ND	1	0.020	0.02
Capacitor, 4.7 µF	Digikey	1276-1056-1-ND	1	0.049	0.05
Capacitor, 10 µF	Digikey	1276-1451-1-ND	3	0.140	0.42
Resistor, 27 Ω	Digikey	311-27JRCT-ND	5	0.002	0.01
Resistor, 1 kΩ	Digikey	311-1.00KLRCT-ND	1	0.003	0.00
Resistor, 10 kΩ	Digikey	311-10KJRCT-ND	1	0.002	0.00
Resistor, 100 kΩ	Digikey	311-100KJRCT-ND	1	0.002	0.00
Resistor, 300 kΩ	Digikey	P300KGCT-ND	5	0.004	0.02
Resistor, 5.1 MΩ	Digikey	541-5.10MHDKR-ND	1	0.017	0.02
10 pin flat flexible cable (FFC) connectors	Digikey	HFN410CT-ND	3	0.840	2.52
10 pin FFC	Digikey	WM14313-ND	1	2.020	2.02
Printed circuit board (PCB) – Mainboard	PCB Universe	Custom	1	0.420	0.42
PCB – Photodiode	PCB Universe	Custom	1	0.420	0.42
Plastic casing	Injection molding company	Custom	1	3.000	3.00
				TOTAL COST	**34.23**

3.3.7 Field trial

Healthcare workers (HCWs) tested freshly obtained fingerprick blood samples from 96 patients in three clinics in Kigali, Rwanda (**Tables 12-13**). After a short 30-minute training, HCWs were able to run a fingerprick blood sample on the smartphone dongle STI test, using the smartphone app for guidance and visualization of results. In parallel, the study team performed the test on the smartphone dongle platform using corresponding venous blood from the same patients.

Test results for detection of each marker were compared with the gold standards of lab-based HIV ELISA, TPHA, and RPR, and presented in terms of signal-to-cutoff of each target relative to its reference test displayed as vertical scatter plots and ROC curves. Cut-off values to determine if a sample was positive or negative for each marker were selected by using receiver-operating characteristic (ROC) curves. Although a final product will offer preset cut-off values, in this development work, we identified cut-off values retrospective to data collection that maximize sensitivity (and thus minimize false-negatives) because our test is targeted towards first-line screening applications.

From fingerprick whole blood testing performed by local HCWs, HIV antibody detection had a sensitivity of 100% (95% CI: 59–100%) and specificity of 87% (95% CI: 78–93%). Treponemal syphilis antibody detection had a sensitivity of 92% (95% CI: 64–100%) with specificity of 92% (95% CI: 83–97%). Anti-cardiolipin antibody detection had a sensitivity of 100% (95% CI: 48–100%) with specificity of 79% (95% CI: 69–87%). ROC curve analysis showed AUCs of 0.96 for HIV, 0.90 for treponemal syphilis, and 0.92 for non-treponemal syphilis (**Fig. 29**).

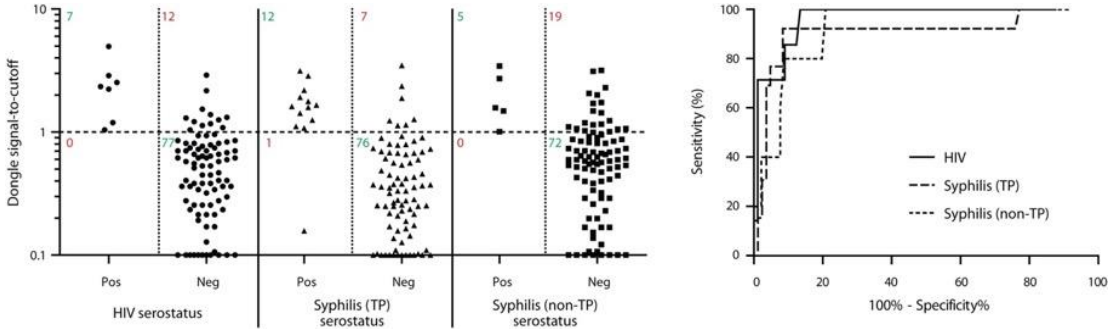

Figure 29. Diagnostic performance of the dongle with clinical fingerprick whole blood samples run by minimally trained healthcare workers. Vertical scatter plots (left) showing signal-to-cutoff ratios of samples positive (Pos) or negative (Neg) for HIV, treponemal (TP) syphilis and non-treponemal syphilis (non-TP) as determined by gold standard tests of HIV ELISA, TPHA and RPR tests. Receiver-operating characteristic (ROC) curves illustrating Area-Under Curve (AUC) for each marker are shown on the right.

From venipuncture whole blood testing performed by the study team, the dongle yielded a sensitivity and specificity of 100% (CI, 59.0 – 100), 91% (CI, 83.0 – 96.0) for HIV, 77% (CI, 46.2– 95.0), 89% (CI, 80.4 – 95.0) for treponemal syphilis, and 80% (CI, 28.4 – 99.5), 82% (CI, 73.0 – 89.6) for non-treponemal syphilis (**Fig. 30**). There is no statistical difference of dongle assay performance on fingerprick and venous whole blood with p-values of 0.45, 1.0, and 0.33 for HIV, treponemal syphilis, and non-treponemal syphilis, respectively using McNemar's test.

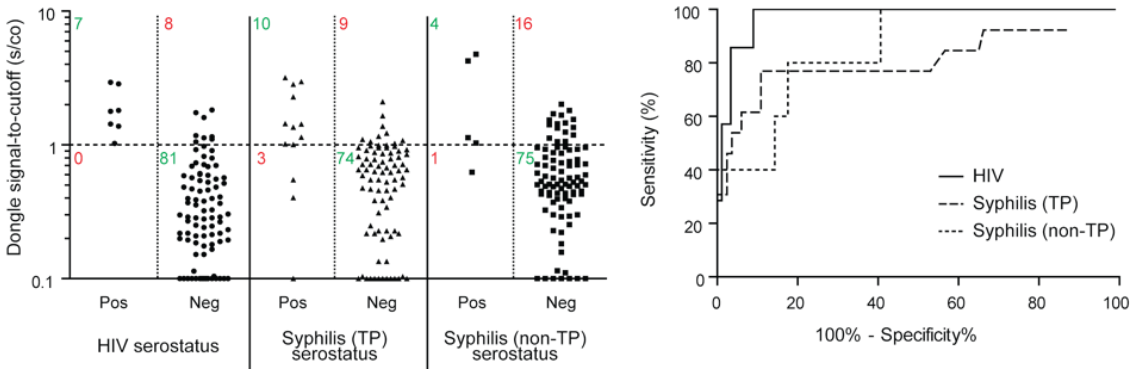

Figure 30. Diagnostic performance of the dongle with venipuncture whole blood samples run by the study team. Vertical scatter plots (left**)** showing signal-to-cutoff ratios of samples positive (Pos) or negative (Neg) for HIV, treponemal (TP) syphilis and non-treponemal syphilis (non-TP) as determined by gold standard tests of HIV ELISA, TPHA and RPR tests. Receiver-operating characteristic (ROC) curves illustrating Area-Under Curve (AUC) for each marker are shown on the right.

3.3.8 User feedback

A third party interviewer administered surveys to gather feedback from both users (HCWs) and patients involved in the study, in order to reduce biases from the study team. Qualitative feedback from surveys of HCWs involved in this study indicated that they appreciated the lack of user interpretation to read the test result or external power to operate. Most thought that the dongle would be most useful in low-volume testing sites such as mobile visits or neighborhood outreach sessions compared to the higher level health centers. When asked how long it typically takes to perform each test (from sample acquisition to issuing results), HCWs self-reported an average of 31 minutes to perform HIV rapid tests, 21 minutes for an RPR test (nontreponemal syphilis) and 16 minutes for the triplex HIV-syphilis test using the dongle. HCWs also noted that the dongle system could be used as a backup test during power outages, which occurred several times during the field trial itself. In concurrence with our own observations and design planning, HCWs felt that the dongle could be made more field ready with reduction of minor peripheral steps currently performed by the study team (e.g. sample dilution).

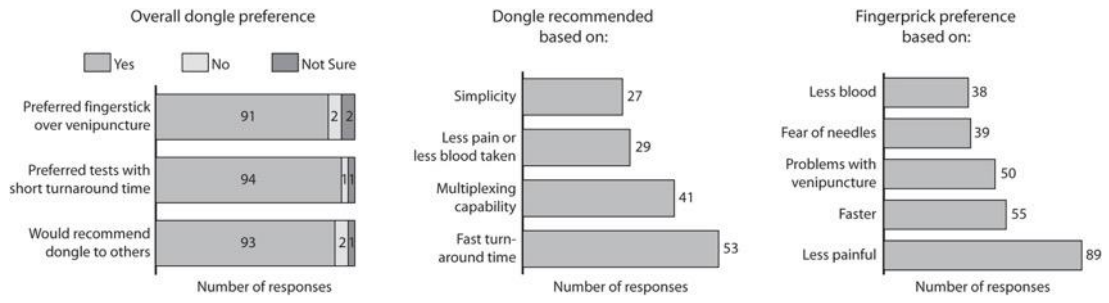

Figure 31. Feedback from patients on their experience with the smartphone dongle testing platform during the field trial.

Table 12. Patient demographics in field trial (Kigali, Rwanda)

Patients	***(n = 96)***
Average age (years)	31 (21-62)
Gender	
Male	40
Female (pregnant)	56 (23)
Clinic	
VCT (Voluntary counseling and testing)	52
PMTCT (Prevention of mother to child transmission)	38
GC (General consultation)	6

Table 13. Healthcare workers in field trial (Kigali, Rwanda)

Healthcare workers	***(n = 5)***
Average age (years)	32 (26-47)
Gender (n)	
Male	0
Female	5
Background	
Average time working in clinical settings (*years*)	10.8 (7-24)
Laboratory technicians, *(n)*	5
Experience with RDT, *(n)*	5
Experience with fingerprick, *(n)*	5
Nursing education, *(n)*	3

Patients were surveyed on their experience with the dongle, preferences for blood sampling methods and acceptability of the dongle as a recommended test method (**Fig. 31**). The vast majority of patients preferred fingerprick over venipuncture blood sampling, preferred a test method with a shorter turnaround time and would recommend the dongle to others. 97% of patients surveyed would recommend the dongle to others because of the fast turnaround time (57%), potential to offer results for multiple diseases (44%), and simplicity of procedure (29%). Fingerprick blood collection was preferred to conventional venipuncture by 95% of patients because it is less painful (98%), takes shorter time (60%), the HCW had trouble with the needle collection (55%), the patient is scared of a needle (43%), and a fingerprick takes less volume of blood (42%). 2% of patients however, stated a preference for venipuncture because they trusted the result more with venous blood.

3.4 Discussion

We demonstrated that healthcare workers could successfully run a multiplexed STI test on our smartphone-dongle platform with minimal training and high patient approval. This was also the first study in which our device was tested with fingerprick whole blood, which showed no significant difference to venous whole blood for HIV, treponemal and non-treponemal syphilis markers on our STI panel. This study allowed us to assess field-performance of the smartphone-dongle STI test, which offers significant differences from testing in the controlled settings of an academic or clinical laboratory. In the field, sensitivities have been reported to be as low as 82% and specificities as low as 85% for the widely used HIV RDTs [106-108]; 64–96% sensitivity and 97–99% specificity for treponemal syphilis antibody tests (Determine, SD Bioline,

Syphicheck, VisiTect, and Chembio) [109-111]; and 85% sensitivity and 96% specificity for non-treponemal syphilis antibody test (Chembio) [111] on whole blood (fingerprick or venipuncture) performed by trained staff. The diagnostic performance of the smartphone dongle was comparable to that of other RDTs in the field. We were also able to gather feedback from target end-users and observe clinical workflow to see where integration of our device could be most useful or applicable.

Testing in the field requires considerations markedly different from testing in controlled academic or clinical laboratories. We built on previous work in creating a dual HIV-syphilis assay with ELISA-like performance to develop an assay platform with considerations for scale-up, stability, reagent pre-packaging and transport to field sites in mind for "plug and play" operation by target end users. By leveraging the portability, processing power and audio-jack powering and data transmission features of a smartphone, we also developed a low-cost device to expand diagnostic testing capabilities in resource and infrastructure constrained settings. While not demonstrated in this study, digitization of rapid test results and ability to upload or link to an electronic medical record (EMR) system further delivers on the value proposition of a smartphone as a platform device for diagnostic testing.

Addition of a non-treponemal test and detection of IgM, performed alongside HIV, moves another step towards a complete POC multiplex antenatal-care panel. Performing three individual commercially-available tests can cost up to $8.50 USD [$0.80-5.00 for HIV RDT [57], $1.00-3.00 for treponemal RDT [49], and $0.50 for RPR [58]]. By comparison, material

and reagents cost per test for our triplex test is about $1.50 USD, leaving room for a competitive market pricing. In addition, compared to RDTs, the dongle offers automation of assay, objective readout of signal, and quantitation, although it requires more instrumentation than RDTs. RDTs present challenges for untrained users to execute precise liquid handling and metering, particularly in multi-step tests. To improve user interpretation of RDT band intensities, researchers have expanded detection methods and integrated the tests with electronic readers [22, 23]. A series of advances have been made on smartphone readers for POC tests by the Ozcan group[112-114], which has led to the creation of a startup company[115]. Most of these technologies remain limited to academic demonstrations and even commercial devices have not yet penetrated widely for global health use cases.

Remaining work to further translate our device for a resource and infrastructure limited setting (Use Case 4) would be to continue optimization with whole blood, reduce peripheral assay operation steps, continue to characterize and improve overall device and assay stability as well as further deliver on the value proposition of the smartphone integration (e.g. software development to connect to medical databases). Finally, a large-scale validation of the assay to determine clinical sensitivity and specificity would be needed to begin any kind of regulatory approval process (WHO pre-qualification program for in vitro diagnostics, CLIA-waiver, CE mark, etc).

HIV Self-Testing Tool for Consumers in Developing Countries

3.5 Background

During the first field trial, we demonstrated user testing of our device in the field with healthcare workers. This work generated a parallel interest from our in-country partner, the Rwandan Ministry of Health/Rwandan Biomedical Center, in assessing the potential to expand HIV diagnostic services even beyond the clinic.

Self-testing has been proposed as the next step in HIV prevention strategy, particularly in the case of high risk groups around the world where uptake of traditional testing and counseling services remains low[116]. Barriers include lack of regular access to clinical testing sites or primary care, fear of stigmatization, discrimination and concerns of confidentiality of results. Alternate strategies such as community outreach HIV counseling and testing (HCT) programs through mobile clinics and door to door testing have improved testing uptake in many areas of Sub-Saharan Africa and Asia[116]. Self-testing could supplement outreach efforts, enabling consumers to find out their status in the privacy of their own homes. A recent meta-analysis of eleven HIV self-testing studies revealed high acceptability of HIV self-testing for risk groups in study populations that spanned resource-limited settings (Kenya and Malawi) as well as high income countries (United States, Spain and Singapore)[116]. Saliva based (OraQuick HIV

Test, Orasure Technologies) and blood based (Determine HIV 1/2 Test, Abbott Laboratories) rapid diagnostic tests (RDTs) were evaluated along with home collection services where clients collected samples for mail-in laboratory testing. Self-testing could be performed accurately by the majority of self-testers, who valued privacy and confidentiality of this option[116].

Several aspects of our platform lend themselves to a self-testing application, namely that minimally trained users can perform the steps needed to run the test and get objective readout results. Integration of a smartphone as a platform device adds further value for future options to manage, store and potentially even share testing data.

Smartphones are becoming more accessible even in resource-limited settings. In Q1 2016, the total number of mobile subscriptions was around 7.4 billion, of which 2.6 billion were smartphone users[117]. By 2020, the projected increase in smartphone subscribers will reach 6.1 billion users[118]. Emerging markets in Asia Pacific, the Middle East and Africa are predicted to be the biggest drivers of growth in mobile subscriptions[117, 118]. The powerful built-in sensors, processors and data transmission capabilities, including Global System for Mobile Communication (GSM), wireless fidelity (Wi-Fi), Bluetooth, and universal serial bus (USB) for long and short range communication make the smartphone an extremely powerful tool for healthcare applications, particularly for diagnostics[119].

To this end, we sought to explore the feasibility of a smartphone-integrated HIV diagnostic test for a home-testing in a resource-limited setting (Use Case 4, consumer use). From our previous

field trial, we identified several areas for improvement and implemented several changes to the assay and device. We designed a follow-up pilot field trial in Rwanda with aims to explore 1) assay performance in the hands of completely untrained users and 2) user-friendliness of a smartphone-interfaced device and 3) preliminary feedback on user acceptability of diagnosis, data storage and potential for sharing sensitive information in a smartphone format.

3.6 Methods

3.6.1 Hardware ("Dongle 2.0") Fabrication

Custom-printed circuit boards were designed in Eagle CAD and printed from PCB Universe. Bluefruit LE Bluetooth Low Energy (BLE 4.0) nRF8001 Breakout Board (Adafruit) was connected to the microcontroller and powered by a CR2032 Lithium 3V coin battery (20.0mm). LEDs and photodiodes were precisely aligned with the cassette slot so that testing zones aligned without manual effort. One-mm pinholes made of 1-mm thick black Delrin (McMaster-Carr) were aligned above each photodiode to prevent stray light. The dongle casing was designed in SolidWorks and printed in-house (Objet 24 3D-Printer, Stratasys). Vacuum chamber was created with a one- way umbrella valve (Minivalve), a rubber bulb from a 60-mL syringe (Becton Dickinson), and a conical spring (Century Spring Corp) inside to aid re-expansion. Silicone rubber o-rings and sheets (McMaster-Carr) were used to connect to outlet and seal the venting port. A bill of materials is provided (**Table 14**).

3.6.2 Smartphone app for self-testing users in Rwanda

The app was written in iOS Swift using the XCode IDE for macOS. The app was manually downloaded by research staff to iPhone 5 device used by participants. Captured results were stored locally on the phone during the study, extracted through SQLite browser and exported as a CSV file for analysis. The app framework consisted of step-by-step instructions with pictures and text instructions that were translated from English to Kinyarwanda (local language in Rwanda) by a third-party translator on the study team.

3.6.3 Assay optimization

3.6.3.a Whole blood

De-identified whole blood samples with known disease status were provided from the Columbia University Medical Center (CUMC), through study approval from Columbia University's Institutional Review Board. Sample reference testing was conducted by CUMC staff, as per the IRB protocol, using plasma separated from whole blood on the Abbott ARCHITECT. Blinded samples with study numbers were then provided to the Columbia development team for assay optimization testing.

Plastic cassettes were functionalized with HIV antigen spotting concentrations at the usual concentration of 2 µg/ml and an increased concentrations of 4-10 µg/ml. Signal performance was assessed after introduction of 2 µl of undiluted whole blood sample, per test. Reagents were pre-loaded as described previously and OD measurements were taken using the benchtop analyzer. Signal performance was compared after testing with 18 clinical whole blood samples.

Table 14. Bill of components for "Dongle 2.0"

Component	Vendor	Part no.	Qty	Cost per ($USD)	Total ($USD)
Photodiode	Digikey	475-2659-1-ND	5	0.514	2.57
Operational Amplifier	Digikey	ADA4505-1ARJZ-R2CT-ND	5	1.980	9.90
Capacitor, 0.1 uF	Digikey	1276-1443-1-ND	5	0.003	0.02
Resistor, 300 kΩ	Digikey	P300KGCT-ND	5	0.003	0.02
Resistor, 100 kΩ	Digikey	311-100KJRCT-ND	1	0.002	0.00
Resistor, 35 Ω	Digikey	1276-3914-1-ND	5	0.002	0.01
Resistor, 10 kΩ	Digikey	311-10KJRCT-ND	1	0.002	0.00
636nm Red LED - 1100 mcd	Digikey	754-1786-1-ND	5	0.182	0.91
Thermistor 10 kΩ	Digikey	490-2414-1-ND	1	0.043	0.04
Capacitor, 1 uF	Digikey	1276-1010-1-ND	2	0.009	0.02
10 pin FFC connectors	Digikey	WM6790CT-ND	1	1.013	1.01
10 pin FFC connectors	Digikey	HFJ110CT-ND	2	1.000	2.00
10 pin flat flexible cable	Digikey	WM14313-ND	1	2.420	2.42
Voltage Regulator	Digikey	296-22681-1-ND	1	0.650	0.65
Pins	Digikey	SAM1000-32-ND	0.3	4.913	1.54
Pin Sockets	Digikey	SAM1104-32-ND	0.3	4.959	1.55
Switch	Digikey	EG4934-ND	1	0.457	0.46
CR2032 Battery	Digikey	N189-ND	1	0.184	0.18
Battery Holder	Digikey	BU2032SM-HD-GCT-ND	1	0.755	0.76
Bluefruit LE Breakout (Adafruit)	Digikey	1528-1199-ND	1	15.960	15.96
MSP430f1611 Microcontroller	Digikey	296-17205-ND	1	10.310	10.31
PCB - Photodiode	Pentalogix	Custom	1	0.420	0.42
PCB - Mainboard	PCB Universe	Custom	1	0.420	0.42
Plastic Casing	Injection molding company	Custom	1	3.000	3.00
Rubber bulb - 60ml	Allegro Medical	561516	1	1.000	1.00
Silicone sheet	McMaster	5787T11	1	0.002	0.00
Switch	Digikey	CKN9838-ND	1	2.460	2.46
Viton O-ring 007 (black)	McMaster	8297T117	1	0.120	0.12
Silicone O-rings 011 (orange)	McMaster	9396K16	1	0.150	0.15
One-way umbrella valve	Minivalve	UM100.014-151.01	1	0.830	0.83
				Total Cost	**58.72**

Addition of an FTA-ABS sorbent (SCIMEDX) was tested for potential effect in reducing non-specific binding (and false positive rate) for the treponemal syphilis assay. Whole blood samples were diluted in a 1:1 ratio with the sorbent (2 µl whole blood mixed with 2 µl sorbent), and 2 µl of the resulting mixture was pipetted into the assay cassette inlet.

3.6.3.b Lyophilized antibody reconstitution

Lyophilization of secondary antibodies was performed by OPKO Diagnostics (Woburn, MA). 9.5 µL of gold-nanoparticle conjugated goat anti-human IgG (0.53 µg/mL) and goat anti-human IgM (0.27 µg/mL) antibodies were lyophilized into 2.5cm of plastic tubing. No detergent or anti-coagulant factors were included in the lyophilization buffer. Lyophilized antibody holders were stored at 4ºC until use. Microfluidic cassettes were prepared at Columbia University using physio-sorption procedures similar to previous studies. Human IgG and rabbit anti-goat IgG were used as capture proteins to test the yield of goat anti-human IgG and human IgM was used as a capture protein to test yield of goat anti-human IgM from the lyophilized antibody holders. It should be noted that rabbit anti-goat IgG was the internal positive control on all HIV-syphilis test cassettes used in previous field work (**3.2.1**).

3.6.4 Pilot field study in Rwanda

This pilot study was designed to get preliminary observations and feedback on the feasibility of a smartphone integrated diagnostic test for self-testing in developing countries. This study was approved by the Columbia University IRB and Rwanda National Ethics Committee. All rules and regulations pertaining to human subject involvement and protection of confidentiality and

sensitive data were followed.

3.6.4.a Participant recruitment and enrollment

The study was conducted at Kimironko (Remera) Health Center in Kigali, Rwanda. Site selection was chosen based on target recruitment from the "Preventing Mother to Child Transmission: (PMTCT) clinic, "Voluntary Testing and Counseling" (VCT) clinic and CD4 clinics for existing HIV-positive persons. Eligibility criteria included enrollment in one of these clinics (thus, already scheduled for HIV and syphilis testing), age 21-65, ability to read and willingness to perform a fingerprick based self-test. Recruitment was done by clinic staff at program specific clinic information sessions using a script developed by the study team. Participants interested in the study were directed to a third-party interviewer fluent in local Kinyarwanda as well as French and English to get more information and undergo the informed consent process.

3.6.4.b Study visit procedures and evaluation methods

After consent, third party interviewer administered a pre-use survey to participants to assess past medical history, risk factors and mobile phone usage. Next, participants underwent a skill demonstration with the device. For training, participants were first shown a short 1 minute instructional video (no audio instructions) on how to operate the device. Participants were then provided with a demo lancet, demo test cassette and the dongle and given the opportunity to go through the directions once with feedback from the study team. After the training, participants were provided with all the test kit components: alcohol swab, lancet, test cassette, dongle and a

smartphone (iPhone 5) with the app already loaded. Participants navigated the app to follow the step by step directions in order to perform the test on their own with no feedback or intervention from the study team (unless they were about to perform a step that was potentially harmful to themselves). Study team used a rubric of steps performed correctly and a timer to assess the participants' skill demonstration. Participants were not given diagnostic results through the device and study visit; instead they received diagnostic results through their normal clinic visit. Afterwards, a third party interviewer administered a post-use interview to assess reactions to the test. Participants were compensated 1000 RWF ($1.33 USD at time of study) for involvement in the study.

Post-use interviews were audio-recorded, transcribed and translated from Kinyarwanda to English by third party interviewers. Three different interviewers conducted the post-use sessions, and transcripts were checked for concordance by at least two individuals.

3.6.4.c Confirmatory testing

Venipuncture whole blood samples in EDTA anticoagulant tubes were collected from each participant as part of their normal clinic visit. After clinical testing was completed, an aliquot of whole blood was used by the study team to test on the device. Another aliquot (~ 500 uL) of plasma was used for HIV ELISA (Vironstika) and TPHA (Spinreact) reference testing at the Rwanda National Reference Laboratory.

3.7 Results

3.7.1 "Dongle 2.0" Hardware

Four main design elements were modified from our previous smartphone accessory: 1) Bluetooth module for data communication (**Fig. 32**), 2) Button cell battery for power, 3) Silver reagent actuation and 4) Smaller overall footprint (casing). Minor changes in fluidic actuation and venting port components were introduced as well.

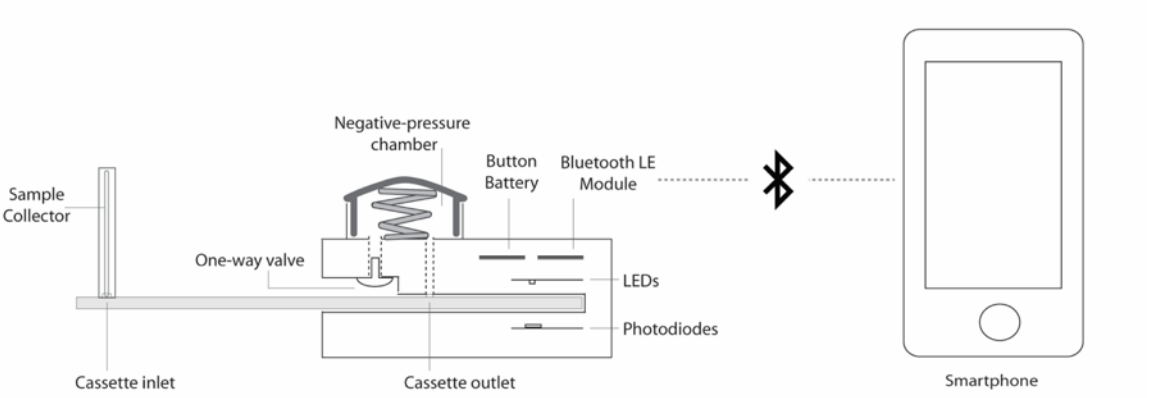

Figure 32. "Dongle 2.0" Schematic. Schematic diagram of the device highlighting a button-cell battery powered Bluetooth module and power-free vacuum generator for fluidic actuation. Once a microfluidic cassette is inserted into the device, each test zone is sandwiched between paired LEDs and photodiodes. Silver development on the cassette results in a proportional decrease in the light sensed by the photodiode and can be quantified by optical density values. The Bluetooth module is connected to a microcontroller (not shown), which is programmed to wait for a signal from the smartphone, take light intensity readings of each zone and then send the information back to the smartphone.

By switching to Bluetooth and adding a button battery for power, we could achieve faster data transfer and a more reliable connection compared to FSK through the audio jack of the phone. Bluetooth allowed for data transfer of five 16-bit integers in under 1 second compared to 90 seconds with FSK. Additionally, we could reduce variability associated with different smartphone models (e.g. varying impendence from audio jacks that affect efficiency of power harvesting). We added a small button cell battery (CR2032, 3.0V) to power the Bluetooth

module, which uses 4.6mW (using the Bluetooth Low Energy Mode) compared to an average 1.5mW used in the FSK audio jack module. While this is a slightly higher power usage, the button cell battery is estimated to last about 760 assays and can be replaced easily. When we designed the first dongle in 2014, the 3.5mm audio jack was a nearly universal feature amongst smartphone versions for both iOS and Android models. In 2016, a year after we switched to Bluetooth, Apple removed the audio jack in the iPhone 7 and all future iPhone models [120]. Google has since eliminated the audio jack in the recently released Pixel 2 and Pixel XL phone models released in 2017 [121].

Using the button cell battery also decreased the CV in replicate optical measurements to 2.8% compared to 10.3% when using the smartphone powered dongle (**Fig. 33**). By increasing the LED intensity, we were able to further decrease CV of signals to 0.23%. These changes allowed for more robust and reliable readings, particularly between devices.

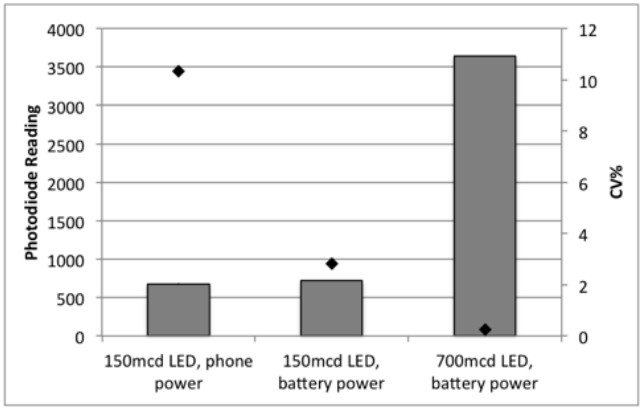

Figure 33. Optical measurement reproducibility. Coefficient of variation (CV %) is shown for photodiode readings from dongles with smartphone power, button cell battery power and with increased LED intensity (150mcd to 700mcd, where millicandela is a unit of luminous intensity).

The casing components were modified to enhance robustness and ease of use. The overall footprint of the "Dongle 2.0" device was almost half (58%) by volume than that of the previous version and untethered from a smartphone for increased portability (**Fig. 34**). For the vacuum chamber, we used a rubber bulb from a 60mL piston syringe, instead of the 140mL bulb in "Dongle 1.0". Previously, the valve opening and associated rubber-o-rings could be jostled out of position with rough handling. The venting port for the one-way valve was moved to the underside of the vacuum bulb chamber, in order to increase device robustness.

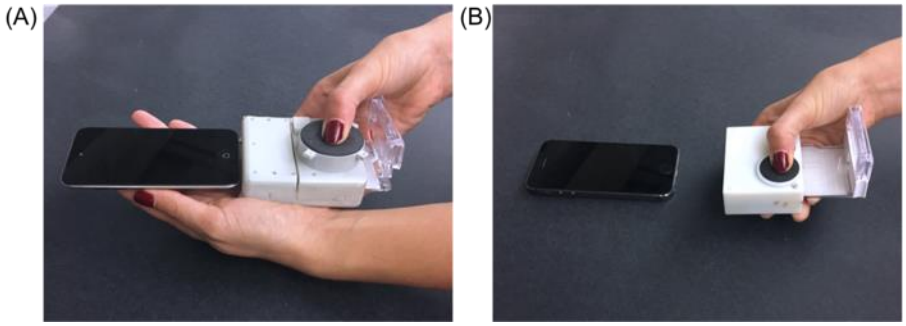

Figure 34. Dongle hardware versions. (A) "Dongle 1.0" was tested in a field trial by minimally trained healthcare workers in Rwanda (citation). Dongle 1.0 measures 7.5cm x 7cm x 5cm, weighs 130g and uses the audio jack of a smartphone for power and data communication through FSK implementation. A 140mL piston syringe bulb was used for the vacuum chamber. **(B) "**Dongle 2.0" was used in pilot study with untrained users in Rwanda as well as a self-testing study in New York City. Dongle 2.0 measures 5.5cm x 7cm x 4cm, weighs 100g, uses a button cell battery (3V) for power and Bluetooth Low Energy module for data communication. A 60mL piston syringe bulb was used for the vacuum chamber.

We also noted that actuation of silver reagents to connect with the microfluidic circuit in our previous design, required users to slide a toggle component that would cover or open a venting port on the reagent cassette. This would initiate the flow and mixing of silver reagents. From our previous field trial, we observed sliding the toggle back and forth for each run, caused the silicone rubber used to seal the port to get worn down easily, and required replacement every couple of days. We designed a manual switch adapted from electrical toggle switches as an alternative to reduce abrasion on the silicone rubber (**Fig. 35**). We removed the electrical contact

from the switch and placed a pin in its place with silicone rubber below. When the switch is pushed in the closed direction, a metal level pushes down the pin, sealing the venting port. This provided a power-free, robust design that allowed us to retain a small form factor.

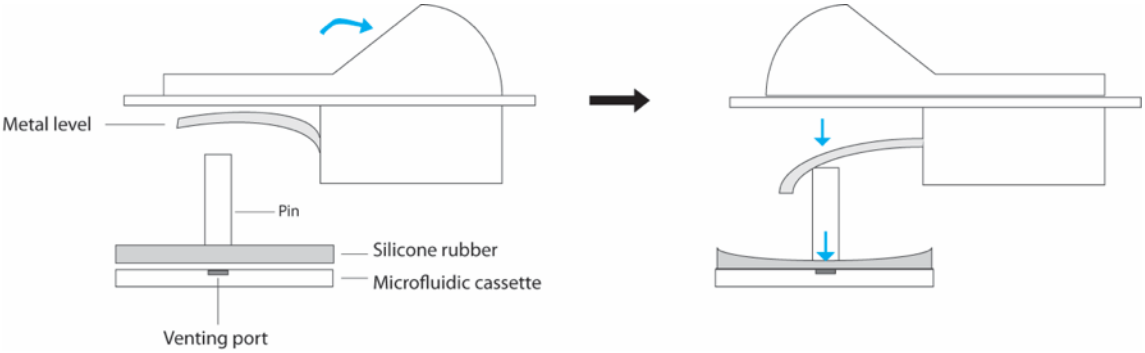

Figure 35. Schematic of dongle switch mechanism. Side view of an electrical toggle switch that was modified such that electrical contacts were removed and a pin placed with silicone rubber below. When the switch is pushed in the closed direction, a metal level pushes down the pin, sealing the venting port of the microfluidic cassette below. The mechanism can be reversed by pushing the switch back to the open position.

3.7.2 Assay improvements to streamline user-operation

In our previous field trial, the study team had to perform companion operation steps, namely in diluting the fingerprick whole blood (and subsequent pipetting back into the mChip cassette), and an incubation step for reconstitution of lyophilized antibodies (Chapter 3.1-3.4 and **Fig. 19**). These steps were necessary to achieve an acceptable signal-to-noise ratio on target zones, both for primary antigen-antibody binding as well as yielding adequate reconstitution of secondary antibodies for signal amplification and internal positive control measurements. To better adapt our product for minimally trained or untrained users, we wanted to reduce the number of peripheral steps needed to perform an assay, particularly those involving other laboratory

equipment such as pipetters. To do this, we wanted to be able to use neat (undiluted) whole blood and eliminate incubation steps using low-shelf life reagents.

We streamlined the assay in two major ways. First, we removed the need for blood dilution. By increasing the surface antigen coating concentration for the HIV detection zone to 10 µg/ml (from 2µg/ml used in the previous field trial, Chapter 3), we were able to improve the sensitivity and specificity of the HIV zone with neat whole blood (**Fig. 36, Table 15**). It should be noted that this optimization was done with venous whole blood samples (with EDTA anti-coagulant).

We also removed the need to pre-fill the antibody holder with buffer to aid with resolubilization. In the previous field trial, the addition of the manual pre-filling step was compounded by using a buffer with limited stability to achieve adequate reconstitution of the secondary antibodies. BSA solutions are typically recommended to be stored under refrigeration and used in 1-2 weeks. PBS and PBS-Tween solutions on the other hand are widely used with long term storage at ambient conditions. In optimizing our reconstitution method, we demonstrated that (1) substitution of a temperature-stable buffer was possible and (2) an incubation period of the reconstitution buffer in the lyophilized holders could be eliminated to achieve comparable end target zone signals (**Fig. 37**).

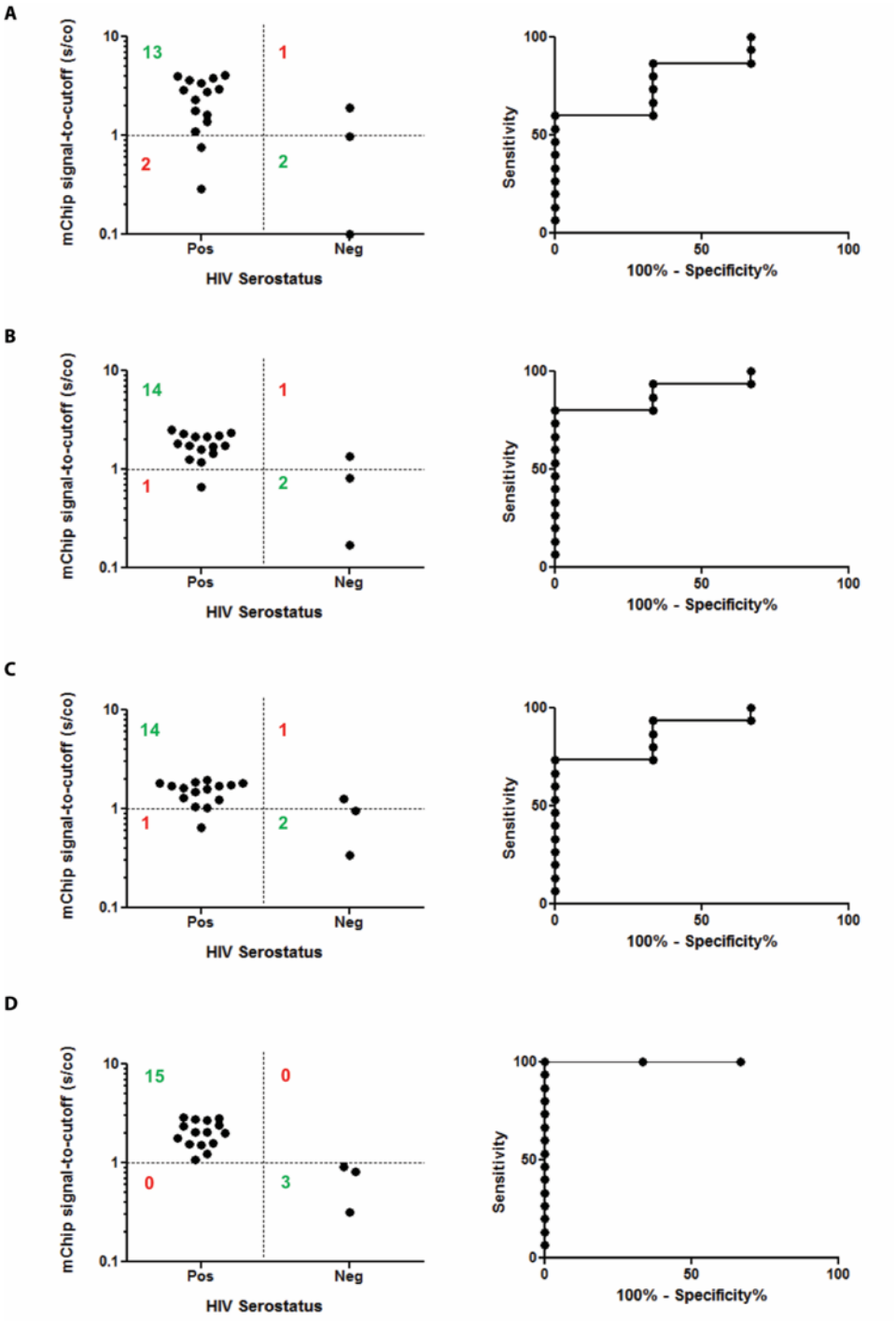

Figure 36. HIV antibody detection optimization with neat whole blood. 18 venous whole blood samples comprising positive (n=15) and negative (n=3) HIV serostatus were tested on microfluidic cassettes with varying HIV antigen capture concentrations of **(A)** 2 µg/ml**, (B)** 4 µg/ml, **(C)** 6 µg/ml, and **(D)** 10 µg/ml. Vertical scatter plots (left**)** show signal-to-cutoff ratios of samples positive (Pos) or negative (Neg) for HIV as determined by gold standard tests of Abbott ARCHITECT HIV ELISA. Receiver-operating characteristic (ROC) curves (right) illustrate Area-Under-Curve (AUC).

Table 15. HIV diagnostic performance with neat whole blood at different capture antigen concentrations

HIV Capture Antigen Concentration	Cutoff	Sensitivity (95% CI)	Specificity (95% CI)	AUC
2 µg/mL	0.1429	86.67 % (59.94-98.34)	33.33% (0.84-90.57)	0.822
4 µg/mL	0.2816	93.33 % (68.05-99.83)	66.67% (9.43-99.16)	0.911
6 µg/mL	0.3710	93.33 % (68.05-99.83)	66.67% (9.43-99.16)	0.889
10 µg/mL	0.3167	100.0 % (78.20-100.0)	100.0% (29.24-100.0)	1.000

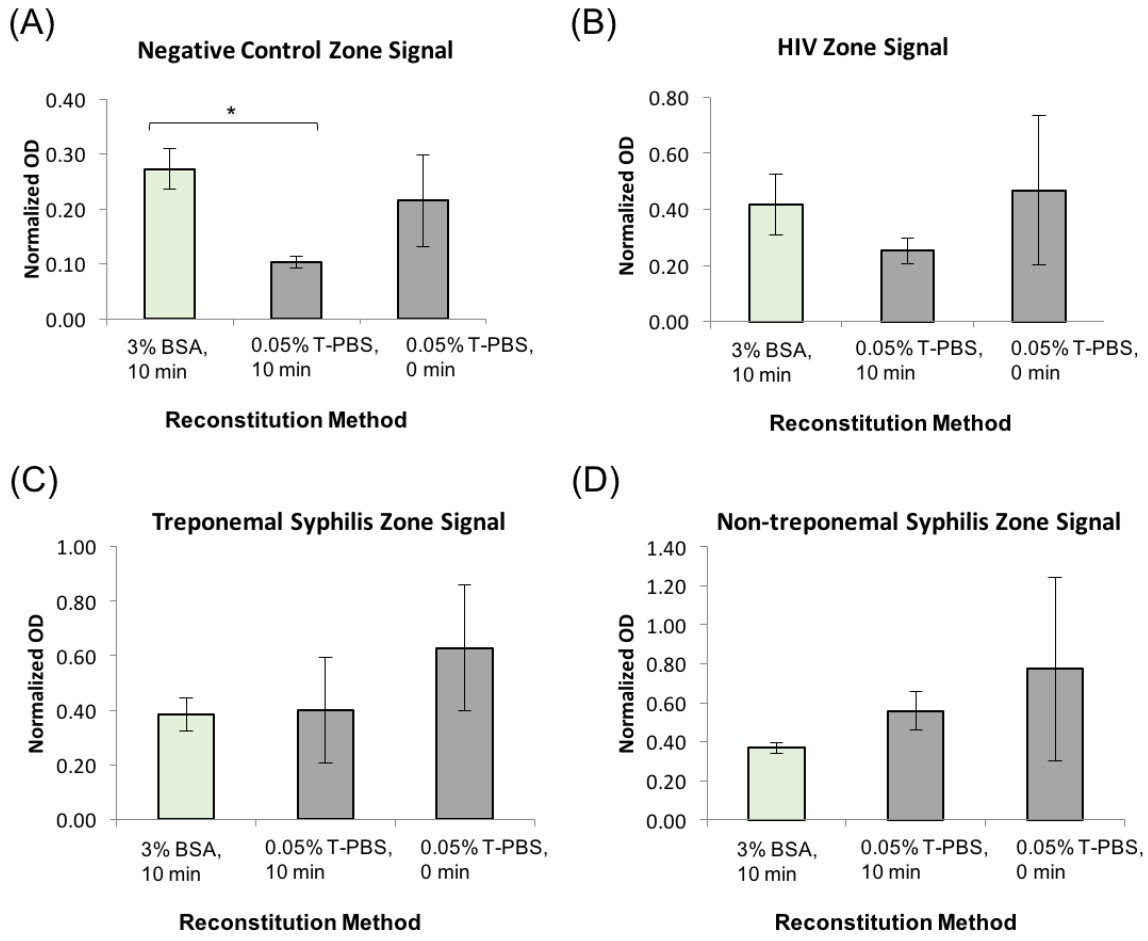

Figure 37. Target zone signals for a dual positive (HIV+/Syphilis+) sera sample using various reconstitution methods for lyophilized secondary antibodies. Field trial condition of using a 3% BSA-0.2% Tween in PBS solution incubated in the lyophilized holder for 10 minutes is shown in green bars. Two additional conditions showing 0.05%Tween in PBS solution incubated for 10 minutes and 0 minutes (direct flow through) are shown in grey bars. **(A)** Negative control zone signals **(B)** HIV target zone signals, **(C)** Treponemal syphilis target zone signals and **(D)** Non-treponemal syphilis target zone signals. Optical Density (OD) measurements were normalized with internal positive control values. Data are averages ± 1 SD (n=3). Asterisk (*) indicates statistical significance ($p<0.05$) using Student's t-test.

Additionally, we wanted to be able to pre-package all reagents into the reagent cassette to create a true "plug and play" experience for minimally trained users in the field. Instead of having to find a way to integrate a relatively large 9 µl bolus into the lyophilized antibody holders, we worked to find a buffer sequence that could work within the constraints of our existing microfluidic circuit (**Fig. 38**). The reagent cassette was designed to hold a maximum of seven-

2µl buffer plugs separated by air gaps (along with compartments for silver amplification reagents).

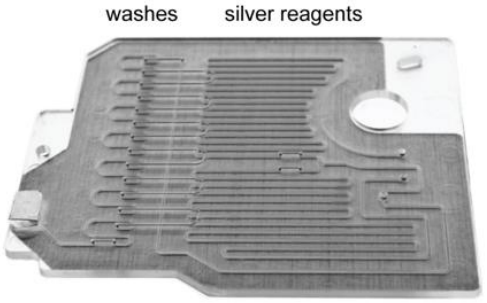

Figure 38. Microfluidic reagent cassette. The reagent cassette allows loading of seven-2µL buffer plugs separated by air gaps and contains two compartments of 50 uL each, for silver amplification reagents A and B.

We demonstrated that reconstitution buffer sequences using the large 9 µl bolus could be substituted with those using only sequences of 2 µl buffer plugs while still achieving comparable antibody yields (**Fig. 39**). Lyophilized gold secondary antibody resolubilization occurs within the first few buffer washes, with the later washes acting to remove non-specific binding.

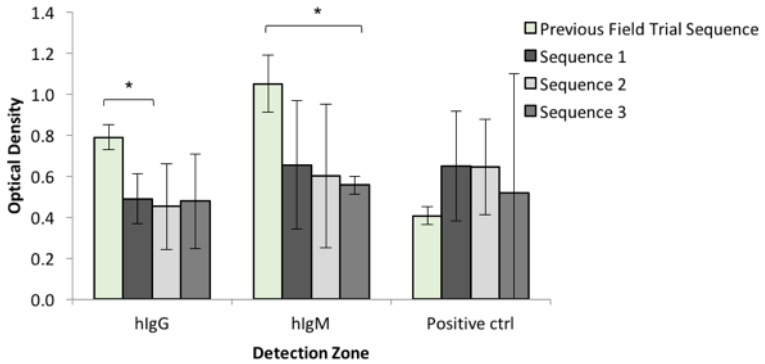

Figure 39. Selection of reconstitution sequence to eliminate pre-filling of lyophilized antibody holders. Detection zones were functionalized with human IgG, human IgM and rabbit anti-goat antibodies (positive ctrl). The previous field trial sequence is shown in green bars (9µL of 3% BSA-0.2% Tween in PBS solution, incubated in the lyophilized holder for 10 minutes, followed by six 2µL wash plugs). Sequences 1-3 used of seven 2µL buffer plugs with no incubation time in the lyophilized holders. Sequence 1 consisted of five 2uL plugs of 0.05%Tween in PBS, followed by two 2uL plugs of water. Sequence 2 consisted of four 2µL plugs of 0.05%Tween in PBS, followed by three 2µL plugs of water. Sequence 3 consisted of three 2µL plugs of 0.05%Tween in PBS, followed by four 2µL plugs of water. Data are averages ±1 SD (*n*=3). Asterisk (*) indicates statistical significance ($p<0.05$) using Student's t-test after ANOVA.

3.7.3 Pilot field study

3.7.3.a Participant background

68 participants were recruited over the study period of 2 weeks (**Fig. 40**). Exclusions based on eligibility criteria reduced the study cohort to 55 and a summary of participant demographics and background is provided in **Table 16**. Approximately two-thirds of participants were female and about a third male. The majority of participants (69%) had a primary school education, with an additional 28% having completed secondary school and only one participant having completed college (undergraduate) education. The majority (86%) lived in an urban area, which correlated with the study occurring in Kigali, the capital city of Rwanda. Median self reported travel time to the clinic was 60 minutes, with the upper end of the range reaching 180 minutes (3 hours). The median annual income was 60,000 RWF (~ $80 USD), with a range of 0 to 2,400,000 RWF (0 to $3200 USD).

3.7.3.b Training and usability

For the first set of participants (n= 15), there was no training process before the skill demonstration with the smartphone based diagnostic device. These participants were simply provided with fingerprick materials (alcohol swab, lancet, gauze, bandage), one disposable test cassette and sample collector, the dongle ("Dongle 2.0") and a smartphone pre-loaded with the custom app. Participants were told to use the app to follow step by step instructions, which included pictures and text (translated in to Kinyarwanda). The study team provided minimal assistance however if participants got stuck or confused on a step for a long time, a study team member would explain the step and move on, while noting assistance on the rubric assessment.

What we observed was that on average, participants in this cohort performed 11.3 out of the 15 required steps correctly (**Table 18).** Difficulties occurred with (1) certain instructions and buttons in the smartphone app, (2) sample collection, (3) connecting the sample holder (Part A) and test cassette (Part B), (4) fully inserting the test cassette into the device, and (5) pushing the bulb to initiate the vacuum. To address some of these issues during the trial itself, we modified text instructions in the app and added a short instructional video (~1:30 minutes) for the next trial set of participants (n= 6, data not shown). For the second set of participants (n= 34), we also added a training sessions for participants to handle the test kit pieces and familiarize themselves with the general test procedure (e.g. understanding the orientation to connect Part A and Part B).

The training took an average of 11 minutes (range: 5-19 minutes) and average time for participants to complete a test was 20 minutes (range: 13-40 min), compared to the first set who took an average of 27 minutes (range: 21-40 min). The minimally trained participant cohort performed 13.7 out of 15 required steps correctly (**Table 18**), showing a statistically significantly improvement ($p < .001$) compared to the first set of participants. While 12 participants performed all steps correctly, only 4 were able to generate a test with a valid positive control. The main reason for the last of valid assay runs seemed to be due to fingerprick blood sample clotting in the channel before flowing completely through, due to lag times in engaging the vacuum. Due to the low number of fully completed runs, we could not compare diagnostic performance in the hands of participants to that of the study team.

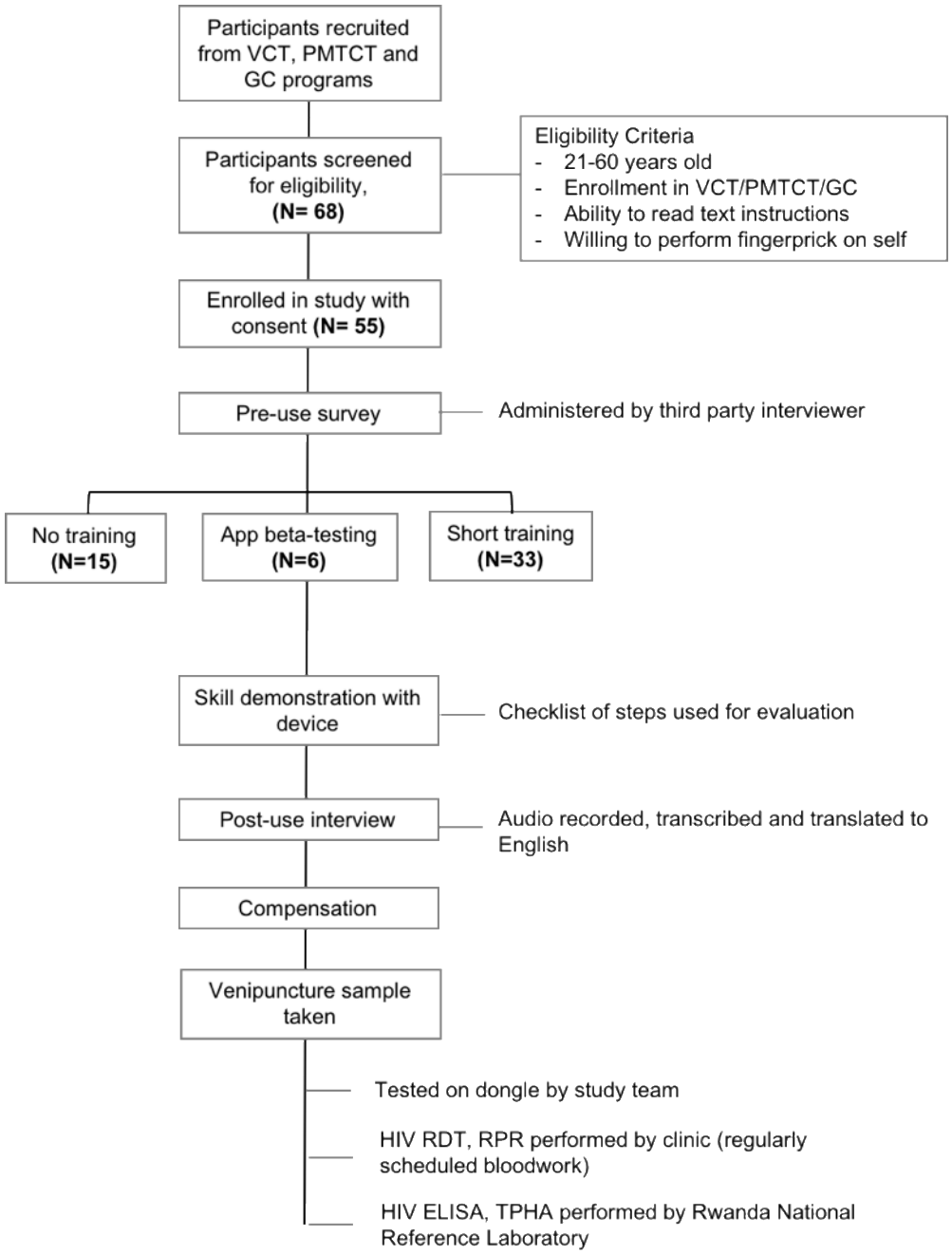

Figure 40. Pilot study field trial design

Table 16. Participant demographics in pilot study (Kigali, Rwanda)

Gender, n (%)		
	Male	21 (38%)
	Female	34 (62%)
Age,		
	In years, median (range)	31 (22 - 54)
Educational Level, n (%)		
	Primary School	40 (73%)
	Secondary School	14 (25%)
	Undergraduate	1 (2%)
Annual income, median (range)		
	In USD	$133 ($0 - 3200)
From Rural or Urban area, n (%)		
	Rural	8 (15%)
	Urban	47 (85%)
Travel time to clinic, median (range)		
	in minutes	60 (2 - 180)

Table 17. Mobile phone usage of participants in pilot study (Kigali, Rwanda)

Do you own a mobile phone?		n (%)
	Yes	48 (87%)
	No	7 (13%)
What type of phone do you own?		n (%)
	Basic	34 (71%)
	Internet-enabled	11 (23%)
	Smartphone	4 (8%)
How do you use your mobile phone?		n (%)
	Texting	40 (83%)
	Phone calls	47 (98%)
	Taking photos	13 (27%)
	Listening to music	16 (33%)
	Watching videos	11 (23%)
	Games	6 (13%)
	Surfing the internet	6 (13%)
	Email	6 (13%)
	Banking	3 (6%)

Table 18. Steps performed correctly by participants in pilot study

	First set	Second set	p-value
1. Cleaned finger with alcohol wipe	15 (100%)	33 (97%)	0.5157
2. Pulled lancet tip off	**7 (47%)**	**31 (91%)**	**<0.001**
3. Used same finger that was cleaned	15 (100%)	33 (97%)	0.5157
4. Pricked finger with lancet	13 (87%)	34 (100%)	0.0251
5. Squeezed finger	13 (87%)	31 (91%)	0.5823
6. Collected blood into Part A	**6 (40%)**	**31 (91%)**	**<0.001**
7. Only filled to the line	9 (60%)	14 (41%)	0.3125
8. Connected Part A to Part B	**6 (40%)**	**31 (91%)**	**<0.001**
9. Inserted chip into device	**8 (53%)**	**33 (97%)**	**<0.001**
10. Pressed bulb	14 (93%)	34 (100%)	0.1188
11. Started Phase 1 (> 1 min)	15 (100%)	33 (97%)	0.5157
12. Switch	14 (93%)	26 (76%)	0.1835
13. Pressed bulb	15 (100%)	32 (94%)	0.3524
14. Started Phase 2 (> .5 min)	14 (93%)	33 (97%)	0.5157
15. Saw Results page	13 (87%)	34 (100%)	0.0251
Total (average)	**11.3**	**13.7**	**<0.001**
All 15 steps correct	**0 (0%)**	**12 (33%)**	**0.0105**
Time to complete test, average (range)	**27 (21 - 40)**	**20 (13 - 40)**	**0.0003**

3.7.3.c Venipuncture blood testing by study team

The study team performed parallel testing of venous blood collected from participants during their normal clinic visit. Neat (undiluted) whole blood as well as blood diluted in a 1:1 ratio with an FTA sorbent was tested on dual HIV-treponemal syphilis cassettes. In both cases, a 2 µl volume of sample (whether neat or diluted) was used. Results of this testing are shown in **Figures 41 & 42**. With undiluted venous blood, HIV antibody detection had a sensitivity of 81% (95% CI: 58–95%) and specificity of 50% (95% CI: 33–67%). Treponemal syphilis antibody detection had a sensitivity of 100% (95% CI: 16–100%) with specificity of 66% (95% CI: 52–78%). With venous whole blood mixed with sorbent, HIV antibody detection had a sensitivity of 90% (95% CI: 68–99%) and specificity of 71.4% (95% CI: 54–85%). Treponemal syphilis antibody detection had a sensitivity of 100% (95% CI: 16–100%) with specificity of 77.4% (95% CI: 64–88%).

While previous optimization (using n= 18 samples) of HIV antibody detection showed 100% sensitivity and specificity with neat whole blood (**Fig. 36**), we observed lower sensitivity and specificity performance with samples (n=55) tested in this pilot study (**Fig. 40**). We did show that HIV antibody detection using whole blood was improved with a dilution, in this case a 2X dilution compared to the 10X dilution used in the previous field trial.

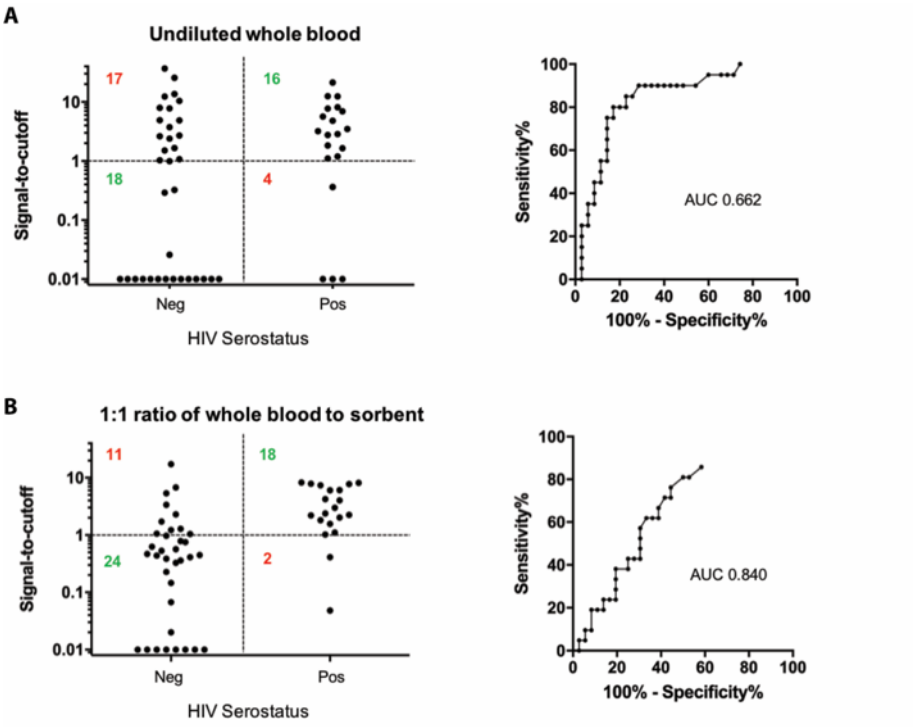

Figure 41. HIV diagnostic performance in pilot study. Venous whole blood was tested **(A)** neat, or undiluted, or **(B)** in a 1:1 ratio of blood mixed with an FTA-Abs sorbent (SciemedX). Vertical scatter plots (left**)** show signal-to-cutoff ratios of samples positive (Pos) or negative (Neg) for HIV as determined by gold standard tests of HIV ELISA (Vironstika). Receiver-operating characteristic (ROC) curves (right) illustrate Area-Under-Curve (AUC).

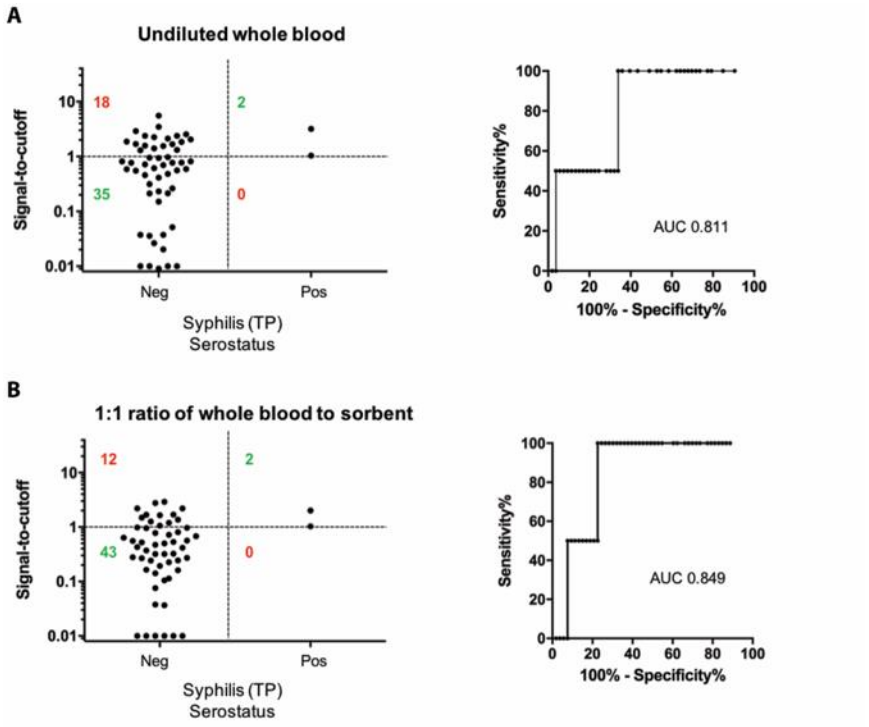

Figure 42. Treponemal syphilis diagnostic performance in pilot study. Venous whole blood was tested **(A)** neat, or undiluted, or **(B)** in a 1:1 ratio of blood mixed with an FTA-Abs sorbent (SciemedX). Vertical scatter plots (left**)** show signal-to-cutoff ratios of samples positive (Pos) or negative (Neg) for syphilis as determined by gold standard tests of TPHA (Spinreact). Receiver-operating characteristic (ROC) curves (right) illustrate Area-Under-Curve (AUC).

With treponemal-syphilis detection, we also saw an improvement in specificity with the addition of the FTA-Abs sorbent. The sorbent is a standard extraction from a Reiter treponeme culture. Sorbent group treponemal antigens are capable of absorbing major non-specific treponemal antibodies to "group treponemal antigens" found in healthy individuals, but not to antibodies against virulent treponema in diseased individuals[122]. These results indicate that further optimization and large scale validation of neat whole blood assays for both HIV and syphilis are needed on our platform before clinical or consumer deployment. The alternative method would be to include a dilution step, in order to maintain diagnostic performance levels previously reported (**3.3.7**).

3.8 Discussion

We demonstrated that with only a short 11 minute training, participants, majority of whom only had a primary school education and all of whom had no technical background in immunoassays or other laboratory techniques, were able to perform the steps required to use a smartphone based microfluidic diagnostic test. Between the two cohorts analyzed in this study, we saw significant improvement in 4 steps: (1) pulling the lancet tip off, (2) collecting blood into Part A (sample collector), (3) attaching Part A to Part B (test cassette), and (4) inserting the chip fully into the device. The addition of an instructional video and opportunity for a brief training period that caused these changes show that most steps for our device are easy to perform. While actual diagnostic results were not provided to participants, a dummy screen with an example of results was incorporated into the app to show that no interpretation of results was required.

Additional improvements in usability can be mitigated through engineered solutions, such as incorporation of more audio-visual cues to indicate if a step was performed correctly. For example, steps like pressing the switch (**Fig. 35**), gave an audible click signaling the step had been completed. One of the most challenging steps we observed was attachment of Part A to Part B, which required the tips of the capillary tubes in Part A to connect with inlet holes of Part B. As there was no cue to indicate when this had been completed, participants struggled to see if more force was required to make the proper connection between the two pieces, often resulting in bent capillary tips of Part A.

In Rwanda, the middle class is roughly classified as people earning 50.000-600,000 RWF per month[123], so participants in this pilot study represented a lower-income sample of the population. This was also reflected in the survey results pertaining to mobile phone usage (**Table 17**). While the majority of participants (89%) owned a mobile phone, most were basic phones. 23% owned internet-enabled phones and 8% owned smartphones. While the majority in this pilot study did not own their own smartphones, the scale of smartphone accessibility is still expanding, particularly in the developing world. Countrywide, mobile penetration rate in Rwanda as measured in 2014 was 68.1%, however internet penetration rate was only 25%[124]. Plans to implement 4G broadband service are underway through the support of the national government[124]. Another barrier to implementation of our device however, is the relatively high cost of the new device ("Dongle 2.0") at ~$58 USD. While this represents current manufacturing costs that we could decrease if produced at scale, in current format it may not be affordable for lower-income consumers in resource-constrained settings.

Additionally, the Rwandan Ministry of Health (MOH) collaborated on this study based on an interest in deploying self-testing services and products for HIV diagnosis. On December 1st 2017 (as part of World AIDS Day commemoration), the Rwandan MOH approved the launch of the OraQuick self-testing kit to provide self-testing opportunities as a supplement to existing testing services in health facilities or mobile VCT programs[125, 126]. Even in the developing world, there is a demand for consumer-facing products that expand access to diagnostics. Understanding the requirements and constraints for product design enables us to better drive innovation and engineering efforts to help achieve this.

Chapter 4

Assessing the potential of a smartphone accessory for HIV home-testing

4.1 Background

Increasingly, there will be interactions of microfluidic diagnostics and untrained users. The microfluidic field has been moving towards more field demonstrations of devices[27, 63, 127]. With more untrained and minimally trained users having access to such devices, it is important to understand how they use devices, what the device failure points are and what the most relevant design features are to spur user adoption and meaningful usage.

Human centered engineering encompasses tailoring technology to human needs and interests and is increasingly being recognized as part of the larger engineering process[128]. Human centered engineering design principles involve framing the user at the center of the process and designing

around them rather than asking human beings to adapt to technological, process, tradition or other constraints. This engineering process is based on an understanding of users, tasks and environments, refined by user-centered evaluation and addresses holistic user-experience[129]. The process puts the user in the center of the design process by integrating the user into each aspect of the process. The user-centered engineering design process is roughly divided into four steps: (1) understand and specify the user context, (2) specify the user requirements, (3) design and produce solutions to meet the user requirements, and (4) evaluate the solution against the requirements[130]. The process encourages iteration until a product or service meets the requirements of the user and does not necessarily require starting at Step 1(**Fig. 43**). For POC developers, the clinical need and potential POC approach have often already been examined and thus the application of this process can focus on engineering design iteration and evaluation phases.

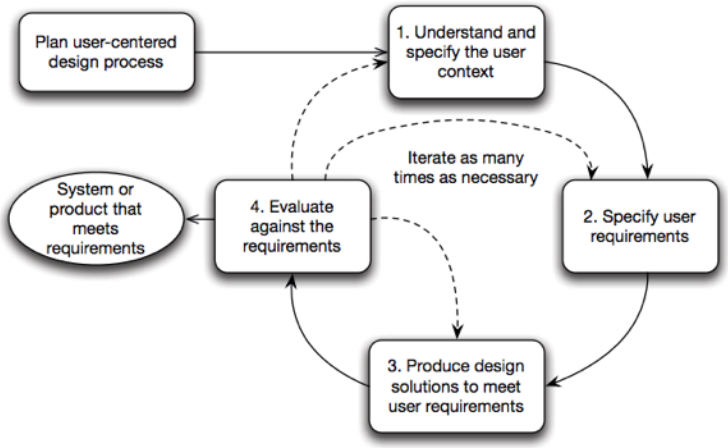

Figure 43. User centered engineering design process based on ISO 9241-210:2010. Taken from [[130]

By employing engineering approaches from interdisciplinary fields, we can better investigate the interaction of people with technology and technical development. We can use this towards biomedical applications in developing interventions and designing new tools to support or prompt positive changes in people's behavior, thinking or awareness. Biodesign and designing persuasive health technologies has been emphasized more in engineering for impact over the last decade as reflected in research and engineering centers across the country (e.g. Biodesign initiative and Persuasive Technologies Laboratory at Stanford University, Harvard Biodesign Laboratory at Harvard University, Department of Human Centered Design & Engineering at University of Washington etc). It emphasizes the iterative design and evaluation strategy, with additional exploration into strategic implementation.

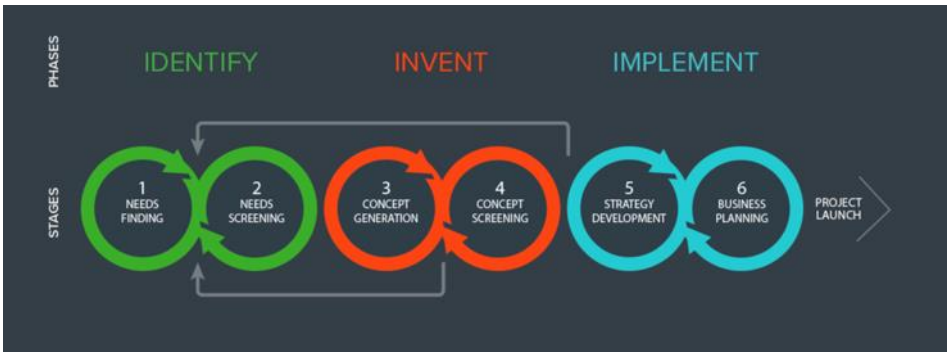

Figure 44. Biodesign innovation process. Taken from [128]

The FDA has also outlined the importance of usability engineering or human factors engineering in an updated guidance document (non binding recommendations) in 2016, stating that medical device developers "should conduct appropriate human factors study, analyses, and tests in the early stages of design process"[131]. Human factors engineering can be seen as a type of systems engineering discipline where analysis of a device entails examining the system boundaries at that device[131]. Users and interactions cause those boundaries to be moved out, so one can now

consider all the interactions within those boundaries. The key elements that affect device use are use-environment (use case setting), the user and characteristics of the users as well as the device interface (hardware/software). All of those elements can interact and research aims can focus on understanding the mechanisms behind that interactions- in sum a basic systems model[131]. Human factor studies and the application of human factors engineering principles to device development have been receiving increased attention from the scientific community, standard-setting bodies, and regulators both in the United States and European Union (EU)[132]. This focus is directed towards mitigating risk in misuse and enhancing effectiveness of use, particularly for consumer (non-health professional) applications.

Usability testing was first widely used in the field of web design to uncover errors and areas of improvement. This was done by observing users who were asked to solve given tasks on the site. To better understand why users' interactions with web sites might succeed or fail, people began to recognize limitations of only examining task related problems. Attention to the holistic user experience began to take shape in human-computer interaction field, which is where Peter Morville, an information architect, came up with the UX Honeycomb model [133] [134]. This was generated from his experience working at an internet training firm in the mid 1990s, teaching clients to use what was, at the time, state of the art internet tools such as Gopher, Archie, FTP and WAIS[135]. Information architecture became a tool for web design to try to anticipate users' major information needs, using both top-down and bottom architecture schemes (organization systems, navigation systems, search systems, labeling systems etc)[136]. Since then, human-computer interaction has grown in importance as a discipline, and encompasses web

and mobile applications[137]. User experience (UX) design, is now commonly used in modern digital technology and design industries to study interactions between users and products. The UX Honeycomb model outlines an expandable evaluation model where separate facets of UX (useful, usable, desirable, valuable, findable, accessible, and credible from the user's perspective) are considered in order to measure success[134]. Though it makes no claims on process, the combination of these elements in product design is thought to deliver maximum value to the user and can be explored in various permutations to iterate on product design. We can apply principles of UX and human centered design towards biomedical applications, particularly with POC microfluidic device engineering, to build products that are better positioned for successful adoption by end-users (whether they are clinicians or patients/consumers).

HIV self-testing is one of the first areas where we will see an increased interaction between microfluidic devices and untrained users. In the United States, while the overall rate of HIV incidence is declining, the population of gay, bisexual and other men who have sex with men (MSM) is disproportionately affected[138]. The Centers for Disease Control and Prevention (CDC) reported that in 2015, MSM accounted for 82% (26,376) of new HIV diagnoses among all males aged 13 or older and 67% of total new diagnoses in the U.S.[138]. The CDC has also reported a 65% increase in syphilis rates in the U.S. between 2011-2015. MSM in particular accounted for two-thirds of all reported syphilis cases[139]. Syphilis and other sexually transmitted infections (STIs) increase the risk of acquisition and transmission of HIV[140]. As a result, the CDC has recommended strengthening STI prevention measures, including yearly

testing for syphilis, gonorrhea and chlamydia, for sexually active MSM and more frequent testing (every 3-6 months) for MSM with multiple or anonymous sex partners. Self-testing products for STI testing could address unmet needs for these target populations, not just in increasing access and frequency of testing but using these products with sexual partners for early diagnosis and potentially even prevention of infection. Currently, the only FDA approved diagnostic product for over-the-counter use by consumers in the U.S., is the OraQuick In-home HIV Test (Orasure Technologies). The OraQuick test is an oral-fluid, lateral flow test for detecting presence of HIV antibodies (HIV-1 and HIV-2), that allows users to self-administer the test at home, obtain results in 20 minutes and interpret results following written instructions[141].

Intellectual framework for structured assessments can be useful for understanding interactions between new microfluidic devices and users. Other fields, such as economics, psychology and behavioral science have rigorous frameworks to study user motivations and behavior. The Behavioral Economics model studies how people make decisions about their behaviors, and aims to identify conditions under which individuals are likely to make systematic decision-making errors or 'biases'[142]. The Behavioral Economics model can be used to explain why motivation does not always translate into healthy behaviors and explore if additional incentives may be necessary to achieve healthy outcomes. The Fogg Behavioral Model (FBM) is another framework used to study behavior change[143]. It asserts that three factors of (1) necessary motivation, (2) sufficient ability, and (3) effective triggers influence whether a user performs a target behavior[143]. In the context of HIV prevention behaviors, this could be applied to analyze and design "persuasive technologies" such as self-testing diagnostic platforms as an

intervention tool. The Fisher and Fisher Information-Motivation-Behavioral Skills (IMB) model was first proposed to understand, predict and promote adherence to highly active antiretroviral therapy among HIV positive individuals[144]. HIV prevention information, motivation and behavioral skills are seen as the fundamental determinants of HIV preventative behavior[145]. Specifically, in cases where "individuals are well informed, motivated to act, possess the behavioral skills required to act effectively, [and thus] likely to initiate and maintain patterns of HIV-prevention behavior", the IMB framework is useful for studying or developing a particular intervention tool[144].

Traditional translational research for therapeutics involves taking knowledge from basic science research and applying it to human clinical trials and eventually patient care. It typically involves a course of multiple observational studies, phases of clinical trials and implementation of evidence based practice. Patient behavior plays a large role in health outcomes and thus an integration of behavioral science into biomedical research has potential to advance and accelerate translational research.

Basic behavioral science	T1: Translation to applied science	Clinical efficacy knowledge	T2: Translation to patients	Clinical effectiveness knowledge	T3: Translation to practice
Examination of patterns of medication nonadherence and resulting drug efficacy and tolerance in animal models.	Observational study of medication adherence in clinical population. Phase 1 feasibility trial of behavioral intervention targeting nonadherence.	Phase 2 clinical trial examining dosing and preliminary efficacy of behavioral intervention for nonadherence in clinical population.	Phase 3 comparative effectiveness trial examining effect of behavioral intervention vs. chronic condition support group intervention on health outcomes of interest.	Dismantling study to examine most effective components of behavioral intervention. Phase 4 clinical trial examining long-term outcomes and cost-effectiveness of behavioral intervention.	Implementation of behavioral intervention for nonadherence across a large number of sites/practices. Use of quality improvement methods to determine optimal approach to implementation and adherence to treatment guidelines across health care system.

Figure 45. Example of research progression through translational stages. Taken from [146]

Using Hommel et al's proposed translational research model[146], we undertook what can be called a "Phase T1" trial where the primary objective was to establish feasibility and acceptability of treatment protocol, which in this case was a potential prevention intervention protocol (smartphone integrated microfluidic self-testing device) using a small sample size of patients/users. Incorporating user testing allows us to identify user requirements and evaluate our design solutions in an iterative development process to enhance user-device interaction. Future studies can then undertake controlled and blinded RCTs involved larger patient samples to evaluate effectiveness of the testing device in engagement towards increased testing as well as towards long term health outcomes.

In this study, we build on our previous work in creating a smartphone-interfaced multiplexed, microfluidic STI test[63] and integrate the IMB theoretical model and elements of UX design to propose a new framework for microfluidic device evaluation that moves beyond traditional characterization of diagnostic performance. We worked with a sample of MSM population in New York City who qualified as a "high risk" cohort for HIV/STI infection based on self-reported sexual behaviors to perform for the first time, a structured assessment of completely naïve users interacting with a smartphone interfaced microfluidic diagnostic device ("SMARTtest").

4.2 Methods

4.2.1 Self-testing study in New York City ("SMARTtest" study)

We conducted the trial in New York City, USA with approval from the New York State Psychiatric Institute and Columbia University's Institutional Review Board. 40 participants who met eligibility criteria were recruited for the study. All study procedures were performed in compliance with the relevant laws and guidelines of the Institutional Review Board and statements of informed consent were obtained for all participants involved in the study.

4.2.1.a Participant recruitment and enrollment

Eligibility criteria included: man or transgender woman who engages in unprotected receptive anal intercourse (URAI); 18 years of age or older; fluent in English or Spanish; HIV-negative by self-report prior to enrollment; not in a monogamous relationship at the time of enrollment; reports having intercourse at least three times per month, on average; reports never or seldom using condoms (no condom use in last 10 occasions for those with 4 or less partners or in less than 80 % of occasions for those with more than 4 partners in the past year); reports at least three episodes of URAI with serodiscordant or unknown status partner in prior 3 months, aware that URAI may lead to HIV transmission and owns a smartphone.

Recruitment and screening took place in person and online using bilingual (English/Spanish) announcements about the study that were distributed at health clinics and gay community events (gay parades, health fairs), posted in authorized areas of gay establishments, reproduced in print media and the Internet, and mailed to participants in prior studies who gave consent to be re-contacted. Study candidates called the research office and responded to a brief eligibility screening questionnaire. Those who qualified were invited to the in-person study visit (Visit 1).

4.2.1.b Study procedure and assessments

Study procedure and assessments consisted of participants completing a consent process, A) Part 1 of a quantitative computer-assisted self interview (CASI), B) skill demonstration using the SMARTtest (mock self test in front of the interviewer, no results shown to participant), C) Part 2 of the CASI and D) a qualitative in-depth interview. Optional FDA approved rapid tests for HIV (OraQuick or INSTI HIV tests) and syphilis (Syphilis HealthCheck), were offered to participants interested in receiving rapid test results and performed by trained research staff. Post-test counseling procedures included information about test limitations concerning window periods of infection, the need for confirmatory testing as well as referrals to facilities for further evaluation and treatment. Participants received $50 in compensation after the study visit.

A. CASI, Part 1: Participants were asked to fill in Part 1 of the Web-based questionnaire using a computer located at the research offices at the HIV Center. The structured questionnaire was computerized and self-administered. Participants were identified in the questionnaire only by an assigned numeric study ID and no identifiers (e.g., name, address, date of birth, social security number) were collected using the questionnaire itself. CiW-500 software program (Sawtooth Software, Inc.) was used to program the questionnaire. Participants received a brief training on how to use the system and responded to questions and response scales shown on the computer screen. Participants were informed that they could refuse to answer any question and could ask for assistance at any point in the interview. The programmed questionnaire and resulting data resided on the study center's internal servers with firewall protection. In Part 1, participants

answered questions related to demographics, smartphone usage, sexual behavior in relation to their HIV and syphilis knowledge, motivation to remain uninfected, substance use and concerns that may affect motivation.

B. Skill demonstration: Participants were asked to use the SMARTtest in a mock testing in which they would not receive the test results. The SMARTtest kit consisted of a disposable blood collector ("Part A"), disposable microfluidic assay cassette ("Part B"), Dongle 2.0 hardware, and fingerprick materials (alcohol swab, lancet, gauze and bandage). An iPhone 5 device (Apple Inc.) with the accompanying SMARTtest app was also provided for the study visit. Participants were video-recorded, with the camera focused only on their hands, in order to assess how well they were able to use the device. If they wished, participants were provided with a mask to obscure their face. Participants sat a table with test kit materials placed in front of them and were asked to use the smartphone app to follow instructions provided therein. Participants were asked to abstain from asking questions to the interviewer until testing was completed. This was done to make sure that participants could follow instructions and could read the results accurately. Once participants indicated they had completed the procedures and the blank test result screen was shown, the camera was turned off. The participant was reminded that if he or she wished to have an HIV or syphilis test, rapid tests could be conducted at the end of the study visit or that study staff could provide a list of places where testing may be obtained. A structured rubric containing a checklist of required steps and open

fields for comments on errors made was used to evaluate the skill demonstration based on in-person observations and video recordings.

C. CASI Part 2: Participants were asked to complete Part 2 of the Web based questionnaire, which consisted of questions about the SMARTtest device, intention to use it and perceived effectiveness and/or difficulty of discussing usage of the device with partners.

D. In-depth interview: Participants underwent an audio-recorded, qualitative interview conducted by a trained study member either in English or Spanish. An in-depth interview guide was developed to structure interviews. The in-depth interview guide focused on a) SMARTtest use with sexual partners; b) sexual decision making based on test results; c) potential content for accompanying smartphone app; d) privacy concerns; and e) conclusions and recommendations.

4.2.1.c Microfluidic test cassettes

Disposable, plastic microfluidic cassettes were functionalized at Columbia University using disease-specific proteins, as described previously[63]. Lyophilization of gold-nanoparticle conjugated secondary antibodies (anti-human IgG and IgM antibodies) was performed by OPKO Diagnostics (Woburn, MA). Lyophilized antibodies were held in capillary tubing within the sample collector. The protruding capillary tip of the sample collector was marked for collecting fingerprick whole blood collection using an orange sticker to indicate directionality and a black fill line for metering 2uL of blood. An additional sticker labeling the sample collector as “Part

A" was attached. Wash buffers (three 2-µl PBS–0.05% Tween 20 and four 2-µl water washes) as well as 60 µl each of silver nitrate (silver A) and silver reducing agent (silver B) were pre-loaded to the reagent cassette by manual pipetting and sealed using an adhesive tape (OPKO Diagnostics). Plastic guidance pieces (OPKO Diagnostics) were soldered onto functionalized, reagent-filled microfluidic cassettes and labeled with "Part B" stickers as well as matching orange stickers to Part A to indicate directionality of attachment. Microfluidic cassettes and sample collectors (also containing lyophilized antibodies) were stored at 4ºC until an hour before use.

4.2.1.d SMARTtest application

The SMARTtest app was written in iOS Swift using the XCode IDE for macOS. The app was manually downloaded by research staff to iPhone 5 device used by participants. Captured results were stored locally on the phone during the study. The app framework consisted of an instructional video followed by step-by-step instructions with pictures and text instructions.

4.2.1.e Data analysis

For qualitative data management and analysis, audio recordings of in-depth interviews were transcribed, reviewed for accuracy and imported into NVivo software for data management and analysis. Coding categories were generated from interview questions and themes identified in participant narratives. Initial set of codes was generated by two research staff, compared, then synthesized to compiled shared coding categories and sub-categories with definitions, inclusion and exclusion criteria and examples. Coders discussed discrepancies until they achieved 80% intercoder convergence. Analysis of coded material will be aimed at progressive identification of

regularities in the data under study and to its organization under a conceptual label. Analysis will include categorization, abstraction, comparison, integration, iteration and refutation.

For quantitative data management and analysis, CASI (Part 1 and 2) and rubric data (skill demonstration) were compiled for analysis and generation of descriptive statistics using Excel or SPSS software.

4.3 Results

4.3.1 UX-IMB framework for evaluation

Using the qualitative and quantitative data analysis we undertook in this study, we propose a framework for evaluation of microfluidic tools towards HIV/STI home testing that integrates behavioral science and UX design (**Fig. 46).** We used the IMB model as the core theoretical frame[141, 147, 148], which asserts that specific informational, motivational, and skill factors are the fundamental determinants of behavior, in this case HIV preventative behavior, and will vary as a function of culture, class, economics, environment, and life circumstances. This model has been extensively validated with diverse populations in over 15 years of research and thus useful for design, implementation and evaluation of preventative interventions[145]. Ultimately, we seek to study the value of a microfluidic POC device for a self-testing use-case setting. To do this, we integrated UX concepts from the Honeycomb Model such as usability, credibility and acceptability of new POC devices for HIV/STI home-testing and consumer use, which influence user knowledge, motivation and behavioral skills towards engaging with a prevention method (i.e. microfluidic POC device). For microfluidic developers, this can

provide a useful framework to assess and analyze mechanisms of user-device interaction, even in prototyping stages.

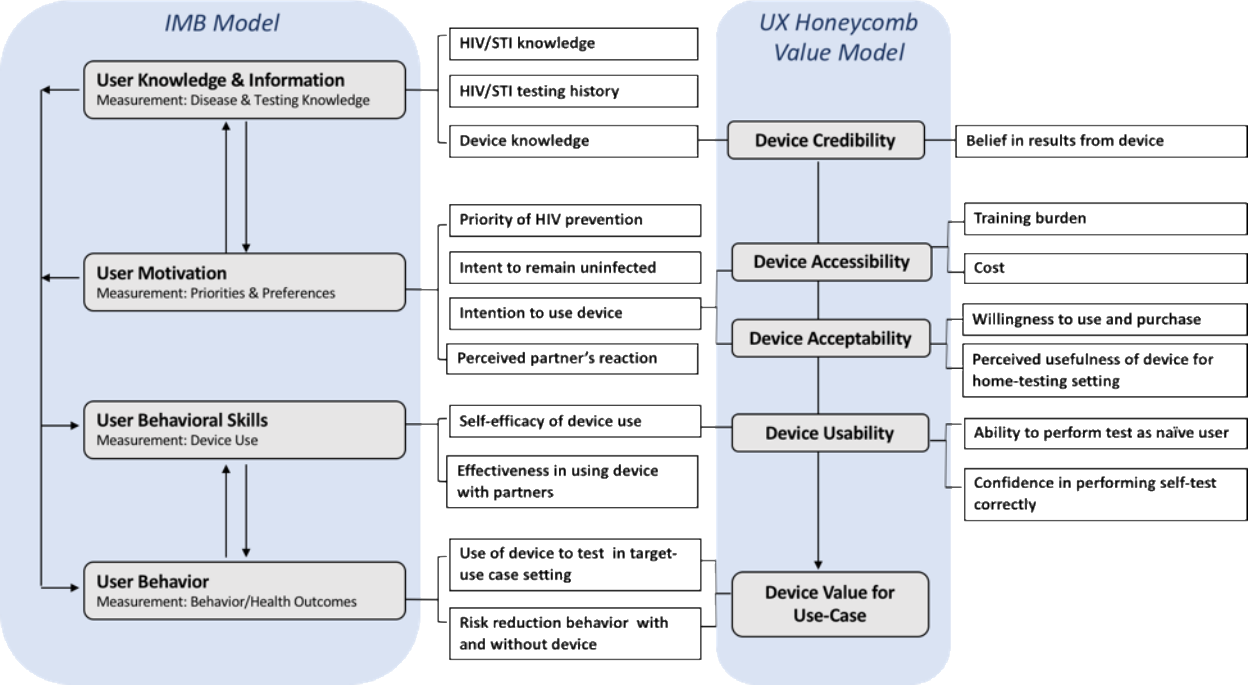

Figure 46. Proposed UX-IMB framework for evaluation of microfluidic devices for HIV home-testing

4.3.2 Description of participant sample

40 participants were enrolled in this study (**Fig. 47**). Recruitment focused on gay and bisexual men, as well as transgender women, though no transgender women ended up enrolling in the study. Eligibility criteria primarily focused on those who qualified as high risk for HIV/STI transmission (including those on Pre-exposure prophylaxis, or PrEP). Full qualitative data was recorded for 39 participants. Participant demographics are listed in **Table 19**, showing diverse backgrounds and range of socio-economic status and education levels in our study cohort. Approximately three quarters (74.4%) of participants identified as gay with the remaining quarter (25.6%) identifying as bisexual or other (no participants identified as heterosexual). The

median income was $30,000, which is somewhat lower than the median income of all New York City households ($93,162) as reported by the U.S. Census Bureau for 2016[149]. It should be noted that income varies significantly across the city boroughs ranging from median income of $37,525 for the Bronx to $77,559 for Manhattan. The median age of participants in this study was 33.5, which is close to the median age of 36.2 for all New York City residents[149]. About a quarter of participants (25.6%) identified as Hispanic/Latino, which roughly mirrors that of New York City (29.2%). 87% of participants reported owning a smartphone and using apps for a variety of purposes (**Table 20**). Primary phone usage activities besides phone calls were texting, email, photography, followed by streaming music and videos and surfing the internet or social media. There were also approximately equal number of iPhone and Android users (**Table 20**).

Though at high risk for HIV/STI transmission based on self-reported sexual behavior (i.e. mainly from limited or no condom use), most participants expressed high interest in avoiding HIV infection. 80% of participants reported high motivation (ratings of 8-10 on a 1-10 scale of motivation) to remain HIV-uninfected. About two-thirds of participants (67.5%) also stated high willingness (ratings of 8-10 on a 1-10 scale of intended action) to enact actions to avoid HIV. This correlates with findings from previous studies that have shown that gay and bisexual men at high risk of HIV transmission are still motivated to remain HIV-uninfected[141]. Our study enrollment criteria included those on PrEP (pre-exposure prophylaxis[150]) if they met remaining criteria to be high risk for syphilis or other STI transmission. We found there was no statistically significant difference in expressed motivations to remain HIV-uninfected between PrEP users and non PrEP users in our study cohort.

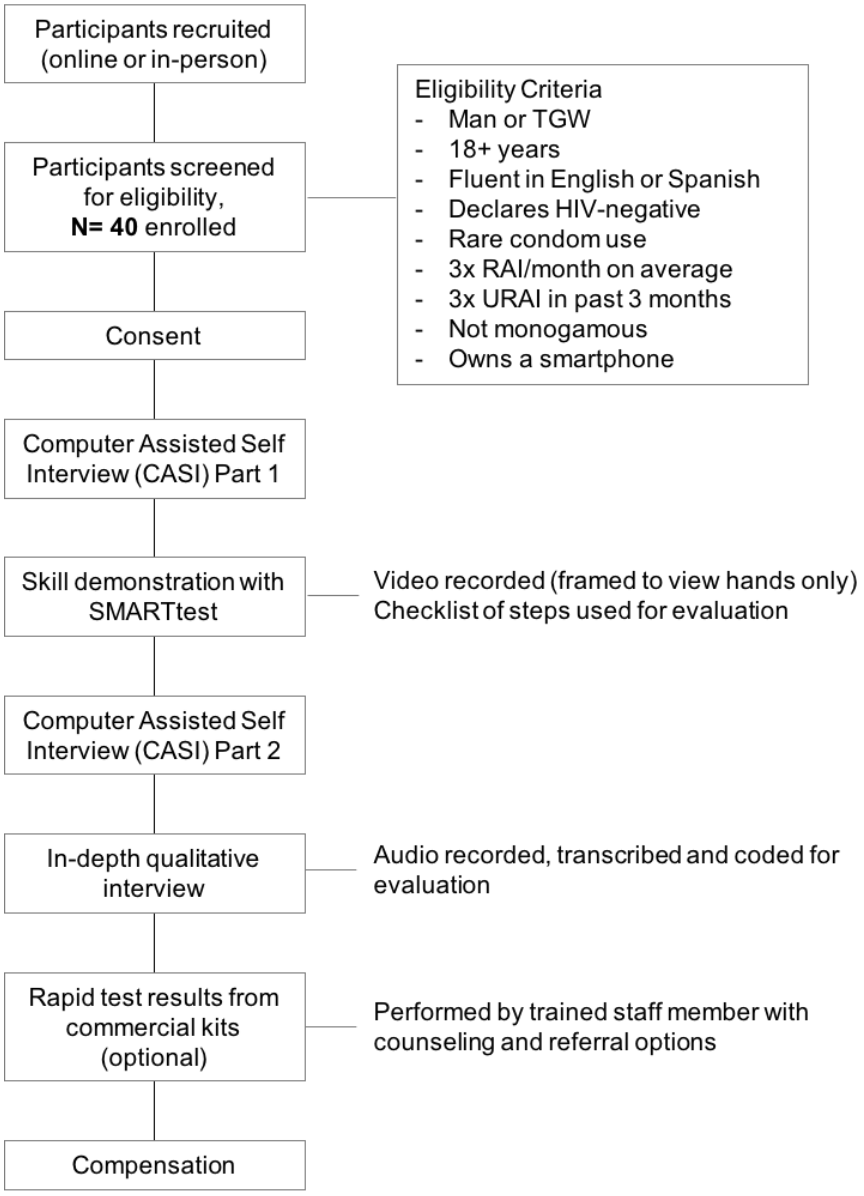

Figure 47. Process flow diagram of study procedures for naïve-user testing (New York City, U.S.)

Table 19. Participant demographics in self-testing study (New York City, U.S.)

Age, mean (range)	38.7 (20- 73)
Income, in thousands (USD)	
Mean	37,501
SD	41,405
Range	0 to 220,000
Race / Ethnicity, n (%)	
African-American	19 (48.7)
White	14 (35.9)
More than one / Other	6 (15.4)
Hispanic/Latino, n (%)	10 (25.6)
Education, n (%)	
Partial/Completed High School	9 (22.5)
Partial/Completed College	21 (55)
Graduate School	9 (22.5)
Sexual Identity, n (%)	
Gay	29 (74.4)
Bisexual / Other	10 (25.6)

Table 20. Phone usage by participants (New York City)

Do you own a smartphone?		
	Yes, n (%)	38 (87%)
	No/Not sure, n (%)	1 (2.5%)
	Prefer not to say, n (%)	1 (2.5%)
What type of phone do you have?		
	iPhone, n (%)	21 (52.5%)
	Android, n (%)	19 (47.5%)
Do you use smartphone applications (apps)?		
	Yes, n (%)	38 (87%)
	No/Not sure, n (%)	2 (5%)
How do you use your mobile phone?		
	Texting, n (%)	40 (100%)
	Phone calls, n (%)	40 (100%)
	Taking photos, n (%)	38 (95%)
	Listening to music, n (%)	37 (92.5%)
	Watching videos, n (%)	36 (90%)
	Games, n (%)	23 (57.5%)
	Surfing the internet (including social media), n (%)	36 (90%)
	Email, n (%)	38 (95%)
	Banking, n (%)	25 (62.5%)
	Other, n (%)	4 (10%)

Figure 48. Image of the SMARTtest kit. An image of the SMARTtest kit containing the device ("Dongle 2.0") with a microfluidic cassette, fingerprick materials of an alcohol swab, lancet, gauze and a bandage, and an iPhone with the SMARTtest app.

4.3.3 Value of device for self-testing

Assessment and demonstration of a microfluidic diagnostic device's value for a particular use-case enables development of new tools that are poised for meaningful adoption by target end users. In order to assess potential value of the new device, in this case for a self-testing use-case, we undertook quantitative and qualitative questionnaires and interviews to expand on we sought to understand how users, specifically gay and bisexual men at high risk of HIV/STI transmission, felt about the microfluidic SMARTtest for a self- or home-testing application. We explored device usability, credibility, and acceptability in our assessment using the UX-IMB framework (**Fig. 46**). We note that these are expandable themes for inquiry that can be customized for a target use-case or trial phase.

4.3.4 Ability of untrained users to perform a diagnostic test with the device

During the skill demonstration, participants were given the "SMARTtest" kit consisting of fingerprick materials, disposable microfluidic assay components (sample collector and assay

cassette), the SMARTtest device ("Dongle 2.0") and a smartphone (iPhone) pre-loaded with the app (**Fig. 48**). We designed the app for a consumer-facing use case by first guiding users to watch an instructional video and then follow step-by-step directions to run the microfluidic test on their own (**Fig. 49**). Users could navigate back and forth between pages describing each step, observe a progress bar as they went through the testing process as well as watch (or skip) the instructional video as many times as they wished. To mimic the home or self-testing experience with an over-the counter (OTC) test, no training or explanations were provided by the study team.

The sequence of steps to run the test consisted of preparing materials for the test (unwrapping gauze, removing the lancet cap etc), obtaining a fingerprick blood sample, collecting a 2-µL metered amount of blood in the sample collector ("Part A"), attaching the sample collector to the microfluidic cassette ("Part B"), inserting the cassette into the SMARTtest device, pushing down on the rubber bulb (negative pressure chamber) to initiate fluid flow, waiting 5 minutes for Phase 1 of the test to run (duration of time for sample and initial set of reagents to flow through), pressing the manual switch and pushing down on the rubber bulb again for silver reagent actuation, and waiting another 5 minutes for Phase 2 of the test to run (flow time for silver amplification reagents and signal reading). Full details on assay sequence and biochemical reactions on the microfluidic chip surface are outlined in our prior work[63]. A final screen showing HIV and syphilis results concluded the test. For purposes of the study visit, only blank results were shown to participants during the skill demonstration. The rubric used for usability

evaluation broke down this process as 16 steps; it should be noted that about half of these are related to fingerprick blood sampling and collection.

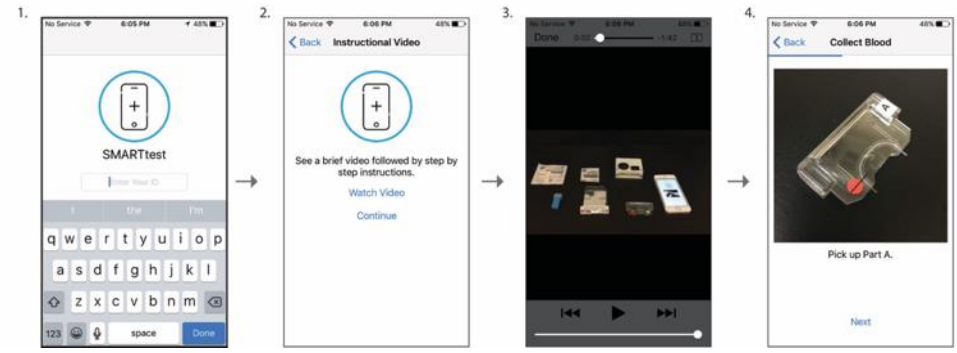

Figure 49. SMARTtest iOS app user interface. General steps of operation are shown: (1) entering log-in information (ID number); (2) a landing screen giving users the option to watch an instructional video and then follow step by step directions; (3) instructional video providing an overview of the test procedures; and (4) step-by-step directions with images.

The majority of steps were easy for participants to perform, with an average 86% of steps performed correctly and average total time of 15.7 minutes to complete the test (**Table 21**). This included a built in 10 minutes of timers and the instructional video, therefore the average total time to complete all manual steps of the test was around 4.6 minutes (**Table 21**). We made some slight modifications to the instructional video between the first and second set of participants (n=20 in each), however observed no statistically significant differences between the two sets of participants in performing each step of the test correctly or overall number of steps performed correctly (88% of steps performed correctly in first set and 90% in the second set) (**Table 21**). 90% of participants felt "pretty confident" or "very confident" in their ability to perform the test correctly when asked for self-evaluation of performance (using a Likert-type scale of 1-5, with 1 being not confident and 5 being very confident). While there was some variability in collecting blood, all participants were able to successfully perform a fingerprick on themselves using the recommended directions in the app. The vast majority of participants (75%) chose to perform the

fingerprick on their left forefinger, matching the finger shown in the instructional video, though no explicit directions were given on choice of hand or finger to sample from.

Table 21. Number of steps performed correctly by participants in self-testing study

	First set	Second set	p-value	All Participants
1. Opened gauze packet	*n/a*	20 (100%)	-	-
2. Cleaned finger with alcohol wipe	20 (100%)	20 (100%)	-	**100%**
3. Pulled lancet tip off	20 (100%)	20 (100%)	-	**100%**
4. Pricked cleaned finger with lancet	20 (100%)	20 (100%)	-	**100%**
5. Squeezed finger	20 (100%)	20 (100%)	-	**100%**
6. Collected blood into Part A	15 (75%)	19 (95%)	0.077	**85%**
7. Only filled to the line	15 (75%)	15 (75%)	-	**75%**
8. Wrapped finger in gauze	*n/a*	20 (100%)	-	-
9. Connected Part A to Part B	20 (100%)	20 (100%)	-	**100%**
10. Inserted chip into device until black lines matched	13 (65%)	11 (55%)	0.516	**60%**
11. Pressed bulb	17 (85%)	15 (75%)	0.429	**80%**
12. Pressed continue to start Phase 1 timer (< 1 min)	18 (90%) *	19 (95%) *	0.549	**93%**
13. Pressed switch	14 (70%)	17 (85%)	0.254	**79%**
14. Pressed bulb	15 (75%)	18 (90%)	0.211	**85%**
15. Pressed continue to start Phase 2 timer	15 (75%)	14 (70%)	0.726	**73%**
16. Saw Results page	11 (55%)	19 (95%)	-	**100%**
Total steps performed correctly (average)	13.2 (88%)	14.4 (90%)	0.513	**13.8**
Number of participants who got all steps correct	6 (30%)	4 (20%)	0.465	**10 (25%)**
Total time of test in minutes, average (range)	16 (11.8-34)	15.3 (13.4-17.4)	0.535	**15.7 (11.8-34)**
Instructional Video (min)	1.05	1.73	-	1.39
Built in timers (min)	10	10	-	10
Time to complete manual steps, average (range)	5.5 (2.0 - 23)	3.6 (1.7 - 5.6)	0.086	**4.6 (1.7 - 23)**

Microfluidic cassette components were modified to enhance visual recognition and handling through stickers and labeling ("Part A" and "Part B"). We also observed that in the previous pilot study, users had to insert the tips of the capillary tube component of the sample collector into matching inlet holes of the microfluidic cassette by eye, causing some difficulty in knowing how much force to apply in pushing the pieces together and in some cases, bending the capillary tube tips out of shape (**Fig. 50**). An additional plastic guidance piece was added to functionalized microfluidic cassettes to guide attachment of the sample collector (Part A) to the microfluidic cassette (Part B) through a simple sliding and locking motion (**Fig. 50**). Once attached, Part A and B are locked irreversibly, containing blood within the closed microfluidic circuit and embedded waste pad. All participants in this study were thus able to attach the two components together.

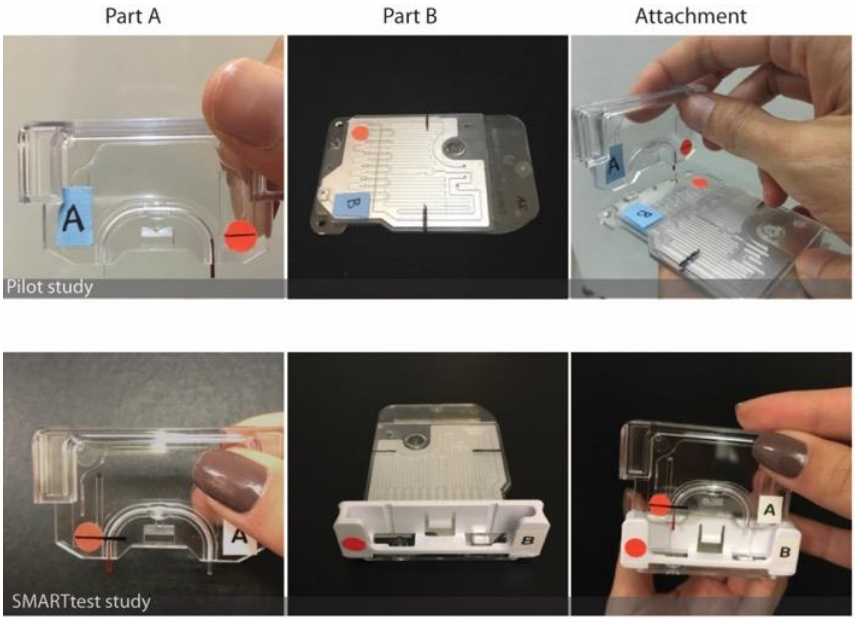

Figure 50. Microfluidic components of self-test kit. Images showing modifications to Part A, the sample collector, Part B, the combined assay and reagent test cassette as well as attachment for pilot study (top row) and naïve-user testing (bottom row).

From the usability evaluation rubric, the most challenging step to perform was inserting the microfluidic cassette into the SMARTtest device, with only 60% of participants able to do this correctly (**Table 21**). The main source of error was not inserting the cassette fully into the device, which affected alignment of the vacuum to the cassette outlet. The next most challenging step was collecting the specified 2-uL volume of fingerprick blood in the sample collector ("Part A"), with participants mostly overfilling past the indicated line on the sample collector or in a few instances, not collecting sufficient blood. The other main source of error was participants who did not use the step-by-step instructions and attempted to perform the test while the instructional video was playing. This resulted in missing steps such as pressing the switch or app buttons that started the built-in timers, or performing them in an incorrect order, particularly for the second phase of the assay. In self-evaluation interviews, most participants indicated that the hardest steps to perform were inserting the microfluidic cassette into the SMARTtest device and collecting the fingerprick blood in "Part A", correlating to the usability analysis by the study team. Interestingly, the next most common step flagged as challenging by participants, was pushing the vacuum bulb, either due to the physical resistance of the bulb itself or feeling unsure about whether this was performed correctly or not.

4.3.4.a Usability analysis

10 participants performed all the steps correctly, however none were able to complete the assay with a valid positive control. When observing the full set of participants, we noted that 70% of the microfluidic tests used showed no blood sample reaching the waste pad of the microfluidic test cassette, indicating the sample had stalled in a prior portion of the channel. We also noted

that 90% of participants took more than one minute between fingerprick blood collection and starting the assay (i.e. engaging the vacuum for fluid flow). While the one minute time period between sample loading and starting the assay was used a rough operational step in prior studies, this was not included in the step-by-step directions in the app.

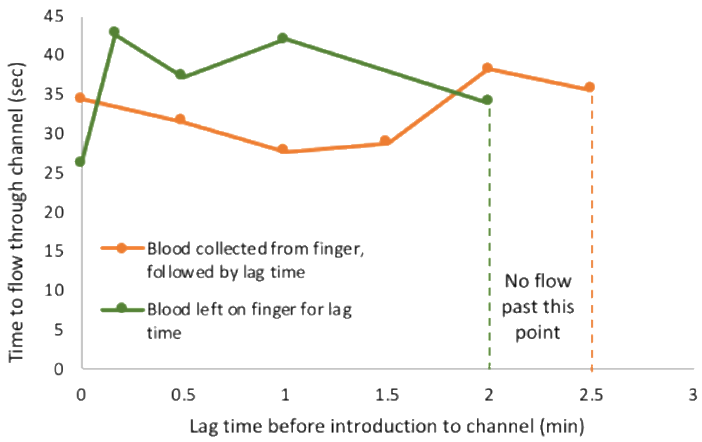

Figure 51. UX-IMB Usability: effect of lag time on fingerprick whole blood flow time through microfluidic channel. Condition 1 consisted of lag time measured in minutes between pricking the finger and subsequent collection and immediate introduction to the channel. Condition 2 consisted of collected the fingerpricked blood sample into the capillary tube of the sample collector, followed by lag time at indicated time points before introduction into microfluidic channel. Time for blood to flow through channel was measured in seconds from inlet to waste pad (end of channel).

We performed a preliminary exploration of lag time between fingerprick blood collection and flow through the microfluidic channel **(Fig. 51**). We examined two conditions that represent user-scenarios with the fingerpricking process: a lag time between pricking of the finger and collection into the capillary tube of the sample collector (Part A) and lag time between sample collection in the capillary tube and introduction to the microfluidic channel (connection with Part B and engaging the vacuum bulb). For fingerprick blood left on the finger, we found we could wait up to 2 minutes to collect and introduce into the channel and still have flow through with no

sample stalling. In the condition of lag time after sample collection, we found we could wait up to 2.5 minutes before the blood would stall in the microfluidic channel. In both cases, we engaged the vacuum prior to sample addition.

We also wanted to assess if there was any difference in occurrence of sample stalling or flow times through the cassette based on method of sample introduction via a standard manual pipette or capillary tube attachment to the microfluidic cassette inlet (**Fig. 52**). In most of our optimization work, we had used a manual pipette for whole blood handling and dispensing into the cassette. With EDTA or heparinized venipuncture blood, we saw little difference between using a pipette or the capillary tube of the sample collector but had not assessed potential difference with fingerprick whole blood with no anti-coagulants. We saw no statistically difference in time for a 2µL fingerprick blood sample to flow through using either method (**Fig. 52)**

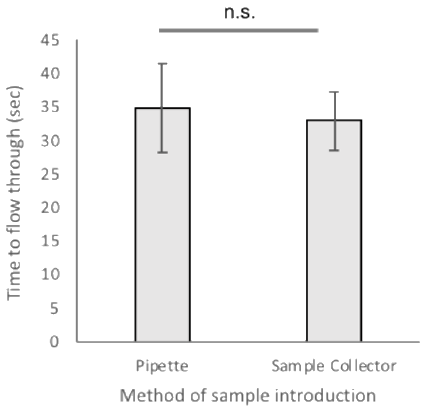

Figure 52. UX-IMB Usability: whole blood sample flow times with different methods of sample introduction to microfluidic chip. A 2µL sample of disease negative whole blood was introduced using two methods (1) collecting in a standard pipette and dispensing into the inlet of the microfluidic chip, and (2) collecting in the capillary tube of the sample collector (Part A) and attaching to the microfluidic cassette inlet. Vacuum was engaged prior to sample introduction into the cassette in both methods. A pre-wash of 2µL plug of 0.05% Tween-PBS was flowed through the channel immediately before sample introduction. Data are averages ±1 SD (*n*=4). Asterisk (*) indicates statistical significance ($p<0.05$) using Student's t-test.

Another assay protocol step that differentiated this usability study setup from prior work (**Chapter 3**) was that channel priming with a 0.05%Tween-PBS wash did not occur just prior to whole blood sample introduction (i.e. a few seconds before blood was introduced to the channel). Microfluidic cassettes were functionalized and prepared for participant use the morning of a study visit session. To more closely mimic user-experience in the intended setting, no additional channel priming was performed after participants received the test kits, which could be up to 6-8 hours after our initial priming step. In Figure 52, the channel priming was performed just prior to sample introduction. We performed another ancillary experiment examining positive control zone signals using microfluidic cassettes that had been primed at various time points prior to sample introduction of disease negative whole blood (**Fig. 53**). We saw a statistically significant decrease in positive control signals after 2.5 hours.

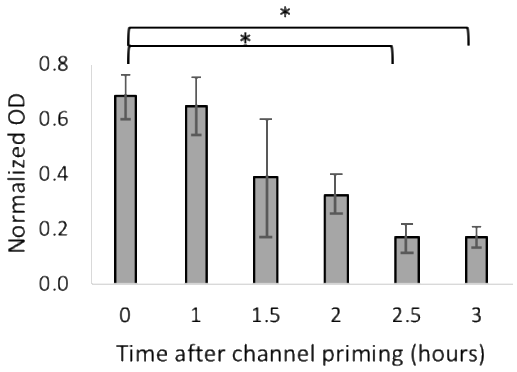

Figure 53. UX-IMB Usability: comparison of positive control signals after channel priming. A 2µL plug of 0.05% Tween-PBS was flowed through the channel. At time points shown after channel priming, a 2µL sample of disease negative whole blood was introduced, followed by the usual reagent sequence for reconstitution of lyophilized gold-labeled anti-hIgG/anti-hIgM antibodies antibodies (three washes of 0.05% Tween-PBS, and four washes of water) and silver amplification. Data are averages ±1 SD (n=3). Asterisk (*) indicates statistical significance ($p<0.05$) using Student's t-test. A comparison of signals at time point 0 and 2 hours after channel priming yielded minimal significance ($p = 0.058$).

What is not reflected in this data is the number of times the vacuum was re-engaged to clear stalled sample or washes (which occurred more frequently in later time points). This suggests that sample stalling and channel priming are somewhat correlated.

Finally, we wanted to assess how changes in the casing and negative pressure chamber in the current Dongle 2.0 design had an impact on user-activated flow. The main changes in the vacuum bulb from previous trials (Chapter 3) were: (1) rubber bulb from a 60mL syringe compared to the 140mL size in Dongle 1.0, and (2) minor adjustments in the height of the 3D printed vacuum chamber casing (**Fig. 54**) surrounding the bulb compared to Dongle 2.0 used in the pilot Rwanda study (which had to be made due to supply chain adjustments to obtain the source 60mL syringe needles). Previously the height of the vacuum bulb chamber was 1cm (total bulb height 2.5cm) and in the current design, the height of the chamber was 0.38cm (with total bulb height 1.5cm).

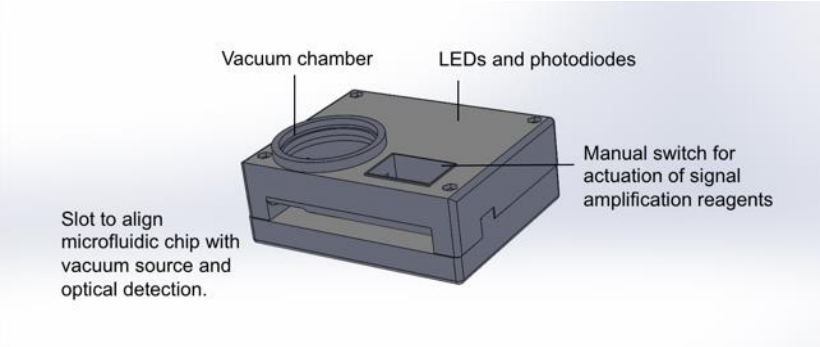

Figure 54. UX-IMB Usability: integrated dongle casing for Dongle 2.0 used in SMARTtest study. 3D printed casing was produced in house, with minor adjustments in vacuum chamber height compared to previous designs.

We performed a follow-up experiment to assess variation in user-activated flow using Dongle 2.0 in this study (**Fig. 55).** Thus, we recruited three additional naïve-users (not included in

SMARTtest Phase 1 cohort), to assess flow times using a standard reagent sequence of washes. We saw a statistically significant difference between flow times for two out of the three users, suggesting variable amounts of pressure applied to the bulb. The 60mL bulb provided higher resistance than the bulb in Dongle 1.0, as was reflected in user-feedback from SMARTtest participants in steps they found challenging. This suggests the slightly smaller footprint enabled by the smaller bulb design may not offset the greater effect of less consistent flow times across users.

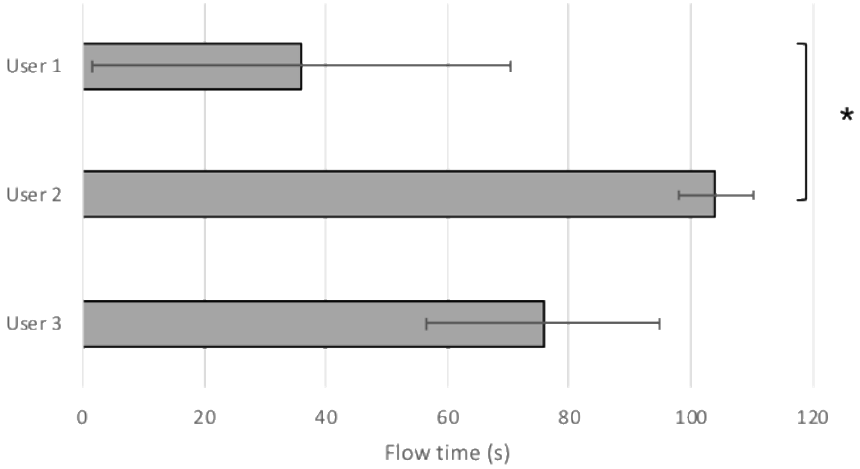

Figure 55. UX-IMB Usability: user activated negative pressure-driven flow. Average time to flow a total sequence of 4 washes: two-2uL washes of 0.05% Tween-PBS and two 2-uL washes of DI water, are shown. All three users were naive users (not enrolled in study, but unfamiliar with device). Data are averages ±1 SD (*n*=3). Asterisk (*) indicates significance, using one way ANOVA followed by pair-wise t-test (P = 0.0248).

We identified critical steps where both device and user-device interaction could be improved: (1) Sample collection and introduction of sample to microfluidic channel, (2) Inserting the cassette fully into the device, and (3) Engaging the bulb of the negative pressure chamber fully for

consistent fluidic actuation. Additional sources of error were mainly due to users using the instructional video instead of step-by-step directions in the app for guidance.

This indicates that various approaches to mitigate the main sources of error are needed, which could include: (1) enhanced user-guidance such as additional prompts in the app (e.g. specifying to wait until the instructional video was over to begin the test or recommended time frames to complete critical steps) or adding more audio-visual cues on the device to indicate if a step was performed correctly (e.g. cassette fully inserted into device, vacuum bulb pushed sufficiently); (2) assay refinement to increase tolerance for the variation in timing of steps performed by users, such as incorporation of anti-coagulant or on-chip dilution to counteract lag time between sample collection and assay start time, or (3) engineering design changes such as modifying the slot for insertion of the microfluidic cassette into the SMARTtest device (**Fig. 54**), adding more powerful negative pressure vacuum system to drive fluid flow, automated microfluidic channel priming step (e.g. running a pre-wash buffer) to mitigate flow issues and modified actuation mechanism of the negative pressure chamber (e.g. larger bulb, changing to squeezing mechanism instead of push to engage etc). Performing usability analysis with target end-users allows us to better characterize friction points and iterate on design choices to increase tolerance for user error or variance in timing of steps.

4.3.5 Device Credibility

We surveyed participants on belief in results generated from the device. Most participants (over 70%) felt "confident" or "very confident" in results received through the SMARTtest when asked to verbally rate on a 1-5 scale. From qualitative data analysis, we learned that many

participants expressed this confidence stemmed from the perception that blood as a sample input would be more accurate than an oral saliva swab. Many also expressed greater confidence in receiving disease negative results, based on their own perceived risk/behaviors, stating that positive results would cause them to pursue confirmation with a second test or at a clinic. Those who expressed less confidence in results generated by the SMARTtest device stated this stemmed mainly from feeling unsure about performing steps of the test correctly (e.g. if the fingerprick blood collection was acceptable) due to the lack of feedback from the device or app. Many also expressed that confidence in results would increase if the SMARTtest had regulatory approval (e.g. FDA approval) or if they were provided information on test accuracy, diagnostic performance statistics and in some cases, how the device worked (i.e. mechanism of action, data transmission from device to smartphone).

4.3.6 Acceptability of device for self-testing

4.3.6.a Willingness to use device

We explored desirability of the device though questions about participants' willingness to use and/or buy the test if it was available. 95% of participants in our study expressed that they would be likely or definitely likely to use the SMARTtest on themselves, if cost was not an issue (**Fig. 55A**). 85% also expressed high likeliness to use with sexual partners. Pearson Chi-Square analysis showed no significant correlation between those who had previously used the Oraquick HIV self-test and willingness to use the SMARTtest for self or partner testing, suggesting that even those who had not been primed with exposure to other STI home-testing products were equally likely to use the SMARTtest.

Participants overwhelmingly expressed that it would be very easy (86.4%) or easy (15.4%) to keep the SMARTtest in their homes (**Fig. 56B**). 74% felt it would be easy to carry it when going out, with the remaining quarter of participants stating they felt it would be fairly hard or very hard to carry SMARTtest when going out (**Fig. 56B**).

Most participants (56.4%) stated a willingness to pay up to $25 USD for one SMARTtest kit, which would include the resuable SMARTtest device ("Dongle 2.0"), 2 sets of disposable, microfluidic HIV/syphilis test cassettes and fingerprick materials (lancet, gauze, alcohol swab, bandage) (**Fig. 56C**). Three participants stated they would not purchase the kit and no participant expressed willingness to pay over $100 USD, however overall preference aligned with cheaper price points. There was a more bimodal distribution in price points that participants were willing to pay for each disposable HIV/syphilis test cassette, around the $3 USD range (~20% willing to pay less than $3 and 31% willing to pay $3-$6) and the $12 USD range (21%) (**Fig. 56C**). There was no significant correlation between self-reported annual income and willingness to pay more for a SMARTtest kit (Pearson correlation coefficient = -0.05, p=.780), or for disposable microfluidic cassettes (Pearson correlation coefficient = -0.14, p = 0.44).

4.3.6.b Perceived usefulness for home-testing

4.3.6.b.1 Circumstances

When asked about potential usage of the SMARTtest, most participants discussed privacy of a home as the primary setting for where they might use it. Participants liked the option of doing

test at home with follow-up options at a clinic or connecting with a doctor, particularly with potential positive test results. While they considered it physically easy to carry with them in the quantitative survey responses, many participants expressed they were less inclined to take SMARTtest on the go, unless it was pre-discussed with potential sexual partners. Some also felt that the SMARTtest was too bulky, though some felt this could be mitigated with a discrete carry-bag or travel kit with less packaging.

4.3.6.b.2 Motivators

When asked about potential prompts or motivating factors to use a device like the SMARTtest, the top two motivating factors expressed by participants were (1) perceived risky sexual behavior or encounters, including the use of drugs or alcohol that might affect judgement and (2) preventative or proactive testing to know or confirm one's status. The ability to get syphilis test results in addition to HIV, was seen as a significant value-add, especially as syphilis information (transmission, symptoms, window period of infection etc) was perceived to be less disseminated or known compared to HIV information. Other prompts that participants discussed towards potential usage of the device was convenience and affordability of getting the SMARTtest, seeing visible symptoms (e.g. syphilis chancres), or using the test to encourage partners towards testing as well.

4.3.6.b.3 Frequency

Participants were asked how frequently they might use the SMARTtest and responses varied between those familiar with the 3 month HIV infection window period and those who discussed

more frequent usage (e.g. once or twice a month). Many of those who stated they would test once every 3-6 months however, expressed they might use it more frequently based on perceived exposure events such as new partners or sexual behavior. Participants who were on PrEP mentioned that they already underwent a recommended quarterly HIV testing regimen under their current medication plan, and may not increase frequency of testing at home. Syphilis results however, were of interest and seen as a prompt to increase testing frequency for many participants.

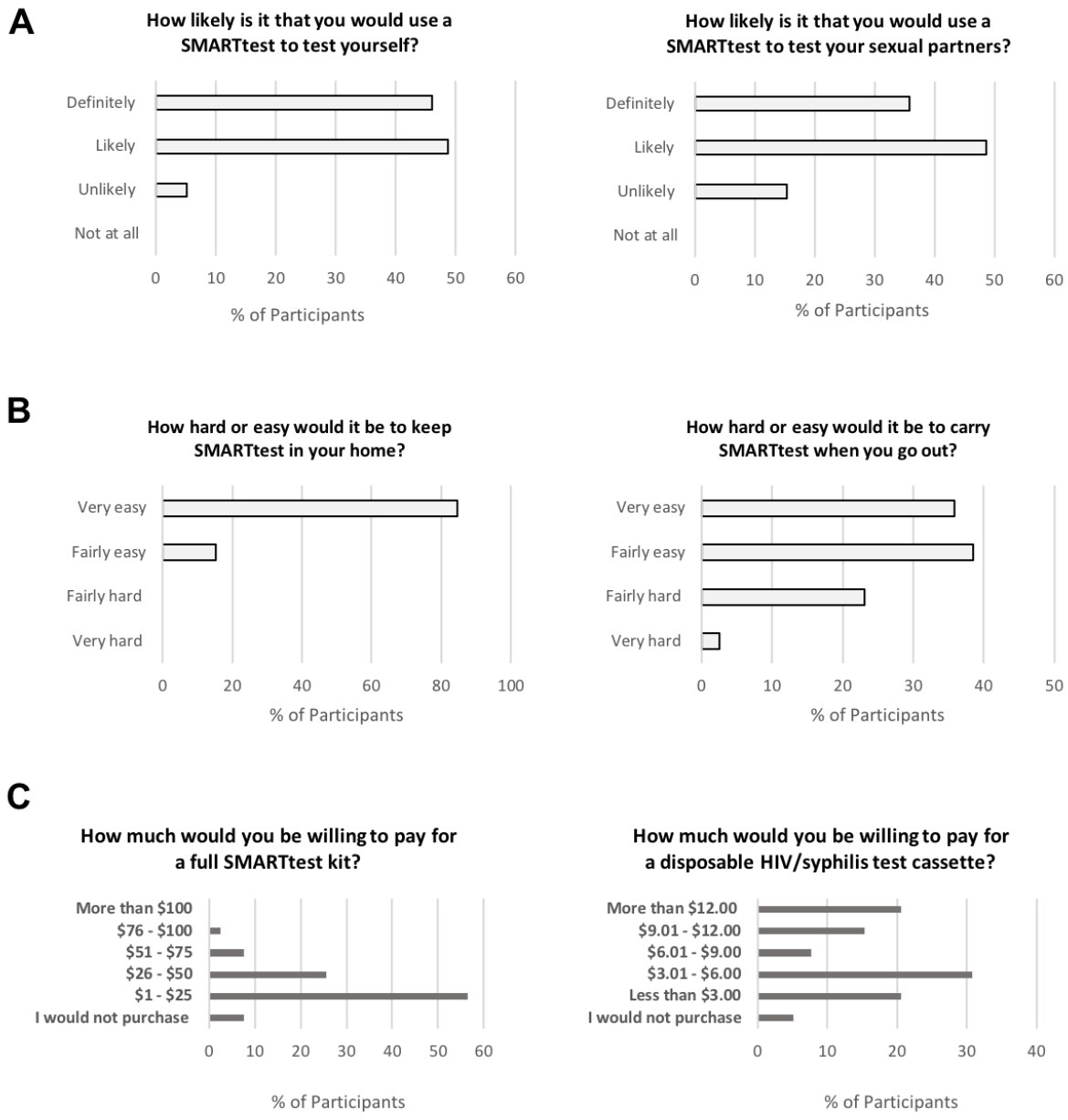

Figure 56. UX-IMB Acceptability: self-use of SMARTtest. (A) Participants' responses on likeliness to use the SMARTtest for self-use and with sexual partners if cost were not an issue. **(B)** Participants' ratings on how easy or hard it would be to keep the SMARTtest at home or carry on the go. **(C)** Price (in USD) that participants were willing to pay for a full SMARTtest kit (device and 2 sets of disposable microfluidic test cassettes and fingerprick supplies) as well as for disposable microfluidic test cassettes alone.

4.3.6.c Partner testing

Both self-testing and partner-testing are seen as key features of harm reduction approach with HIV transmission[141]. We explored acceptability and perceived effectiveness of the SMARTtest for use with partners (**Fig. 57**). Most participants (>74%) expressed high likeliness to use test kits with a range of sexual partner types including one-night stands and spouse-equivalent type of partners (defined here as those with whom participants shared an emotional connection with or who were repeat partners) (**Fig. 57A**). 92.3% of participants indicated a higher likelihood to test spouse-equivalent type of partners, compared to 74.4% who would use it with one-night stands and 87.2% who would use with all other types of partners (**Fig. 57A**).

When asked about perceived use with potential partners, over 70% of participants felt that it would fairly easy or very easy to raise the idea of using SMARTtest with a partner, and to ask partners to be tested with it (**Fig. 57B**). All participants stated they would find it easy (fairly easy or very easy) to show a partner how to use SMARTtest (**Fig. 57B**), with about 69% of participants feeling comfortable performing the fingerprick HIV test themselves on a partner (**Fig. 57C**). When asked how they imagined typical partners would feel about having a fingerprick HIV test performed on them, participants' responses were more split with slightly less than half (41%) envisioning partners feeling uncomfortable and the remaining half stating they imagined partners feeling comfortable with it. As a result, there was an approximately equal number of participants who would choose to test partners themselves or have the partner test himself with the fingerprick based SMARTtest (**Fig. 57C**). Another source of concern was usage of test with partners under the influence of alcohol or drugs. Participants expressed it would be

slightly easier to use the test while they were under the influence (62%) compared to partners being under the influence (51%), though both cases revealed alcohol/drug use being a potential barrier to using the test (**Fig. 57D**).

85% of participants also expressed they would find it easy or very easy to avoid intercourse (both RAI and AI) with partners who refuse to test, suggesting potential impact on sexual behavioral choices could be made through a self-test product. 85% participants also expressed high confidence in their ability to judge whether partners could become violent over using it and over 87% of participants stated they would find it easy or very easy to avoid violent situations that might arise out of using a test.

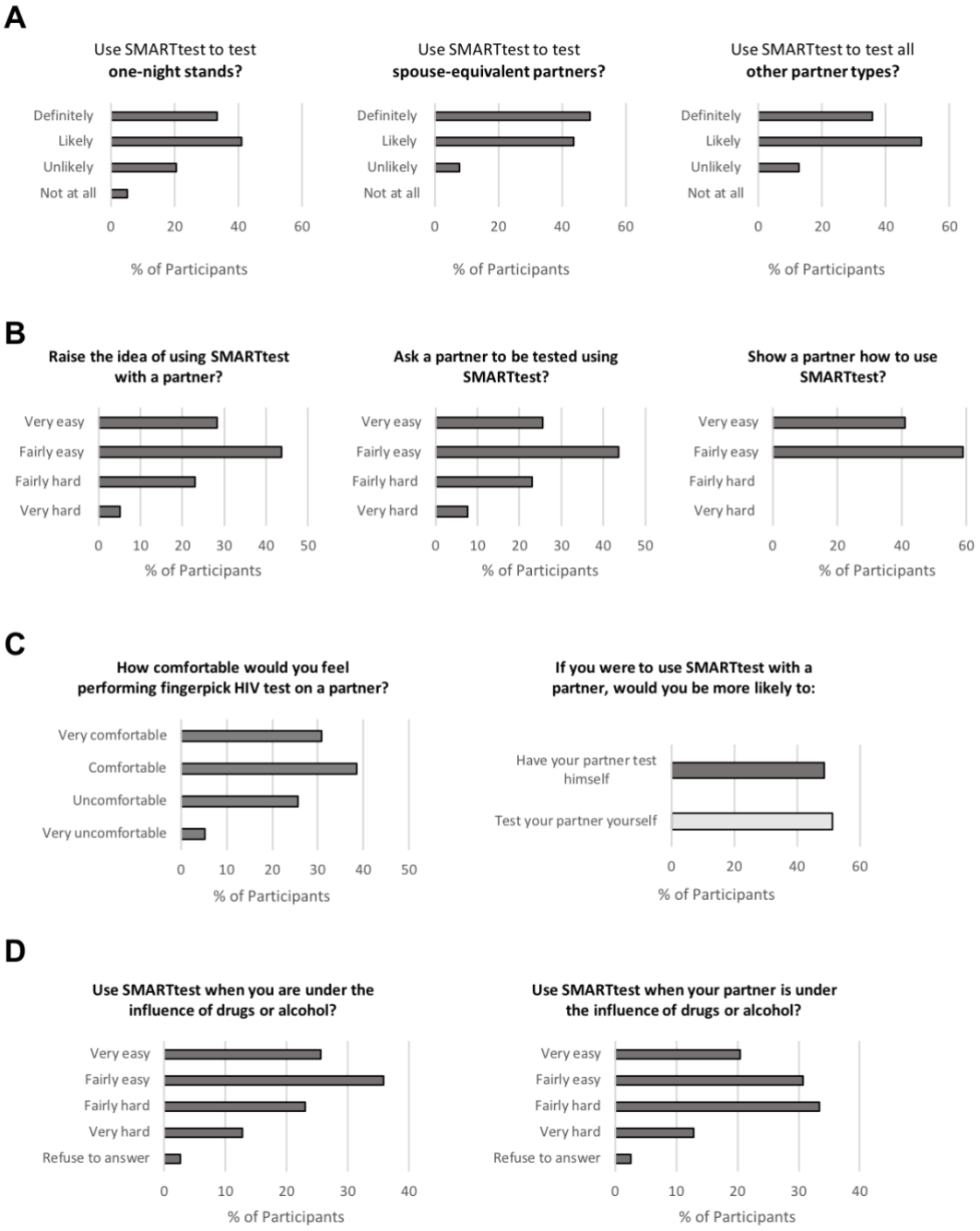

Figure 57. UX-IMB Acceptability: using SMARTtest with partners. (A) Participants' likeliness to use SMARTtest with different types of sexual partners, ranging from one-night stands spouse-equivalents (defined as those participants felt an emotional connection with) and all other partner types, if cost were not an issue. **(B)** Participants' responses on approaching partner-testing with the SMARTtest including how hard or easy it would be to raise the idea of the test, asking a partner to be tested and showing a partner how to use it. **(C)** Comfort and ease of using SMARTtest with partners including how comfortable participants would feel performing a fingerprick on partners and preference on who would perform the test. **(D)** Perceived influence of drugs or alcohol on ease of using SMARTtest with partners when participants were under the influence or when partners were under the influence.

4.3.7 Overall perception of device (advantages & disadvantages)

The top two advantages participants perceived about the SMARTtest was the ability to get instant results (i.e. not have to wait to go to a clinic or make an appointment for testing) and the smartphone integration. Instant results were seen as an advantage for greater flexibility in timing and location of testing as well as using with partners. Participants discussed various perceived advantages of smartphone integration including the ubiquity of smartphone usage and opinion that people enjoyed new technology and apps, the ability to store and track test results and perceived future functionalities such as sharing results with partners (including social media/online dating profiles) or with providers for followup. Other advantages that many participants discussed was that the SMARTtest felt simple to use, was portable and provided both HIV and syphilis results. Most participants liked the video and step by step instructions in the app, and felt the in-app guidance made the testing process easier to follow along.

Several participants compared the SMARTtest to their experiences with the OraQuick and expressed perceived advantages included greater portability compared to the commercial OraQuick testing kits, the ability to get dual test results, smartphone integration and faster time to result (10 minutes of waiting time in the SMARTtest versus 20 minutes with the OraQuick). A few participants also expressed that the SMARTtest was easier to use than the OraQuick and that it felt more accurate due to the removal of interpretation of visual lines for the result, use of blood over saliva as a sample input and reduced possibility of contamination (e.g. with saliva sample vial in the OraQuick).

When discussing perceived disadvantages of the SMARTtest, more participants expressed concerns with potential usage with partners than using it on their own, such as how partners might react, and what to do if a partner tested positive (e.g. providing counseling). One participant mentioned that having a self-testing product like this could add to "politicization" of people's decisions to test or not test, and subsequent stigma or social pressure based on these decisions. In terms of self-usage, some participants mentioned factors such as the use of blood and questions about test accuracy. Participants also expressed that while the device felt portable, making it even more compact would enable and encourage carrying it on the go. Some also discussed cosmetic refinements that correlated with translation of prototypes to commercial products (e.g. visually pleasing colors, sleeker form factor, making cassette insertion easier etc).

We explored concerns about a blood based self-test further. As with general concerns about the SMARTtest, there was a dichotomy between usage on self versus with partners. Most participants stated that they themselves had little to no concerns about a fingerprick blood based test, however could imagine others who might be concerned. Blood sampling was seen as a bigger "ask" from partners than an oral swab. Some participants expressed that they felt more apprehensive about handling their own blood, however the majority of participants also expressed the perception that a blood based test inspired more confidence and accuracy than an oral saliva method for HIV/STI test results. A few participants discussed that dealing with the fingerprick and bandage application after testing, might be disruptive to subsequent sexual activities, but the majority of participants did not see it as a significant barrier.

4.3.8 Re-visiting UX-IMB Framework

We used both analytical approaches to review and assess user interactions with the device (task analysis) as well as empirical approach deriving data from users' experiences (qualitative interviews and quantitative surveys). We examined three major components of the UX-IMB framework (**Fig. 46**) in this study: credibility, acceptability and usability of our microfluidic device, where each component can be mapped onto the three major constructs of the IMB model (information, motivation and behavioral skills) (**Fig. 58**). This model posits that performing a health promotion behavior is a function of the extent to which someone is well informed about the behavior, motivated to perform the behavior, and has the requisite skills to execute the behavior and confidence in their ability to do so across various situations[151]. This was an initial feasibility trial, so user-behavior (usage of device for self-testing and sexual behavior decisions) was not directly measured, however using this framework allows us to understand theoretical device value for the intended use-case, here for HIV home-testing for a target population of gay & bisexual men in the U.S.

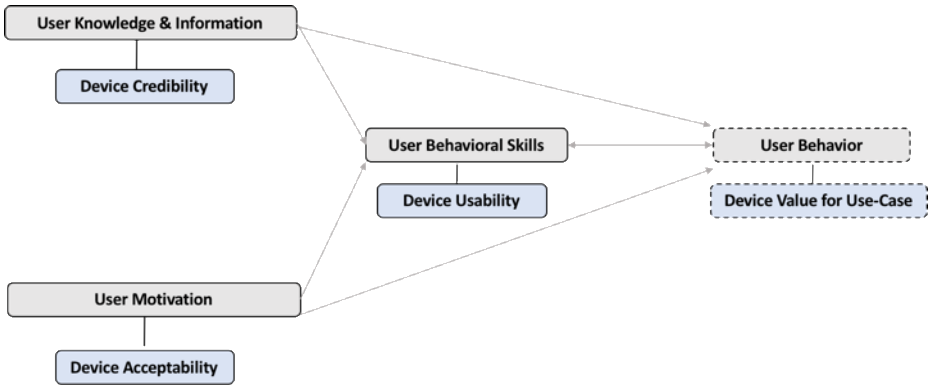

Figure 58. Linkage of SMARTtest study measurements to key parameters of IMB framework.

Usability analysis allowed us to evaluate a completely naive user's skill in using the device. Many of the critical errors lie at the intersection of user and device interaction. We were able to evaluate the device on some key design parameters of: (a) simple and intuitive use, (b) tolerance for user-error, (c) physical effort needed, (d) perceptible information, and (e) flexibility in use for diverse users and settings. Our results showed that the SMARTtest was relatively simple and intuitive to use by users with varying levels of education and income (in this study cohort), however had a low tolerance for error and high physical effort needed for critical operation steps (e.g. cassette insertion and vacuum bulb engagement). Perceptible information was high in the sense that participants found the video and step-by-step directions in the app useful in conducting a microfluidic assay, however was counterbalanced by the commonly expressed feeling that the device did not provide sufficient informative feedback on steps that were performed. Participants generally felt the device was portable enough to carry outside of the home (particularly by those who compared it to current commercial OraQuick kits), though multiple components (e.g. microfluidic cassettes and fingerprick supplies in addition to the dongle device itself) was seen to require a more compact form factor for transport.

Device credibility analysis showed us that in this cohort, a blood based test was perceived as more accurate for HIV and syphilis test results. The smartphone form factor can enhance information delivery, which could address remaining gaps in user knowledge and information on test information (e.g. diagnostic accuracy) that would enhance device credibility as well as provide additional resources on disease and testing information (e.g. window period of infection). Most participants indicated that follow-up testing or confirmation of positive results

(more so than negative results) in regular clinical settings was desired, supporting the need to integrate linkage to care elements in the device platform.

Device acceptability accesses user-motivation for engagement with the device for self-testing. This was measured through coding category themes that were derived from qualitative interviews: willingness to use the device, perceived usefulness for home-testing and potential use with partners. Key features of the SMARTtest device that participants identified as motivators for using was multiplexed results (syphilis and HIV), and opportunities to use the smartphone platform for results sharing and linkage to care.

4.4 Discussion

To maximize impact on patient care, the pace of translational research must be accelerated; integration of behavioral science into biomedical research has the potential to do this due to the large role of patient behavior in health outcomes[146]. We can use these frameworks to better understand the mechanisms of interactions between users and POC devices, that extend beyond physical interaction with the user-interface (hardware and software) and explore potential uptake and effectiveness of use based on user characteristics, environment Our work aims to provide a user-centered framework for testing and evaluation of microfluidic devices, especially those targeted towards home- or self-testing (**Fig. 46**). Such a framework can enable microfluidic POC developers to integrate user-based evaluations earlier in the development process to refine design features as well as build products that motivate generation of consumer-driven health data and improve health outcomes.

This is the first structured assessment, to our knowledge, of completely naïve users using a continuous flow, microfluidic POC test. We demonstrated feasibility of adapting microfluidic POC devices for consumer use with generally high acceptability from target end users. We used interdisciplinary analytical approaches to assess user interactions with the device and derive data from users' experiences from qualitative interviews and quantitative surveys. We examined three major components of the UX-IMB framework in this study: credibility, acceptability and usability of our microfluidic device, where each component can be mapped onto the three major constructs of the IMB model. From this, we were able to characterize friction points for naïve users in device handling and identify critical user-device interaction mechanisms. We also expanded understanding of mechanisms that drive device usage and effectiveness of use.

This was an initial feasibility trial, so user-behavior (usage of device for self-testing and sexual behavior decisions) was not directly measured, however using this framework for evaluation allows us to understand theoretical device value for the intended use-case, and future studies can assess implementation to evaluate device uptake for self-testing and health/behavior outcomes.

Our previous work demonstrated the use of the smartphone dongle as tool for clinicians or healthcare workers to use in resource constrained health settings[63]. Here, we made some changes to the device and accompanying smartphone app to study the potential of the platform

for a consumer-facing application. The modifications to the app included integration of a short instructional video, and step by step directions with photos and text for naïve users. Three main hardware design elements were modified from our previous smartphone dongle device[63]: 1) Bluetooth module for data communication, 2) Button cell battery for power, and 3) Smaller overall footprint (casing) (detailed in Chapter **3.7**).

One challenge of adapting the device for consumer use was providing instructions to perform a fingerprick blood draw for users who may have never handled lancets before. From observing the in-person skill demonstration of the first set of participants (n=20), we observed that additional guidance was needed even after blood was collected, as many participants were unsure about when to use provided gauze and bandages or how to handle the device if their finger was still bleeding. We modified the instructional video and in-app directions for the second set of participants (n=20) to provide explicit recommendations and cues on using gauze and bandages, which mitigated much of the friction points of handling the device and smartphone after the fingerprick process. This however, also increased lag time between fingerprick blood collection and pushing down on the vacuum bulb to begin flow of the blood sample through the microfluidic channel, often resulting in clogged channels. One modification could be to adjust the assay order of operation to insert Part B into the device, engage the vacuum first and then proceed to fingerprick blood collection and attachment of Part A to Part B to counteract user-introduced lag time.

We had marked a black fill line on Part A (sample collector/lyophilized antibody holder) to provide visual indication of sample metering of 2 μL. While this provided visual guidance, participants found sample collection into a capillary tube one of the more challenging steps of the test. To mitigate this in the future, we could redesign the capillary tube of the sample collector to have a maximum specified fill volume or provide additional guidance on collecting blood with a capillary tube (e.g. directionality of holding the sample collector, recommended time frame between generating a droplet of blood and collecting it etc). We also marked lines on the assay cassette and the dongle device to provide a visual cue to know when insertion of the device was completed, however the tight seal between the device and cassette (which was needed to ensure proper alignment between the vacuum port of the device and cassette outlet), resulted in higher resistance in pushing the cassette fully inside. Similarly, variation in force applied to the vacuum bulb resulted in both inconsistent (in some cases, insufficient) fluidic actuation and higher physical effort needed by participants. As a result, many participants in this study found these steps challenging. Binary steps with audio-visual cues (e.g. hearing a clicking sound) such as attaching the two microfluidic components together or pressing the switch were easier for participants to perform.

None of the participants in this study could generate a diagnostic result, however we have previously demonstrated an evaluation of diagnostic performance in the hands of minimally trained users compared to trained users, showing no statistically significant difference in sensitivity or specificity results of the assay. In this study, we observed that completely naïve participants of varying educational levels could all, for the most part, navigate the use of a

microfluidic POC test using a smartphone app. We characterized friction points for naïve users in device handling and test instructions, which can inform future engineering and assay development efforts. Credibility of results produced through the SMARTtest was moderately high and perceived to increase if provided with test accuracy statistics (requiring a large-scale characterization of diagnostic performance with clinical samples), or more feedback if steps in running the assay were performed correctly. Moreover, we were able to better understand target end-user needs such as designing a POC test that could be easily used with sexual partners and provide options for linkage to care. We found that acceptability of a device like the SMARTtest for home testing was generally high, in that participants saw value in a product that was relatively easy to use, portable, provided both HIV and syphilis test results, and utilized the smartphone as a platform device.

In this feasibility study, we did not explore device accessibility to users at length, as several stages of development are needed before the SMARTtest is commercially available. Our usability study did reveal that there was a low training burden through the in-app video and step by step directions and familiarity of a smartphone interface. The SMARTtest re-usable device costs approximately $60 USD under current in-house manufacturing protocols (**Table 14**). This is higher than the $25 price point many of the participants in this study stated they would be willing to pay for a full test kit, which includes the disposable fingerprick supplies and microfluidic cassette components. One OraQuick home-testing kit retails for ~$40-45 USD (in New York City) and consists of a single use lateral flow device, one test tube filled with 1-ml of liquid reagent, testing directions attached to a plastic box, pre- and post-test booklets,

disposal bag and pencil. A timer for measuring 20-40 minutes is also required but not provided in the kit. Our system is more compact, integrates instructions and provides a platform for storing, saving or sharing results if desired, counseling, referral and linkage to care options (features of the app that will be explored in future studies). Smartphone integration was seen by participants in this study as one of the biggest advantages to our rapid test platform; in the context of accessibility, the ubiquity of the smartphones offered a familiar format and thus low training burden through the accompanying app. Additional value proposition of app features suggest smartphone integration may play a large role in adoption of new devices and tests.

Some studies have shown participant preference for oral sampling over fingerprick blood[152] however other studies have shown that acceptability of blood-based testing increases if other STIs can be detected[153]. In our study, many participants expressed that while fingerprick blood sampling might be a greater ask with partners, they would be comfortable performing it on themselves. Many also shared the perception that blood-based testing was seen as more accurate than oral saliva-based testing, suggesting acceptability of fingerprick blood based tests.

A number of new companies are seizing on the business opportunity of at home STI testing, as evidenced by a number of new online and app based products[154]. Products vary from mail-in testing, telemedicine consultations and follow-ups, to enabling in-person visits at partner laboratories. Prices vary from $45 for an at home-test (Biem), $79-$350 for mail in testing (myLAB Box), $219-$349 for STI panel testing (STDCheck.com)[154]. Some aspiring

startups such as Safe are attempting to integrate physician networks, traditional diagnostic testing companies and electronic health records into one app to facilitate testing at partner sites and storage of STI results[155]. These companies are capitalizing on the draw of upfront pricing, app-based linkage to care options and data storage to attract millennials and early adopters of "healthcare 2.0". While the business models of many of these early stage companies are still unproven, the amount of venture capital funding[155] for them is one indication of the high interest in consumer-facing health care products and services in the United States.

The growing availability and development of consumer-facing devices suggests that the potential for consumer led health has never been greater[156]. The attainment however, of desired clinical sensitivity and specificity is insufficient to motivate consumer use of health devices and hence gathering of large scale data. Thus far, deployment of consumer-led health devices has been ad hoc without structured assessment of user experience, which can lead back to appropriate design and technical parameters. We have created an integrated, user-based framework of behavioral and design evaluation which can be applied to microfluidic device development process. This in turn, will accelerate translation of new technologies in creating POC tools that are poised for more impact and widespread adoption by target end-users in diverse settings.

Chapter 5

Conclusions

Microfluidic technology has long held the potential for miniaturizing high-performance diagnostic tools and expanding access to healthcare; our work shows several advances in realizing that potential through translational engineering. In this dissertation, we examined the development pipeline from proof-of-concept assay development in a microfluidic POC platform, to field testing prototypes and exploring user experience, for intended end-use settings that range from U.S. based primary care clinics, rural health centers in low-resource settings as well as self-testing environments in both.

We showed that translation of integrated microfluidic diagnostic devices from the bench to clinical use is analogous to climbing a development ladder, starting from the many parameters in assay development. Our Lyme Disease and Leptospirosis assay development work illustrated some of the considerations for the first stages of developing miniaturized ELISA-based

immunoassay in a microfluidic format. We started with choosing appropriate capture antigens, optimizing the capture antigen concentration for antibody capture, multiplexing multiple markers on one microfluidic chip, and obtaining baseline clinical characterization of diagnostic performance using "cleaner" sample inputs such as diluted serum/plasma.

The second stage of assay development moves beyond the initial proof-of-concept parameters in translating an established diagnostic technique into a miniaturized format. We illustrated these efforts with our work in developing an integrated triplex HIV-syphilis assay. This stage of development involves considerations for scale-up, stability/storage and adaptation of the assay to handle additional, unprocessed sample inputs such as venous and capillary whole blood. The latter remains one of the most challenging aspects of POC assay development as whole blood contains many components that present challenges to achieving high analytical reproducibility, sensitivity and specificity as well as uniform flow conditions in a microfluidic device. These components include immunoglobulins, lipoprotein particles, serum albumin and various other proteins, in addition to blood clotting factors, electrolytes, blood plasma proteins and nutrients (e.g. glucose, amino acids, fatty acids) that may be dissolved or bound to plasma proteins. We explored some strategies to handle whole blood as a sample input in a microfluidic device including dilution, increasing surface capture marker concentrations and exploring various blocking agents to reduce non-specific binding of analytes. Though we saw promising results with some of these strategies towards diagnostic performance, on-chip plasma separation or on-chip dilution of whole blood could be better strategies to streamline and/or automate the number of manual steps needed to run the test by a target end-user while still achieving clinically relevant performance statistics.

Integration with hardware and software to run the assay, acquire data and interpret results is the next rung of the microfluidic POC device development ladder. We leveraged silver enhancement for signal amplification, allowing us to use simpler and lower cost optical components for detection. This gave us more flexibility with companion hardware devices needed to run our microfluidic POC assays, from a benchtop instrument suitable for a physician's office or urgent care setting in the U.S., to a handheld dongle for field usage or self-testing. In designing a product for the former setting, we envisioned greater access to refrigeration, electricity and less importance of portability and ability to withstand rough handling. For the latter setting, these parameters were flipped, and our hardware design criteria focused on developing an extremely portable and rugged form factor that required no other peripheral equipment, leveraging the ubiquity of smartphones as the source for device powering, data processing and communication.

This led us to re-examine previous frameworks such as the ASSURED acronym [157], which has been widely used as criteria for building POC tools for global health applications or low resource settings. While initially useful for researchers, its introduction in the early 2000s came before the age of smartphones and widespread data connectivity. The letter "E", standing for "Equipment-free", did not take into account the recent explosive rise of consumer electronics and availability of hardware components with increasing capabilities at falling prices. By revisiting our revised 2x2 matrix model of defining four distinct POC use-cases, we can now summarize how each of these settings imposes specific constraints on device design based on the relative importance of translational engineering parameters (**Fig. 59**).

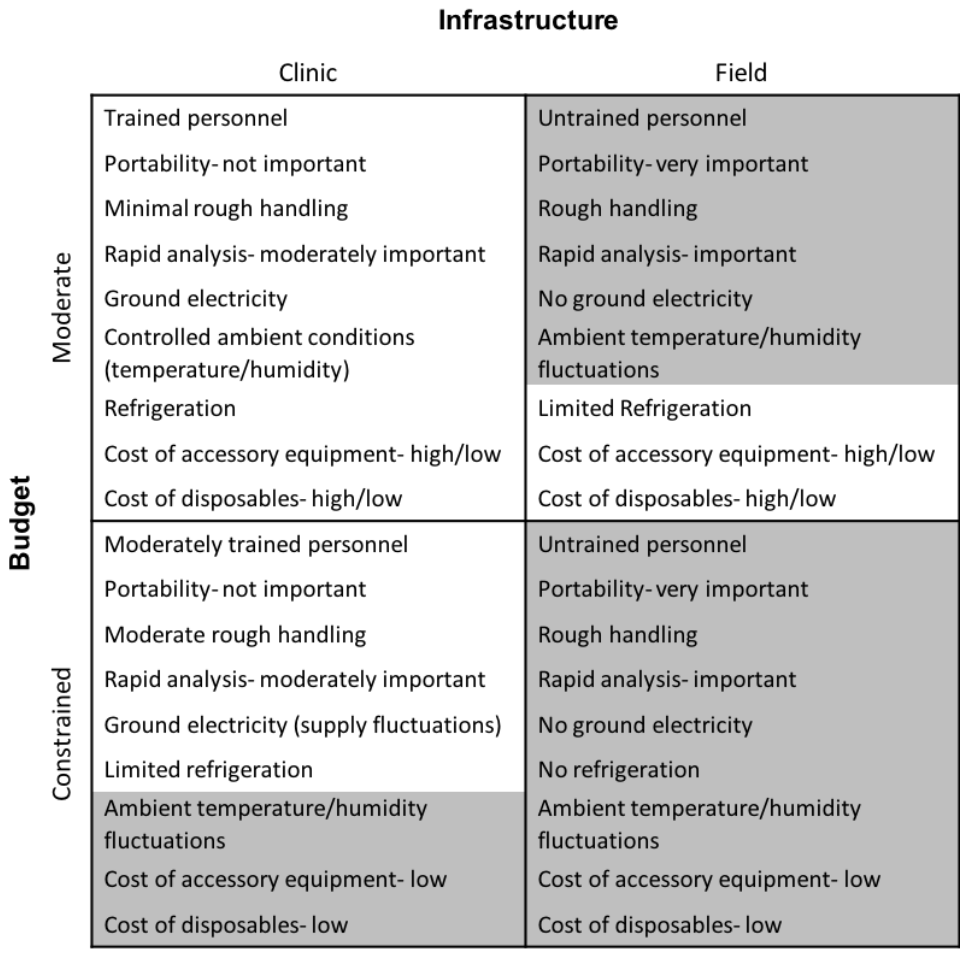

Figure 59. POC use cases where each setting imposes specific translational design constraints.

Use Case 1. Clinic level with moderate budget

Use Case 1 encompasses settings with access to a moderate budget and some level of clinical infrastructure such as hospital emergency rooms, operating rooms, or intensive care units. Here, there are personnel and resources such as electricity and refrigeration which support operation of POC devices, as well as a moderate budget which can accommodate accessory equipment necessary to help run the test. (At Columbia University Medical Center, the number of different

platforms used for POC testing grew from 7 in 1995 to 29 in 2015, and the number of patient tests grew from about 500,000 in 1995 to more than 2,000,000 tests as performed by 4000 users in 2014[158].) In these settings, a POC diagnostic device can be used at the patient's bedside and return rapid results to help direct treatment. Moderate throughput in testing of samples is sometimes desirable as a feature of the POC device.

Use Case 2. In the field with moderate budget

Use Case 2, as in the previous use case, allows for a relatively flexible and moderate budget for the POC device, but it is set apart by differences in infrastructure available to run the test. In this use case, there are often no trained users and no accessory laboratory equipment, such that a self-contained, portable device is vital, with the ability to withstand rough handling and provide rapid analysis. POC devices in this use case are intended for the field, and can contain sophisticated POC technologies to accomplish their analytical functions. For example, POC devices used at home, what we commonly refer to as consumer electronics or consumer devices, may cost a significantly greater amount to the insurer or patient (compared to other field settings such as global health), but need to be operable by untrained users and are self-contained in terms of reagents and disposal. This use case also encompasses many devices currently on the market and sold in pharmacies in the U.S., Europe, and other developed settings.

Use Case 3. Clinic level with constrained budget

Use Case 3 presents settings with access to laboratory facilities and trained personnel, but where the resources and budget are more constrained than Use Case 1. Examples include primary care clinics in developing countries, and nonprofit and non-governmental organization health centers.

For researchers intending to develop POC devices for this use case, significant design considerations must be given to cost; in return, full integration (e.g. sample preparation steps), portability, and sample throughout may not be as critical. In addition, while the goal remains to still provide diagnostic information within a single clinical visit to prompt a treatment decision, the turnaround time for results could be extended from minutes to hours.

Use Case 4. In the field with constrained budget

Use Case 4 settings represent the greatest set of constraints for POC diagnostics. These have tight budget constraints, as exemplified by field settings such as lower-level clinics and health posts, mobile health and community health outreach, and self-testing settings. A number of design choices, in cost, portability, and automation, must be made to ensure the POC device is usable in such settings. It should be noted that, with modifications and further iterations, devices listed in other use cases could also be appropriate for this use case.

Table 22 illustrates specific design choices that can thus be made depending on the intended use-case setting. For example, an early design choice in our work towards developing an HIV-syphilis diagnostic device for low resource settings (e.g. Use Case 4), was the use of thermoplastics as a microfluidic cassette material choice[61]. This enabled easier scale-up and large scale fabrication (thus a relatively low-cost platform), while retaining features and channels to perform multiplexed assays on one chip (in contrast to paper based lateral flow tests).

Table 22. Range of POC device design considerations for Use Cases 1-4.

	Use Case 1 **Moderate Budget, Clinic**	**Use Case 2** **Moderate Budget, Field**	**Use Case 3** **Constrained Budget, Clinic**	**Use Case 4** **Constrained Budget, Field**
Material Choice	Glass Silicon Plastic	Glass Silicon Plastic Flexible electronics	Paper Plastic	Paper Plastic
Reagent Stability	Short term or long-term storage	Long-term storage needed	Short term or long-term storage	Long-term storage needed
Sample pre-treatment	Off-chip by trained technician	None	Off-chip by minimally trained technician	None
Fluidic Actuation	Electrokinetic Pneumatic, Magnetic	Pneumatic Capillary	Electrokinetic Pneumatic Magnetic	Pneumatic Capillary
Fluidic Control	Machine/power Pipetting by technician Valves	Passive On-chip valves	Machine/power (minimal) Pipetting (minimal) Valves	Passive On-chip valves
Signal Detection	Fluorescent (larger machines) Colorimetric Electrochemical	Colorimetric (by eye, smart-phone, or hand-held device)	Fluorescent (small, cheap machines only) Colorimetric Electrochemical	Colorimetric (by eye, smartphone, or hand-held device – must be cheap)
Disposal	Not needed	Self-contained	Not needed	Self-contained
Sample type / acquisition	CSF, amniotic fluid, whole blood, urine, saliva, plasma, sera, swabs, tears	Whole blood, urine, saliva, swabs, tears	Whole blood, plasma, serum, urine, saliva, swabs, tears	Whole blood, urine, saliva, swabs, tears

While some design considerations such as long term stability and storage at ambient conditions or whole blood processing require additional development for our device to be used in actual clinical practice, our field studies in Rwanda showed the smartphone dongle based HIV-syphilis assay could achieve comparable performance to other rapid tests run under field conditions. Moreover, minimally trained healthcare workers who received only 30 minutes of training with the device, performed the test themselves on patients coming into local clinics. Assessing the performance of a test under real-world conditions, which can be markedly different than those of a laboratory, is critical in the POC design and development process.

As POC diagnostic devices are becoming increasingly integrated, and tested with clinical specimens, we have presented our developments in the field through the lens of different use-case scenarios, which impose different design constraints on all aspects of the POC diagnostics device, from material choice to signal detection (**Table 22**). Unlike previous classifications of use-case scenarios, we propose a framework which decouples the infrastructure available to run the test, from the budget allocated to purchase the POC device. This decoupling presents use cases which pose distinct constraints for researchers (**Fig. 59**), including different design constraints for use in a clinic or in the field which would depend on the budget available. This framework presents the constraints across a more diverse set of POC settings than just global health.

While access to peripheral equipment and trained personnel to use centrifuges and pipetters may not be a barrier to all POC settings, user-experience and consequently work flow, behavior and ultimately adoption of a new device are affected by such design choices. Our work in testing

prototype devices in the hands of healthcare workers illustrated that in addition to portability and fast turnaround time that all POC tools offer, a device that relied on no other peripheral equipment or ground electricity and was relatively simple to operate was seen as more likely to integrate quickly and effectively with existing clinical work flow patterns for mobile health outreach efforts, our target use-case setting. Potential opportunities to automate and digitize testing data, particularly outside of large urban centers, was also seen as valuable to public health administrators in Rwanda. We learned that new POC devices must present value to the end user over existing methods, whether in incorporation of sample processing, streamlining the number of manual steps to produce a diagnostic result or integration with data systems and health records.

Self-monitoring via fitness trackers and smartphone apps reveal a growing demand by patients and consumers for managing and seizing control of their own health. While the increasing availability of diagnostic information to non-healthcare professionals presents questions on how diagnostic information should be handled and shared, the successful deployment of at-home glucose monitoring and home pregnancy tests[159] shows that self-testing (or home-testing) can supplement traditional clinical visits in meaningful ways. Whether by promoting preventative behaviors, drawing in first time users or triggering linkage to care, self-testing provides one avenue of increasing engagement between patients and providers.

We assessed potential value of our smartphone-interfaced, microfluidic device for a self-testing application in our user-based evaluations with both patients in Rwandan clinics as well as MSM consumers in the U.S. In both cohorts we observed an interest and willingness to receive

potentially sensitive health information outside of traditional clinical avenues. The smartphone was a key element in reducing the training burden needed for a completely naïve user to perform a diagnostic assay and also one of the features most likely to engage users in adoption through the potential for tracking and managing health data, as well as opportunities for linkage to care. In our self-testing evaluation with MSM consumers, we were also able to assess usability, accessibility, acceptability, and perceived effectiveness for home-testing in the U.S. This included use with sexual partners, which is seen as a key element of HIV harm reduction in this population.

Naïve user testing also allowed us to characterize friction points in the overall testing process that can inform future design iterations of our POC tools. Steps to address these can range from increased user guidance on fingerprick blood sampling or feedback on steps performed, assay changes to increase tolerance for variable timing in users performing blood-based assays, as well as hardware design changes to enable more binary manual steps and automate troubleshooting (e.g. microfluidic flow issues). We also accessed mechanisms that affect user-device interaction through expanded human factor engineering analysis with frameworks from behavioral science models. Future implementation studies can be performed with participants taking devices home over a period of time for evaluation. Such studies can provide more longitudinal data on device performance, stability and usability as well as preliminary information on testing frequency, motivation for use and preferences with health data management.

Future directions

Broader technological and non-technological components in the POC ecosystem (**Fig. 1**) will also shape the path of new microfluidic tools for real-world use. This includes components like regulatory guidance, legislation and reimbursement, which affect patient health information protection, boundaries of direct to consumer diagnostic services and whether POC products will be reimbursed by public and private insurers or aimed for consumers and patients paying out of pocket for POC health products and services.

Data Analytics

Smartphones and accompanying fitness trackers have also enabled the collection of multimodal data. In the near future, devices equipped with "Internet of Things" technologies will stream other types of health data from diverse sources. For POC diagnostic devices, the value of health biomarkers could be augmented by interpreting the information alongside these ever expanding data sets. Health information collected from consumers (from Internet, mobile phones and social media platforms) are increasingly being entered into a central database for analysis. For a patient's health, information being collected into the ResearchKit (a database from Apple)[160] could link users to studies on Parkinson's disease, diabetes, asthma, breast cancer and heart disease[161]. Towards public health and epidemiology[162], search engine queries and page views to websites could be used to infer behaviors associated with medical conditions (such as cardiovascular disease, sexually transmitted infectious, pregnancy and mental health conditions[163], or outbreaks of communicable diseases[164]). However, care must be taken to avoid over interpretation, by using multiple data sources and analysis methods and cross-

validation with public health data[164], and by addressing vulnerabilities in algorithms for overfitting[165]. Future analysis of large sets of health and medical data will be augmented by artificial intelligence (for example by leveraging IBM Watson Health's cloud-based healthcare analytic platform[166]). Already, interesting concepts are being proposed for how POC systems can be integrated with machine learning, such as the generation of a predictive model capable of assessing risks for heart attack and heart failure patients[167]. An intriguing (but less tested) approach for analyzing large sets of data is crowdsourcing [168].

Clinical workflow

As healthcare delivery becomes increasingly decentralized, healthcare professionals and patients are adapting their workflows to fit in the use of POC diagnostic devices. For pathologists and clinicians, adoption of POC testing continues to grow[169]. Effective implementation of POC testing eliminates transport, processing, and aliquoting processes that take place in core laboratories, thereby creating a more streamlined and faster workflow[170] and enabling more face-to-face interactions between providers and patients to understand test results and plan treatment options[170]. Nevertheless, because more CLIA-waived tests means expansion of POC tests in diverse settings[171], it is often difficult to ensure proper oversight and quality assurance[170] over minimally trained or untrained users. Some hospitals and clinics appoint point-of-care coordinators or management teams to ensure consistent procedures, regulatory compliance, correct documentation of results, and end-user assistance[170, 172]. It is important for developers of POC devices to take advantage of advances in wireless connectivity and electronic health records to achieve ease of use and quality assurance.

Integration into healthcare system

Patients and consumers are increasingly adapting POC tests. Examples include genetic testing services (such as 23andme)[171]. They are also expanding their use of on-demand home visits by doctors [173], which are being offered by a number of startup companies (including Heal, Pager, Doctors Making Housecalls). Many of these visits entail the use of a large range of connected smart devices for POC or remote diagnosis[173], in the context of primary and urgent care as well as wellness assessments and annual physical exams. Examples include POC assessments of sexually transmitted infections, and measurement of diabetes and cholesterol levels[174]. The use of such POC tests will only increase, spurred by the growth of more CLIA-waived POC diagnostic tests, increased self-monitoring via fitness trackers and smartphone apps, and the growth of on-demand clinical services and telemedicine. How consumer driven usage of POC devices will intersect within the larger continuum of healthcare and delivery of clinical services remains an open question. For developers, it will be important to understand how new interventions will affect target populations, how linkage to care can be addressed and whether health outcomes can be measurably improved.

It is also important to note that not all clinical diagnostics may be appropriate for POC application if they place excess burden on health workers or patients or do not provide actionable clinical benefits[175]. Additionally, recent events with consumer data breaches and unauthorized sharing of data[176, 177], illustrate the attention that must be given to safeguarding the collection and management of sensitive health information (e.g. HIV/STI or genetic testing data).

Nevertheless, the rapid progress of non-traditional components in the new POC ecosystem – such as consumer electronics, connectivity, and data analytics -- has infused POC diagnostics with new energy, perspectives, and risk capital. While this thesis has focused on POC immunoassays, other classes of diagnostic assays are also poised for deep clinical and consumer impact such as molecular or cellular testing applications. An application of human centered engineering design towards POC development, through a holistic integration of target-user and use case setting involves integration of interdisciplinary fields from biochemistry, materials science, electrical engineering and mechanical engineering to medicine and behavioral science. In the short term, both technological and non-technological challenges may persist before a large set of new POC devices will be introduced to the market. In the long term, the field is on track to deliver POC diagnostic devices across all appropriate decentralized settings as the remaining technological hurdles are addressable, and the social benefits too rich for governments, clinicians, insurers, and consumers to ignore.

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