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Editors

Frugal Innovation in Bioengineering for the Detection of Infectious Diseases

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Frugal Bioengineering: Preface and Introduction

Recent advances in biomedical engineering have paved the way for the development of low-cost technologies that can aid in diagnosing diseases or improving global health outcomes. Rapidly deployable, portable, and low-cost diagnostic platforms have the potential to impact millions of people around the world, especially those living in extreme poverty. In 2014, Manu Prakash and colleagues at Stanford University invented an ultralow-cost microscope, which they termed "foldscope," to be used in the field in resource-poor settings [1]. Coupling principles of optical design and origami, the microscope was able to achieve over 2000× magnification with submicron resolution all the while costing <$1 in parts [1].

Because of its low cost, diverse applicability, and potential for widespread adoption, the impact and benefit of the foldscope is enormous; early test data demonstrated successful high-magnification bright-field images of unicellular microorganisms such as *Leishmania donovani* and *Trypanosoma cruzi*, deadly pathogens that are causative agents of neglected tropical diseases [1]. At the heart of the foldscope invention is an implementation of "cost-conscious" or "frugal" science – an affordable product with simplified design and durability that is easily scalable [2]. Prakash and colleagues write, "Our long-term vision is to universalize frugal science, using [the foldscope] platform to bring microscopy to the masses." Thus far, 50,000 foldscope kits have been shipped to over 130 countries with a goal to ship one million more [3].

Frugal innovations, like the foldscope, which maximize value while minimizing resources (or advances that allow for "doing better with less resources for more people") [4] provide an opportunity to address the needs of marginalized communities. More than half of the world's population – around four billion people – live on less than $9/day [5] constituting the base of the economic pyramid [6]. They face significant unmet needs in terms of healthcare, electricity, water, sanitation, financial services, education, and food [4, 6]. Meeting these basic needs is critical to improving the overall welfare, productivity, and income of those at the base of the pyramid, thus allowing households to engage with the formal economy and find a path out of poverty [6]. As an example, "M-Pesa," a mobile phone-based money

transfer service, allows for millions of people to gain access to the formal financial system. A frugal solution that was first launched in Kenya and soon spread to many countries across the developing world including India and South Africa, M-Pesa provides a means for people to pay for goods and services with a mobile device. Besides M-Pesa, there are many similar examples of affordable technologies and ideas being developed across industries such as an energy-efficient charcoal stove (ashden.org) and a nonelectric water purifier (nesta.org.uk) that serve basic needs at scale. In the context of healthcare, General Electric's MACi portable battery-operated electrocardiogram (ECG) deployed in rural parts of India allows for patients to get tested for less than 20 cents [7].

Beyond having affordable price points for consumers at the base of the economic pyramid, all of these innovations – from the foldscope to the MACi – have key design criteria at heart: they are built for scarcity, scalability, and sustainability [8]. First, frugal designs must recognize that material and human infrastructure may be severely limited in rural settings [8]. Second, in order to create a sustainable and scalable solution, there must be a demonstration of need as well as evidence for efficacy and improved outcomes at the community and national levels [8]. Engaging with consumers early in the design process and making certain that any product born out of the frugal innovation process is socially appropriate and suits local user preferences will be critical to the long-term applicability of the solution [8, 9]. Finally, simplicity and durability should be prioritized to allow for extended use of the product [8, 9].

In a recent article published in *BMC Medicine*, epidemiologists Tran and Ravaud classified frugal innovations in medicine along four different subtypes, which we found to be useful and informative [10]. The authors define these as (a) lean tools and techniques, (b) opportunistic solutions, (c) contextualized adaptations, and (d) local bottom-up innovations. First, lean tools, of which the foldscope and GE MACi are examples, are extremely cost-efficient versions of existing technologies used in developed countries. They are portable, durable, easy to operate/maintain, and able to function in harsh environments and may use locally sourced parts [10]. Second, opportunistic solutions target the use of mobile phones and the Internet to address challenges in healthcare. For instance, the authors classify the use of SMS-based identification of counterfeit medication as one example in this category ([10]; Sproxil.com). Third, contextualized adaptations use existing materials repurposed for a novel medical use. An example is the Solarclave – a device consisting of a bucket, a pressure cooker, and 140 mirrors – that achieves temperatures of 120 °C in the sun and is used for sterilizing surgical equipment [11]. Finally, local bottom-up innovations are simple solutions born out of necessity and adapted to local settings where other types of interventions may not be feasible [10]. Here, Tran and Ravaud suggest that solar disinfection of water is one frugal solution that fits neatly in this category. In a controlled field trial targeting children aged 5–16 years, the simple disinfection of water using only sunlight showed significant reduction in diarrhea in a rural province in Kenya where other forms of water treatment such as by chemical means, filtration, or boiling were not feasible [12]. In short, the four

subtypes of frugal innovations identified by Tran and Ravaud are a mixture of simplifying high-tech toolkits and developing "homegrown fixes" to be able to address significant unmet needs with regard to health [10].

Our initial motivation for putting together this edited book was to learn about various types of frugal innovations that are currently being developed and deployed in resource-limited settings around the world. We were very inspired by the simple and elegant design of foldscope, and being bioengineers ourselves, we wanted to study the range of frugal technologies in the bioengineering space that were addressing unmet needs in global health. We sought to focus our efforts on frugal innovations for diagnosing infectious diseases such as malaria and HIV/AIDS, especially given their devastating burden on populations of many developing countries. Although we tried to be as comprehensive as possible in inviting contributors from a variety of different research areas and backgrounds, we only scratch the surface in this book in terms of innovations in "frugal bioengineering," which is now a rapidly developing field.

The chapters in this book will introduce readers to novel frugal bioengineering innovations that are broadly applied to address challenges facing global health – from diagnosing diseases to developing "on-the-field"/"point-of-care" (POC) basic screening solutions. The low-cost technologies covered here have the potential to tremendously benefit resource-limited developing country settings without compromising on the high-sensitivity and high-fidelity standards common to the expensive technologies currently used in developed nations. The chapters covered in this book mostly fall under the "lean tools and techniques" classification from the Tran and Ravaud framework. Below, we provide a brief overview of individual chapters and also list relevant literature and additional reading pertaining to the area of focus for each chapter.

Overview of Chapters in the Book

Chapter 1 – Advances in Point-of-Care Diagnostics for Infectious Disease

In the first chapter, Rebecca Richards-Kortum and colleagues set out to review various types of diagnostic technologies developed for POC testing. To begin, the authors detail the ASSURED guidelines from the World Health Organization – a set of seven criteria that a POC diagnostic test should ideally meet in order to be deemed useful in a resource-limited setting (stands for affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, and delivered). While the guidelines serve as a useful benchmark for developing frugal innovations, POC devices can include a wide spectrum of diagnostic tools that (a) vary in complexity, (b) can be used at home or in the clinic, and (c) can be operated by people with or without technical skills [13].

Useful Additional Reading

Drain et al. Evaluating diagnostic point-of-care tests in resource-limited settings. *Lancet Infect. Dis.* 2014

- John and Price. Existing and emerging technologies for point-of-care testing. *Clin. Biochem. Rev.* 2014
- Pai et al. Point-of-care testing for infectious diseases: diversity, complexity, and barriers in low- and middle-income countries. *PLoS Medicine*. 2012
- Wu and Zaman. Low-cost tools for diagnosing and monitoring HIV infection in low-resource settings. *Bulletin of the WHO*. 2012

Specifically, Richards-Kortum et al. review technologies related to three classes of biomarker targets, namely, cell-based assays, protein detection tests, and nucleic acid tests. An application of cell-based assays is in detecting and identifying white blood cells (e.g., monitoring CD4+ T-cell counts for managing HIV infection). In this category of assays, the authors review underlying methods such as flow cytometry, static imaging, as well as electrical impedance and discuss examples of diagnostic tools including Partec CyFlow miniPOC (sysmex-partec.com), PointCare NOW (pointcare.net), Alere Pima Analyser (alerehiv.com), HemoCue WBC (hemocue.com), Daktari CD4+ cell counter (daktaridx.com), and the Visitect CD4+ strip (omegadiagnostics.com). A drawback is that many of the instruments listed here can be very expensive, some costing in the thousands of dollars [14].

Useful Additional Reading

- Boyle et al. Emerging technologies for point-of-care CD4 T-lymphocyte counting. *Trends Biotechnol.* 2012
- Rowley. Developments in CD4 and viral load monitoring in resource-limited settings. *Clin. Infect. Dis.* 2014
- UNITAID Technical Report. HIV/AIDS diagnostics technology landscape. 2012
- Wang et al. Microfluidic CD4+ t-cell counting device using chemiluminescence-based detection. *Anal. Chem.* 2010

In detailing the second category of assays related to protein detection tests, Richards-Kortum et al. focus on lateral flow assays, agglutination assays, microfluidic paper-based devices, and 2D paper networks that may be used to quantify an antigen in a sample of interest. The low-cost, easy-to-use, and quick readout time attributes of these assays make them particularly attractive from a frugal bioengineering standpoint. A few examples of tools in this category of assays include CareStart Malaria RDT (accessbio.net), SD BIOLINE Dengue Duo (standardia.com), and a paper microfluidic platform for malaria antigen detection [15]. The third chapter of this book delves into greater detail on paper microfluidics.

Lastly, nucleic acid diagnostic tests seek to detect pathogen DNA or RNA and are highly sensitive and specific but require means for sample preparation and signal amplification. Some examples of tools include SAMBA (drw-ltd.com) and Xpert MTB/RIF (cepheid.com). Finally, Richards-Kortum et al. conclude by highlighting technology gaps in current POC diagnostic tests for HIV, malaria, and tuberculosis that need to be addressed in the future.

Useful Additional Reading

- Byrnes et al. Progress in the development of paper-based diagnostics for low-resource point-of-care settings. *Bioanalysis*. 2013
- Chin et al. Microfluidics-based diagnostics of infectious diseases in the developing world. *Nature Medicine*. 2010
- Chin et al. Low-cost microdevices for point-of-care testing. In *Point-of-Care Diagnostics on a Chip*. 2013
- Sharma et al. Point-of-care diagnostics in low resource settings: present status and future role of microfluidics. *Biosensors*. 2015

Chapter 2 – Lab-on-DVD: Optical Disk Drive-Based Platforms for Point-of-Care Diagnostics

In the second chapter, Banerjee and Russom describe a novel invention, Lab-on-a-DVD, which converts a consumer DVD drive into a laser scanning microscope for diagnostic testing at the POC. The approach combines the low-cost optics of a DVD drive with centrifugal microfluidics. The authors detail bio-applicability of the platform particularly in the context of cellular-level and molecular-level diagnostics, corresponding to the various types of assays outlined by Richards-Kortum et al. in the previous chapter. At the cellular level, Lab-on-a-DVD can be used to capture and enumerate CD4+ cells from whole blood [16]. And, at the molecular level, applications range from printing DNA microarrays to conducting loop-mediated isothermal amplification of DNA in a miniaturized microfluidic platform.

Useful Additional Reading

- Fang et al. Loop-mediated isothermal amplification integrated on microfluidic chips for point-of-care quantitative detection of pathogens. *Anal. Chem.* 2010
- Gorkin et al. Centrifugal microfluidics for biomedical applications. *Lab on a Chip*. 2010
- Graydon. Bioimaging: Lab on a DVD. *Nature Photonics*. 2013
- Ramachandraiah et al. Lab-on-DVD: standard DVD drives as a novel laser scanning microscope for image based point of care diagnostics. *Lab on a Chip*. 2013

Chapter 3 – High-Performance Paper Microfluidic Malaria Test for Low-Resource Settings

In the third chapter, Liang and Fu describe an immunoassay for malaria detection in low-resource settings using paper microfluidics. The authors first discuss gold standard methods for malaria diagnosis such as high-magnification transmission light microscopy, enzyme-linked immunosorbent assay (ELISA), and lateral flow tests. Although highly sensitive, the first two methods are laboratory-based methods requiring equipment and trained personnel to perform multiple sample processing steps. Meanwhile, lateral flow tests may suffer from poor sensitivity even though they are very easy to use. Therefore, Liang and Fu propose paper microfluidics as a solution that is (a) capable of achieving high sensitivity of antigen detection and (b) appropriate for on-the-field use. The authors also provide a side-by-side comparison of gold standard methods versus paper microfluidics across a number of different parameters such as cost, assay time, assay sensitivity, ease of use, and shelf life/storage requirements. When contrasted against conventional microfluidics using glass, silicon, or polymers, the material costs for paper-based systems (e.g., using cellulose) are lower and more environmentally friendly. Specifically in this chapter, Liang and Fu focus on important non-instrumented tools (such as device geometry, dissolvable fluidic barriers, and absorbent fluidic shunts) that enable automation of multistep sample processing in paper-based devices and effectively mimic pump controls and valves used in conventional microfluidics. The authors describe the high sensitivity achieved using the paper-microfluidic platform in detecting a malaria antigen (comparable to the gold standard ELISA), thus providing an excellent example of a frugal diagnostic for detecting malaria.

Useful Additional Reading

- Li et al. A perspective on paper-based microfluidics: current status and future trends. *Biomicrofluidics*. 2012
- Mao and Huang. Microfluidic diagnostics for the developing world. *Lab on a Chip*. 2012
- Martinez et al. Diagnostics for the developing world: microfluidic paper-based analytical devices. *Anal. Chem.* 2010
- Park et al. Smartphone quantifies Salmonella from paper microfluidics. *Lab on a Chip*. 2013
- Yang et al. Paper-based microfluidic devices: emerging themes and applications. *Anal. Chem.* 2017

Chapter 4 – Microfluidics for Fast and Frugal Diagnosis of Malaria, Sepsis, and HIV/AIDS

Building upon topics raised in the first three chapters, in the fourth chapter, Warkiani and colleagues provide a comprehensive review of recent advances in microfluidic technologies at the POC for the diagnosis of malaria, HIV/AIDS, and sepsis. In particular, for the case of malaria diagnosis, the authors classify various microfluidic innovations based on five methods of detection: (a) cell deformability, (b) electrical signatures, (c) molecular analysis, (d) optical means, and (e) magnetic properties. They rank the performance of these detection methods (as "good," "fair," or "poor") across the ASSURED guidelines (see also first chapter by Richards-Kortum et al.). To conclude the chapter, Warkiani et al. list several commercially available diagnostic tools and offer a future outlook suggesting that many of the microscale assays discussed throughout the chapter can be applied to other fields related to food and the environment.

Useful Additional Reading

- Chin et al. Commercialization of microfluidic point-of-care diagnostic devices. *Lab on a Chip*. 2012
- Lee et al. Nano/microfluidics for diagnosis of infectious diseases in developing countries. *Advanced Drug Delivery Reviews*. 2010
- Lu et al. Low cost, portable detection of gold nanoparticle-labeled microfluidic immunoassay with camera cell phone. *Electrophoresis*. 2009
- Myers and Lee. Innovations in optical microfluidic technologies for point-of-care diagnostics. *Lab on a Chip*. 2008
- Rivet et al. Microfluidics for medical diagnostics and biosensors. *Chemical Engineering Science*. 2011

Chapter 5 – Perspective from Industry: AROMICS

In the fifth chapter, Plasencia builds upon the future outlook of Warkiani and colleagues in the previous chapter and introduces the readers to AROMICS (Applied Research using OMIC Sciences), a privately owned development stage pharmaceutical company that is developing a number of different diagnostic products for detecting pathogens in food and in the environment. The products include "Bugcheck" (a rapid handheld analyzer for detecting gram-positive bacteria in meat products and water for use in the food industry), "Immunolegio" (a magnetic biosensor for detection of *Legionella* in environmental water samples), "Pathfinder" (an optical biosensor for detection of pathogens in milk), "Cleanhive" (a lateral flow

device for the detection of pathogens in honey bee colonies), and "Hilysens" (a lab-on-a-chip device for disease monitoring and treatment of Lyme disease). The author also includes technical aspects and specifications for many of the devices mentioned. Lastly, the author concludes the chapter by offering readers a very unique perspective on lessons learned in the process of launching a biotech startup focused on diagnostics relevant to global health.

Useful Additional Reading

Jaroslawski and Saberwal. Case studies of innovative medical device companies from India: barriers and enablers to development. *BMC Health Services Research*. 2013

Chapter 6 – Funding Frugal Innovation in Global Health: Philanthropy, Aid, and Industry

In the sixth and final chapter from a contributing author, Neal provides readers with an excellent overview of "frugal innovation" – from the term's historical origins to its current application in the context of global health. Neal points out that "frugal innovation" as coined by Carlos Ghosn, Chairman of Renault and Nissan, likely may have initially meant "how to do more with less." But, she argues that in order to save and improve lives in developing country settings, "doing more with less" cannot necessarily imply building simpler, cheaper versions of existing solutions, but rather it must also be a call for constructing solutions with direct relevance to end consumers and with locally sourced materials. Furthermore, Neal makes two very important arguments in the chapter that may enable success of frugal innovations in the long-term: (a) the need to evaluate health impact of potential frugal diagnostics, and (b) the need to build better bridges between philanthropy and industry.

Useful Additional Reading

- Mahmoud et al. Product development priorities. In *Disease Control Priorities in Developing Countries* (2nd edition). Jamison, Breman, Measham et al. (editors). 2006
- Moran et al. The role of product development partnerships in research and development for neglected diseases. *International Health*. 2010
- Olliaro et al. A changing model for developing health products for poverty-related infectious diseases. *PLoS Neglected Tropical Diseases*. 2015

Chapter 7 – Concluding Remarks

To conclude, we provide a brief summary of some recent advances in frugal bioengineering including the hand-powered ultralow-cost paper centrifuge, which has immense potential as a diagnostic for infectious diseases such as malaria and African trypanosomiasis [17]. Ultimately, we hope that the material we have put together in this edited book can serve as a useful and informative starting point for readers to dig further into the many aspects of frugal science currently under research and development for the diagnosis of devastating infectious diseases in resource-limited developing country settings.

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13. Pai, N.P., et al.: Point-of-care testing for infectious diseases: diversity, complexity, and barriers in low- and middle-income countries. PLoS Med. 9, e1001306 (2012)
14. Rowley, C.F.: Developments in CD4 and viral load monitoring in resource-limited settings. Clin. Infect. Dis. 58, 407 (2014)
15. Fu, E., et al.: A two-dimensional paper network format that enables simple multi-step assays for use in low-resource settings in the context of malaria antigen detection. Anal. Chem. 84, 4574 (2012)
16. Ramachandraiah, H., et al.: Lab-on-DVD: standard DVD drives as a novel laser scanning microscope for image based point of care diagnostics. Lab Chip. 13, 1578 (2013)
17. Bhamla, M.S., et al.: Hand-powered ultralow-cost paper centrifuge. Nat. Biomed. Eng. 1, 0009 (2017)

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Contents

Advances in Point-of-Care Diagnostics for Infectious Disease

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1 Introduction

Modern biomedical diagnostic technologies have the potential to save many lives each year by enabling earlier and more accurate detection of infectious disease, better selection of appropriate treatment, and more effective monitoring of therapeutic response. Global health is improving, and Diagnostic testing of specimens collected from patients at the point-of-care (POC) (e.g., blood, sputum, urine, stool) can help by ensuring that patients receive appropriate treatment quickly [1, 2]. Most commercially available diagnostic systems are designed for the developed world, with an expectation of basic laboratory infrastructure such as reliable electrical power, clean water, controlled temperature storage, access to supplies and replacement parts, and trained personnel to operate and service the instruments. However, the global burden of infectious disease falls disproportionately on populations in low- and middle-income countries. In these settings, quality laboratory infrastructure is often limited and rarely extends to lower-tier health facilities such as health clinics and district hospitals [3–6]. Therefore, a major challenge in the biomedical engineering community has been to develop POC diagnostic tests that are inexpensive, effective, and minimally reliant on laboratory facilities in order to meet the needs of healthcare workers and patients in the developing world. In this chapter, we review some general requirements and considerations for diagnostic tests in resource-limited settings and highlight several key emerging technologies in this area.

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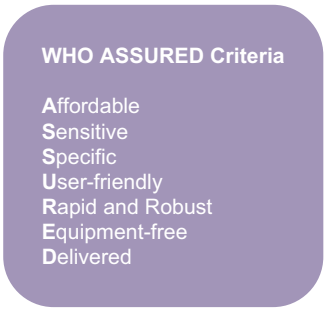

Fig. 1 ASSURED criteria set forth by the World Health Organization

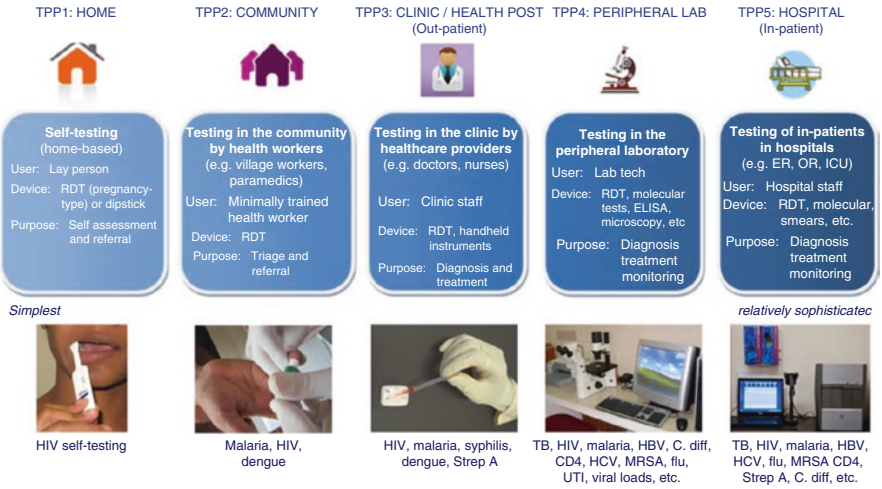

Fig. 2 Diversity of target product profiles (*TPP*), users, and settings within the spectrum of POC testing. RDT stands for rapid diagnostic test (Reprinted with permission from Pai et al. [9])

In 2004, the World Health Organization (WHO) introduced a set of seven criteria that a POC diagnostic test should meet in order to be useful in a resource-limited setting [7]. These guidelines are known as the ASSURED criteria (Fig. 1). They state that a device should be (1) affordable, (2) sensitive (few false negatives), (3) specific (few false positives), (4) user-friendly, (5) rapid and robust (e.g., does not require refrigeration), (6) equipment-free, and (7) delivered (accessible to end users). While this is a useful framework, there is considerable variation in the way these requirements are interpreted in the context of different diseases, countries, and healthcare settings [8]. POC testing can be viewed as a spectrum of technologies that are suitable for implementation in a range of settings by a variety of potential users ranging from home-based self-testing to in-hospital testing at the bedside (Fig. 2) [9]. For example, pregnancy tests and other dipstick assays that use a small volume of bodily fluid such as saliva, urine, or blood as a sample are suitable for home use and are usually the end goal for all POC diagnostics. On the other end of

the spectrum, devices such as flow cytometers and thermal cyclers are suitable for hospitals equipped with steady electricity, trained personnel, and sufficient funding. As defined by Pai et al. in a study published in *PLoS Medicine*, POC testing is the successful use of a technology to guide clinical decision-making at the POC, and this definition is consistent with many types of devices [9]. Affordability is a key and often overlooked requirement in any resource-limited setting, particularly in terms of per-test cost.

In low-income countries, nearly four in every ten deaths are among children under 15 years, while only two in every ten deaths are among people aged 70 or older. Infectious disease accounts for almost one third of all deaths in these countries and resulted in a loss of 423 million disability-adjusted life years (DALYs) worldwide in 2012 [1, 10]. Developing appropriate diagnostic measures for infectious diseases in resource-limited settings remains a considerable challenge. Several high-impact diseases that are successfully managed in high-resource settings still do not have satisfactory POC tests appropriate and affordable for low-resource settings. These include tuberculosis (TB), infant human immunodeficiency virus (HIV), and sepsis [11]. For these and other diseases, latent infection, drug resistance, and coinfection add complexity to diagnostic needs and present challenges difficult to overcome at the POC. In some cases, rapid diagnostic tests (RDTs) are available, but their performance in resource-limited settings has been unsatisfactory. For example, malaria RDTs suffer from varying performance, poor regulation, and limited adoption of diagnostic results [12].

In addition to technical barriers, the development of suitable and effective diagnostic tools for the developing world faces commercial, logistical, and organizational hurdles. In many cases, a limited commercial market means there is little incentive for private investment and thus inadequate funding to perform research and development [7, 13]. Furthermore, researchers developing tests in a high-resource setting may poorly understand the requirements of low-resource settings. Access to clinical trial sites with appropriate gold standards can be limited and unreliable, making it difficult to proceed with translation of a diagnostic. Finally, a lack of clarity in international regulatory processes impedes commercialization of diagnostic technologies. Without harmonized global regulatory standards, counterfeit and ineffective tests often appear on the market, and government health officials may lack the resources to distinguish between legitimate and counterfeit tests [12, 14–17].

The following sections discuss diagnostic tests currently being developed or implemented for POC testing for three different classes of biomarker targets in resource-limited settings: (1) cell detection tests, (2) antigen/antibody detection tests, and (3) nucleic acid tests.

2 Cell Detection Tests

Assays to detect cells at the POC fall into three main approaches: flow cytometry, static imaging, and electrical impedance. Recently, lateral flow technology has also been used. Cell-based tests are often used to detect and identify white blood cells

(WBCs) as part of a complete blood count (CBC) or in managing HIV (CD4+ count) [18–20].

Flow cytometry is frequently used for cell detection. In flow cytometry systems, cells are optically labeled and flowed through small-diameter tubing, typically with a hydrodynamic sheath. This allows cells to flow in a single-cell stream, so that the fluorescent emission, forward scattering, and side scattering of each individual cell can be measured. Forward scattering gives information about cell size, side scattering gives information about cell contents, and fluorescent labeling can be used to give information about cell surface receptors, e.g., using a fluorescently labeled anti-CD4 antibody to detect CD4+ cells. A schematic of a typical flow cytometry system is illustrated in Fig. 3. While these devices are typically used in peripheral or hospital laboratory settings, several groups have attempted to design POC flow cytometers more appropriate for health posts. Shi et al. created a small, portable flow cytometry system capable of performing a WBC count and four-part differential (lymphocyte, monocyte, neutrophil, and eosinophil) by staining the WBCs with a three-dye cocktail and detecting fluorescence [21].

Becton, Dickinson and Company (BD) has developed a benchtop flow cytometer, the BD FACSCount, which is capable of performing a CD4 count and calculating %CD4 (Fig. 4). This device requires little sample preparation, is more compact than standard flow cytometry systems, and has no need to interface with external computers [18, 22]. Other devices designed to perform a CD4 count at the POC include the Partec CyFlow miniPOC and the PointCare NOW [18]. These devices have several advantages, including accuracy, high sensitivity and specificity, and portability. However, they have high fixed costs, are hard to repair, and require sample processing, expert users, and wall power. Because of these limitations, they are likely only appropriate for peripheral or hospital labs, and work is still needed to develop flow cytometry-based cell detection systems appropriate for community clinics.

In attempts to overcome the challenge of developing affordable flow cytometry systems, static imaging systems have been proposed for cell detection at the POC. These systems commonly use a disposable cartridge to disperse and stain cells, and a reader to capture an image and process it to obtain a clinically useful result. The HemoCue WBC (Fig. 5a) uses a small disposable cartridge to automatically stain 10 μL of whole blood, which is then imaged (Fig. 5b) in a small portable reader that returns the WBC count. This device is rapid, accurate, well characterized, portable, and requires no sample preparation, making it an ideal POC cell detection system. However, it has a high per-test cost ($3/test in the United States), and it does not provide WBC differential data [23]. To address this shortcoming, HemoCue recently released the HemoCue WBC DIFF. This device is a similar imaging-based system that is capable of performing a WBC count and five-part differential (lymphocyte, neutrophil, monocyte, basophil, and eosinophil) at the POC. This test has many of the same advantages as the HemoCue WBC, but it also has a high per-test cost of about $5/test [24].

A recently developed proof-of-concept system can perform a red blood cell (RBC) count, WBC count with three-part differential, and platelet count; in this system, the whole blood is diluted and added to a cartridge that spreads the blood into a

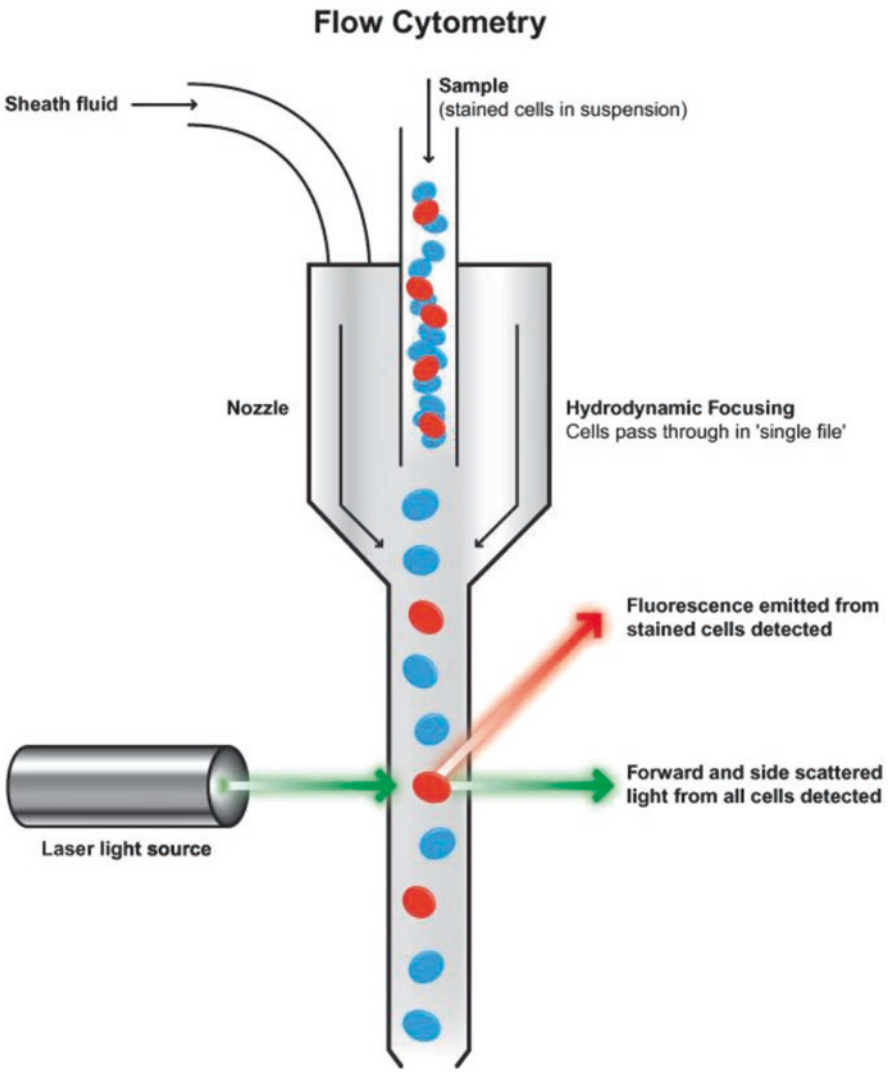

Fig. 3 Cells flow through a flow cytometer, which gathers information about each cell through a combination of forward- and side-scattered light and fluorescence emission (Image provided courtesy of Abcam Inc. Image ©2015 Abcam)

thin film. Once prepared, both fluorescence and dark-field imaging techniques are deployed to capture images used to calculate the concentrations of various blood components. However, due to high instrumentation cost and significant sample preparation requirements, this technique is not yet feasible for use at the POC [25]. Several other imaging-based systems have also been developed to perform a CD4 count, including the Alere Pima and MBio CD4 [18, 26]. These imaging-based

Fig. 4 The BD FACSCount is a compact flow cytometer capable of measuring CD4+ count and %CD4 cells (Courtesy and © Becton, Dickinson and Company. Reprinted with permission)

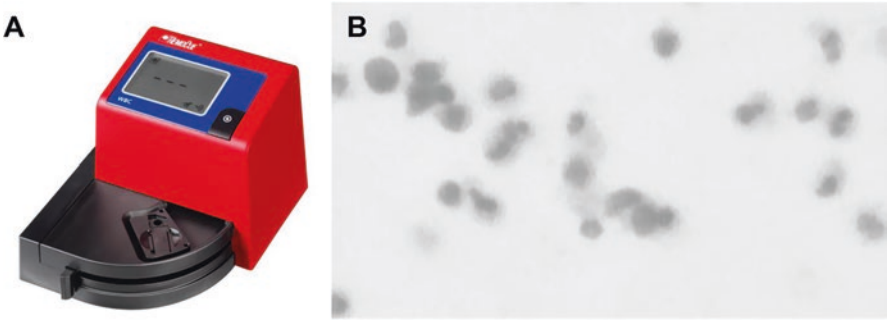

Fig. 5 (**a**) The HemoCue WBC. (**b**) Image of HemoCue WBC cartridge taken on a conventional microscope (Reprinted with permission from Osei Bimpong et al. [23])

systems return results faster than flow cytometry-based systems and typically require less user expertise. However, imaging-based systems have lower throughput and typically have a higher per-test cost than flow cytometry systems due to the cost of staining agents, antibodies, and other consumables [18].

Coulter counters are perhaps the most famous devices to use electrical impedance to count cells. In a Coulter counter, an intact cell passing through a small opening produces a measurable change in impedance across the opening. However, few devices using the Coulter principle are appropriate for low-resource settings. The Daktari CD4+ System by Daktari Diagnostics (Fig. 6) uses electrical impedance in a novel way to quantify CD4+ WBCs. The system uses antibodies in a disposable chip to capture CD4+ WBCs, discarding the RBCs and CD4-WBCs. Captured cells

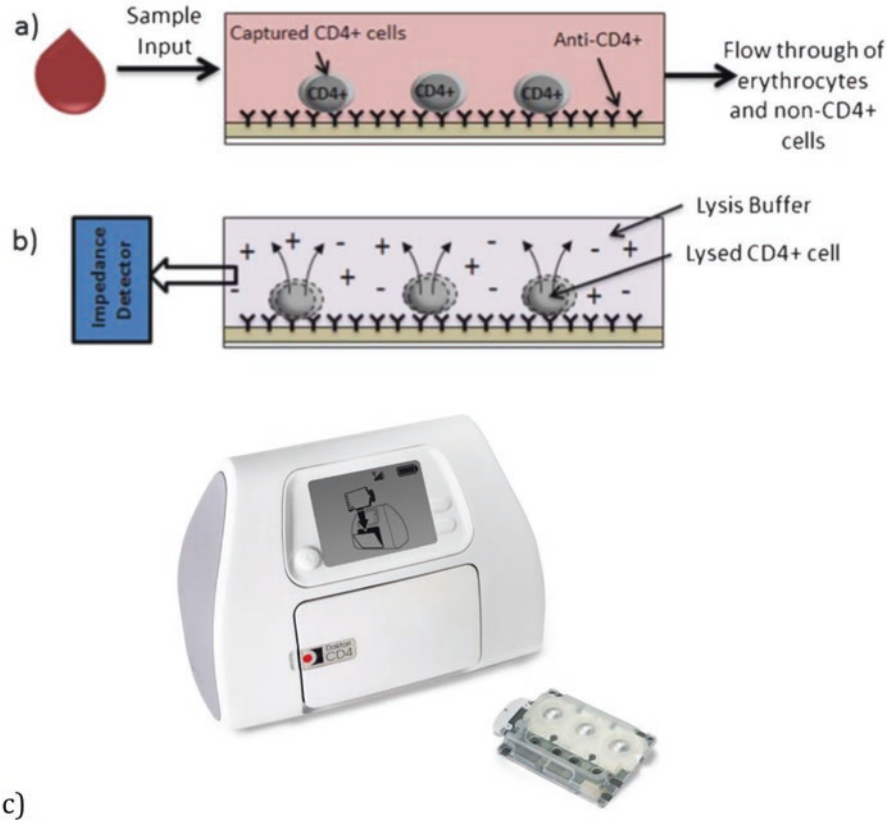

Fig. 6 The Daktari CD4+ System counts CD4+ cells using electrical impedance. (**a**, **b**) Schematic of assay process (Reprinted from [18] with permission of the Royal Society of Chemistry). (**c**) Photograph of the Daktari disposable cartridge and associated reader device (Image used with permission of Daktari Diagnostics Inc.)

are lysed with a hypotonic solution, releasing intracellular ions and changing the electrical impedance of the solution inside the chip [27]. The impedance is measured by a portable reader and is used to calculate the approximate number of CD4+ cells. Preliminary data for the Daktari system indicate good agreement with flow cytometry (100% sensitivity and 88% specificity for a cutoff of 350 cells) [28]. The measurement requires few user steps, is simple to operate, and is rapid (14 min to answer [29]) and robust (storage temperature from 2 to 40 °C) [18]. However, the high cost of the instrument (<$8000) and the disposable (<$9) will likely present difficulties to widespread use in the lowest resource settings [18], though it may be appropriate for some peripheral labs and hospital labs. By changing the antibodies present in the disposable, this approach could be used to quantify other types of cells.

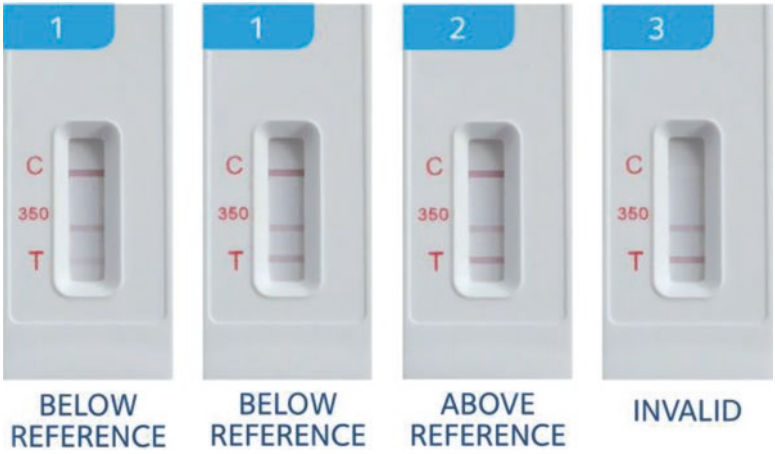

Fig. 7 The Omega Visitect CD4 system. The control line must be present for the test to be considered valid. To determine the CD4+ count, the intensity of the test line "T" is compared to the reference line "350" indicating the intensity corresponding to 350 CD4+ cells. "Below reference" is a presumptive result to offer treatment (Image used with permission from Omega Diagnostics Group PLC)

More recently, lateral flow technology has been used in cell detection tests. The Visitect CD4 strip (Fig. 7) provides a semiquantitative result to help clinicians determine the urgency of initiating antiretroviral treatment in HIV+ patients. The user adds whole blood and a buffer solution to the device. Biotinylated antibodies against CD4 bind to the cells, the cells are lysed, and the CD4-antibody complexes are captured by additional anti-CD4 antibodies at the test line. Finally, a second buffer solution releases streptavidin-labeled gold, which binds to any biotinylated anti-CD4 antibodies present at the test line as well as immobilized proteins at both the reference line and the control line. By optimizing the amount of gold released and the amount of biotin at the reference line, the test developers have calibrated the intensity of color at the reference line to match the intensity of the line when 350 CD4+ cells are present in the sample. Thus, comparing the intensity of the test line to that of the reference line indicates whether a patient's count is above or below this threshold. Alternatively, an optical reader can be used to compare the intensity of the test and control lines. The strips have good performance (100% sensitive, 83% specific) [18], are robust (storage up to 40 °C), and are simple to operate. Though the 40-min run time is longer than many POC CD4+ counters, the test can still be completed during one patient visit. The per-test cost is currently $5.00 [18]. This device meets many of the ASSURED criteria, with its main limitations being the lack of quantitative results and reduced specificity. It would be appropriate for use in communities and in health posts. The method may be generalizable to detecting any type of cell based on its surface markers.

3 Antigen/Antibody Detection Tests

Four major types of POC antigen/antibody detection tests have the potential to meet ASSURED criteria: lateral flow assays (LFAs), agglutination assays, microfluidic paper-based devices (μPADs), and 2D paper networks. Often, these diagnostics detect antigens via sensitive and specific binders, usually antibodies coupled to a colorimetric label, through either spectroscopic or enzymatic methodology. Each type of assay has benefits and disadvantages for use at the POC and may be more appropriate for qualitative, quantitative, or semiquantitative tests.

Lateral flow assays (LFAs) are becoming popular as POC diagnostic tools both in low- and high-resource settings because they are rapid, cost-effective, and easy to use. Using direct and sandwich immunochromatographic techniques, proteins of interest are captured by antibodies or aptamers and detected using colorimetric detection labels [30]. Commercially available LFAs exist for various infectious diseases, and data on these LFAs have been published including their sensitivities, specificities, detection times, and sample requirements [31]. LFAs typically run within 10–25 min and require little training to collect the sample and perform the test. While traditional LFAs such as pregnancy tests screen for a single marker, LFAs can be multiplexed using colorimetric reporter molecules and separate capture lines. Latex beads of different colors have been used in this format, and silver nanoparticles were recently used to distinguish between proteins common in dengue fever, yellow fever, and Ebola [32]. The CareStart Malaria RDT and the SD BIOLINE Dengue strips are two examples of multiplex LFAs that detect infectious agents [33–35]. While multiplexing holds promise to increase speed and lower costs of screening, nonspecific binding and crossover can lead to false-positive results [36].

The principle behind agglutination testing is to form an optically detectable cross-linked network of labeled nanoparticles when the analyte of interest is present. As depicted in Fig. 8, if the analyte is present in the sample, it will cross-link with detection reagents, forming a visually distinguishable product. If the analyte of interest is absent from the sample, the particles will not aggregate. For example, a latex agglutination test has been developed to detect antibodies to HIV [37]. In this assay, recombinant HIV protein is coated onto latex beads. These protein-coated beads are then mixed with patient serum on a card. If antibodies to the HIV protein are present, they will bind to the latex beads and form a matrix. This matrix produces visual clumps, indicating a positive test. If no antibodies are present in the patient serum, the card will lack these visual clumps, since a cross-linked matrix was not formed. Benefits of the test include its long-term stability in high heat (44 weeks at 37 °C), high sensitivity (99.72%), specificity (99.47%), and speed (10 min) [38]. User-friendliness can be further improved because technicians must mix the reagents by hand and interpret results. However, agglutination tests like this are useful for qualitative assessments in the clinic or in a peripheral lab. Clinically relevant HIV agglutination tests include capillus HIV-1/HIV-2 latex agglutination (LA) test and Serodia HIV-1/HIV-2 particle agglutination (PA) test, although LFAs are more commonly used in the field for HIV testing [39–43]. In addition, the

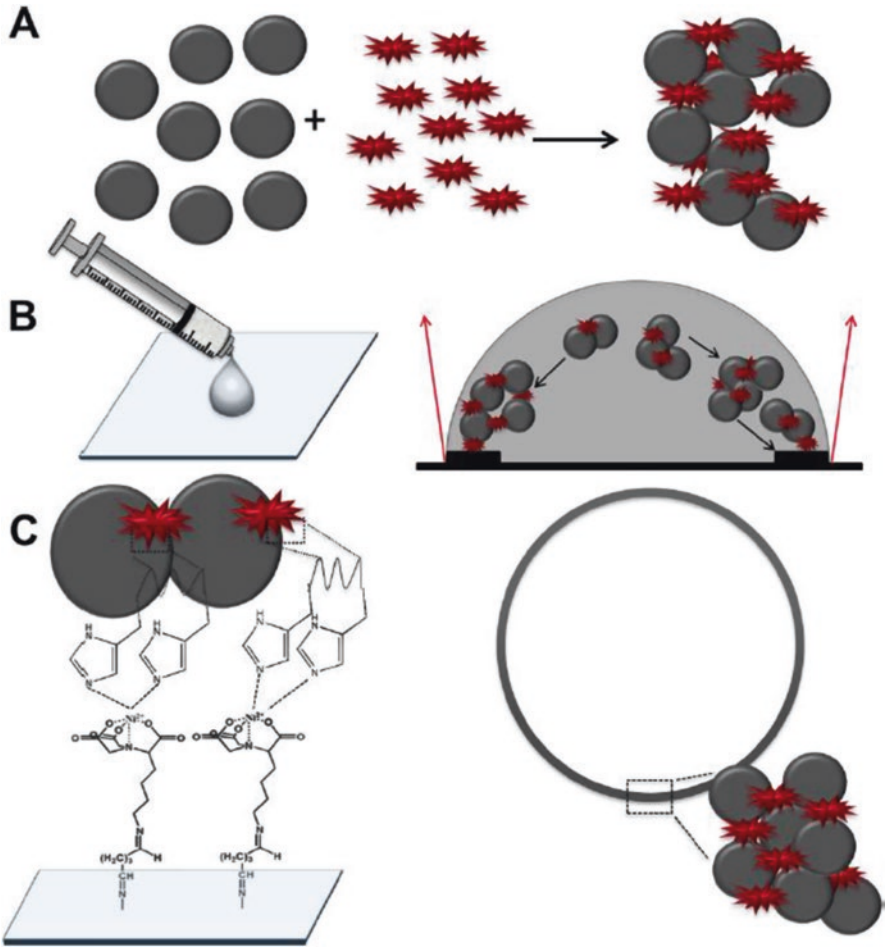

Fig. 8 (**a**) Functionalized gold nanoparticles bind to malaria HRP2 protein. (**b**) A droplet of solution is placed onto a glass slide, and complexes accumulate at the surface of the drop during drying. (**c**) After all evaporation has occurred, a spectroscopic ring with gold and bound HRP2 can be visually observed (Reprinted with permission from [75]. Copyright 2014 American Chemical Society)

ACON hepatitis C virus agglutination test, is the most used immunochromatographic rapid test for hepatitis C virus in Cameroon hospitals [44].

Antigens and antibodies can also be detected at the POC using microfluidic paper-based devices (μPADs). The Whitesides group at Harvard University has integrated hydrophobic and wax barriers into μPADs to control fluid flow and contain enzymatic and small molecule colorimetric reactions for diagnostics [45, 46]. Figure 9 shows a design to assess concentrations of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) to monitor liver function [47].

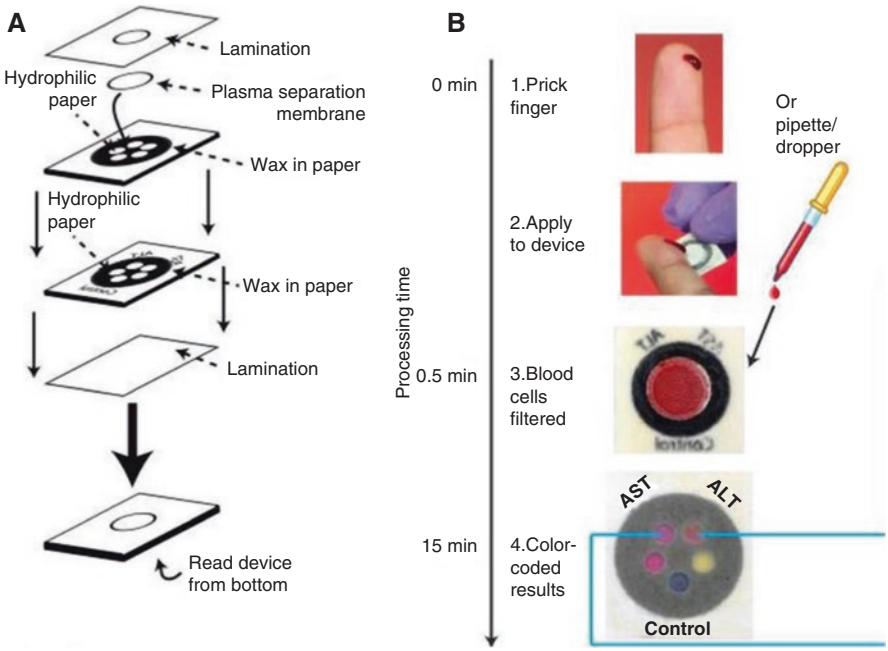

Fig. 9 (**a**) A microfluidic paper-based device (μPAD) is created by melting wax into hydrophilic paper and integrating into one device. (**b**) Application of one blood drop onto AST/ALT device allows for multiplexed diagnosis for liver functioning with minimal user input (Reprinted from [47] with permission from AAAS)

In this "lab-on-a-stamp" method developed by Whitesides and published in *Analytical Chemistry*, wax is melted into hydrophilic paper to produce a precise pattern [48]. Different buffers and reagents are added to each printed detection zone so that multiple analytes as well as negative and positive controls may be tested at once. Additionally, plasma separation membranes to separate plasma from whole blood can be incorporated to minimize sample preparation. While dried reagents are stable over several months, running tests in varying temperatures may affect assay run time, and readout in the form of color bars or color gradients may be difficult for users to interpret without proper training [49]. At room temperature, the AST/ALT assay works within 15 min using one drop of whole blood. The technology is simple, inexpensive, and rapid, and can easily be applied toward diagnostics in a clinical setting [49]. However, typically only one-step reactions can be performed. The two-dimensional format of μPADs limits the sensitivity and sophistication of diagnostics. Three-dimensional devices have been created, but no complex diagnostic reactions have yet been tested [50].

In addition to qualitative protein tests, which provide a yes/no result, semiquantitative and quantitative tests are often desirable in low-resource settings. For semiquantitative assays in which a range of concentrations is sufficient, ladder assays are a user-friendly option [51]. In a ladder assay, multiple drops of the same capture

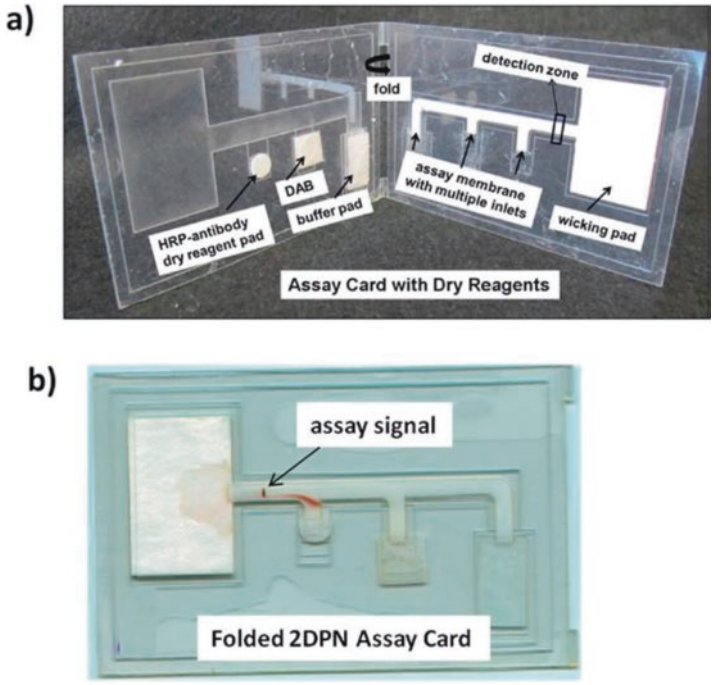

Fig. 10 An example 2D paper network is assembled (**a**) and folded, and an enzymatic reaction produces a signal in the detection zone if protein is present (**b**) (Reproduced from [54] with permission of The Royal Society of Chemistry)

agent are spotted onto nitrocellulose, and the number of spots that change color correlates to the concentration of protein in the sample. Therefore, the user simply needs to count the number of spots to determine protein level. Ladder assays have similar advantages to LFAs, including their characterization as affordable, user-friendly, rapid, and sustainable in harsh environments over long periods of time. Sensitivity and specificity vary depending on the capture and detection agents used.

LFAs are known for their simplicity and ease of use, yet often they are not as sensitive as the gold standard 96-well plate enzyme-linked immunosorbent assays (ELISAs). Traditional ELISAs provide quantitative results but are expensive and require infrastructure, machinery, and user expertise not available in many low-resource settings. Furthermore, they require at least 4 h to perform. Although paper-based 96-well zones have been developed [52], these only lower the cost of traditional ELISAs without improving ease of use.

Recently, the Yager group at the University of Washington demonstrated paper-based ELISAs in the form of two-dimensional paper networks (2DPN) that meet most of the ASSURED criteria [53]. As shown in Fig. 10, the 2DPN consists of a hydrophilic nitrocellulose network (right), a cellulose absorbent pad (right), and glass fiber pads (left) in a plastic housing. The glass fiber pads hold ELISA reagents, and studies have shown that reagents are stable when dried in these pads and stored

at 37 °C over several months [54]. To run the assay, a user simply needs to rehydrate reagent pads, add sample, and fold the plastic housing in half. Reagents are delivered sequentially via capillary action down the nitrocellulose to a detection zone, where capture reagent is spotted. No further user input is required because the cellulose absorbent pad drives capillary flow of the fluid across the nitrocellulose. As a result, any assay that can be performed in a 96-well plate can be performed in a 2DPN. This design allows for complex reactions and signal amplification mechanisms that ensure sensitive detection of protein. For example, in the paper network in Fig. 10, if malaria protein HPR2 is present in a sample, it will bind detection antibody at the capture region. The detection antibody is conjugated to horseradish peroxidase (HRP), which in the presence of hydrogen peroxide oxidizes the substrate diaminobenzidine (DAB) and produces a brown precipitate. Adding enzymatic detection greatly increases the sensitivity of the assay, and this 2DPN format can be altered to detect any infectious protein that has a comparable 96-well assay. These paper networks are sensitive, specific, low cost, robust, and relatively easy to use, although they might require sample preparation such as plasma separation, depending on the protein. The 2DPN format can be used to achieve colorimetric detection with gold enhancement, to employ enzymatic detection for greater sensitivity, and to alter and control fluid flow with sugar time delays and other mechanisms [55–57].

Lateral flow assays and two-dimensional paper networks can produce quantitative results at the POC but require readout equipment to do so accurately. Low-cost battery-powered readers have been developed to measure the strength of the colorimetric signal at the test line and report analyte concentration [58]. Readers can be used to read multiple assays, with the only consumables being batteries.

4 Nucleic Acid Tests

Nucleic acid tests (NATs) are used to diagnose infectious diseases by detecting the genomic DNA, genomic RNA, or messenger RNA of pathogens [59]. These tests are highly specific and sensitive because they recognize encoded sequences and may detect very few copies of genetic material. In addition to detecting the presence or absence of pathogenic nucleic acids, NATs may be used to monitor disease state by quantifying the concentration of pathogenic nucleic acids in a sample. NATs generally require three steps: sample preparation, amplification of target, and detection of amplicon. Recent work has focused on developing methods to perform each of these processes at the POC.

During sample preparation, the first step in a NAT, nucleic acids are extracted from their native environments (i.e., cells or viruses) and purified from sample components that may interfere with downstream processing [6]. Standard laboratory methods for nucleic acid sample preparation often involve treating the sample to lyse cells and denature proteins, binding the nucleic acids to a solid phase, washing the immobilized sample to remove impurities, and releasing the nucleic acids into a

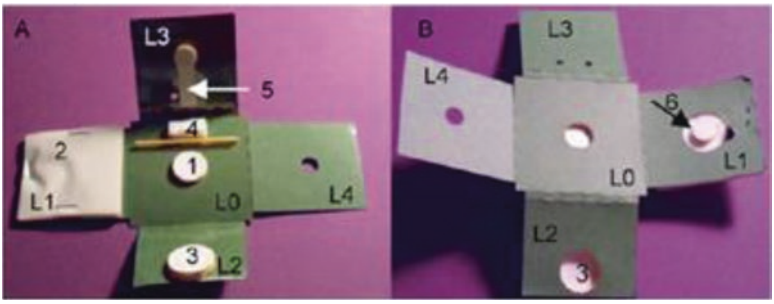

Fig. 11 Paper origami device for sample preparation of DNA. (**A**) Front side of device, (**B**) Back side of device

solution that is compatible with the next step (i.e., amplification) [6, 59]. Sample preparation may require equipment including micropipettes, a heater, a centrifuge, and a vacuum manifold.

To eliminate the need for equipment, a paper origami device has been developed for sample preparation of DNA from sputum [60]. Figure 11 shows the front side (A) and back side (B) of the device, which contains a DNA filter (1), waste absorption pad (2), sample loading cup (3), buffer storage pad (4), fluid channel (5), and a pad to facilitate contact between layers. By folding these layers in a predefined sequence, the entire process of sample preparation is performed in a simple, self-contained device.

The second step of a typical NAT is amplification of the target sequence. Because only a few copies of nucleic acid may be present in the sample, the genetic material must typically be copied 10^8–10^{13}-fold. The standard method for amplification is polymerase chain reaction (PCR), during which enzymes and complementary oligonucleotides called primers are employed to copy DNA. If RNA is the target, reverse transcription is first employed to generate cDNA. Then, during PCR, the sample is heated to melt apart double-stranded DNA, cooled below the melting temperature of the primers to allow the primers to bind to their complementary sequences on the target DNA, and heated again so that polymerase enzymes can extend the primers, creating a copy of the DNA. This process is repeated many times to generate enough DNA for detection. PCR requires pipettes, expensive thermal cycling equipment, and several hours of incubation. In order to perform amplification at the POC, researchers have developed isothermal enzymatic amplification methods [61]. These methods require a shorter incubation period and eliminate the need for thermal cycling. One example is recombinase polymerase amplification (RPA), which employs recombinase enzymes in order to unwind the DNA and anneal primers without heating to high temperatures. RPA requires only 30 min of incubation and a simple heater for operation [62].

Detection of amplified nucleic acids is often the last step in a NAT. In all NATs, the presence of amplified nucleic acids is detected by the production of an optical or electrical signal. In some assays, detection occurs simultaneously with amplification

(i.e., real-time detection). Fluorescent dyes that bind selectively to double-stranded DNA are often used to detect PCR products in real time. Fluorogenic oligonucleotide probes that bind to PCR products through base-pair complementarity offer a more specific approach. For end point detection, PCR products may be captured on a solid support, such as nitrocellulose or plastic, and detected in a sandwich assay. Probes in a sandwich assay may be conjugated to fluorophores, nanoparticles, or microparticles. Optically absorbent microparticles and gold nanoparticles are often employed in POC applications because they are visible to the unaided eye when aggregated. For example, a method has been developed for detecting the RNA products of an isothermal amplification reaction on a lateral flow strip. By dispensing many different oligonucleotide sequences in small capture spots on the strip, several RNA sequences may be detected simultaneously using blue microparticles [63, 64]. This lateral flow format is well suited for rapid visualization of results without the need for equipment.

One major hurdle for performing NATs at the POC is the requirement for heating equipment. Most heating equipment requires electricity, which is often unavailable in low-resource settings. In addition, thermal cyclers required for PCR may cost over $10,000 USD. These machines, designed for benchtop use, are large, heavy, and difficult to transport. To adapt this technology for low-resource settings, researchers have developed miniature, battery-powered thermal cyclers. Other approaches have been tailored for isothermal amplification to provide non-instrumented nucleic acid amplification (NINA) [66]. Figure 12 shows a heater that employs an exothermic chemical reaction to produce heat and a phase-change material to ensure that the temperature remains within the required range for amplification. Other work has shown that human body heat may be harnessed to power RPA reactions for POC applications [67].

Although NATs are the most sensitive and specific method available for diagnosing and monitoring infectious disease, no commercially available NAT currently meets the ASSURED criteria. So far, all NAT tests require instrumentation, failing to meet the “equipment-free” and “deliverable” criteria. The best available technologies are suitable for district hospitals but cannot be employed in more remote settings [19, 68]. One example is the SAMBA semi-Q (simple amplification-based assay semiquantitative test for HIV-1) [69]. This assay distinguishes between viral loads above and below 1000 copies/mL in 90 min. In a study conducted in London, Malawi, and Uganda, the SAMBA semi-Q was 97.3% concordant with the gold standard test, the Roche TaqMan v2. Figure 13 shows the fully integrated sample preparation and amplification instruments (Fig. 13a) and the visual readout of the test (Fig. 13b). Although easy to use, the system requires electricity and benchtop equipment, limiting its use to settings with significant infrastructure.

Another technology that may be suitable for centralized hospitals but not for community settings is the Xpert MTB/RIF, an NAT that detects TB and drug resistance in less than 2 h. While this test provides quick results and requires minimal training to run, its unit cost of $17,000 USD and POC per-test cost of $9.98 still limit its utility [70, 71]. These examples illustrate that current technology for NATs is complex and difficult to implement in low-resource settings. Further work is needed to adapt NATs for the POC.

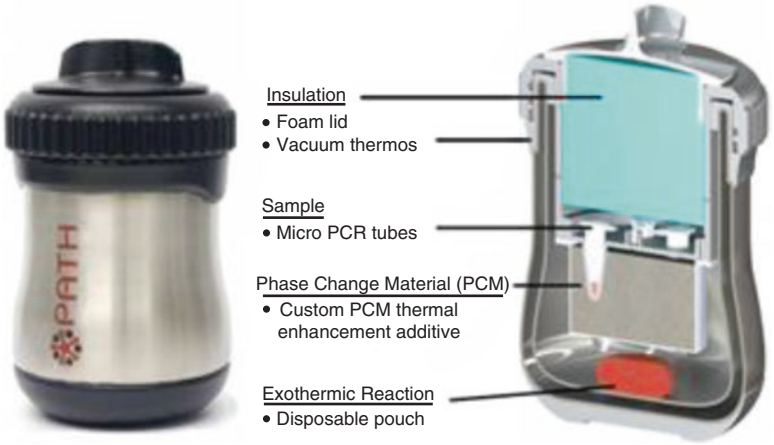

Fig. 12 A heater that uses an exothermal chemical reaction to produce the heat required for nucleic acid amplification (Reprinted from [65] (open access))

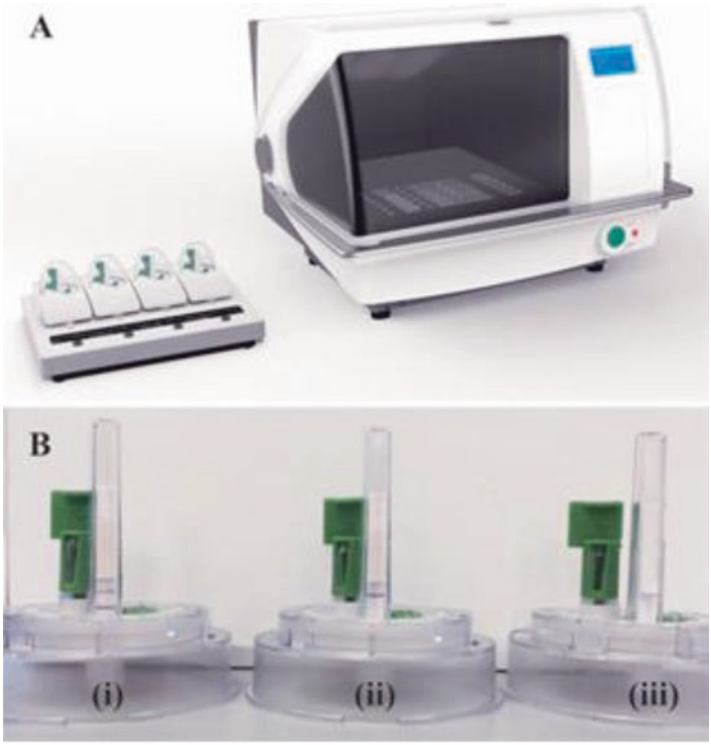

Fig. 13 Fully integrated SAMBA semi-Q sample preparation and amplification instruments with test readout. (**A**) SAMBAprep (*right*) and SAMBAamp (*left*) instruments. (**B**) SAMBAamp cartridge showing results for (i) >1,000, (ii) <1,000 copies/mL, and (iii) invalid results

5 Summary

Biomarker	Summary
Cell	Cell-based tests are used to detect white blood cells or in managing HIV infection by performing a CD4+ count. These tests are accomplished using flow cytometry, static imaging, or electrical impedance. Most cell-based detection methods still require expensive equipment, trained personnel, and a continuous electrical supply.
Antigen/antibody	These tests use antibodies and colorimetric detection strategies to identify or quantify antigens in a sample. Lateral flow assays, agglutination assays, microfluidic paper-based devices, and 2D paper networks are promising approaches due to their low-cost, quick readout time, and disposable nature. Several assays are now approved for diagnosis of infectious diseases including malaria, dengue, and HIV.
Nucleic acid	Nucleic acid tests are highly sensitive and specific but require sample preparation, amplification of target, and detection of amplicon. To avoid the high costs and energy requirements associated with melting and annealing nucleic acids, isothermal methods are currently being investigated. To detect the amplicons, fluorescent probes or aggregation with nanoparticles in a lateral flow format is currently being used.

6 Technology and Infrastructure Gaps

Over the past two decades, there has been a surge of research interest and investment from the public and private sectors in developing POC diagnostic tests that are appropriate for resource-limited settings [72]. There has been progress in some areas, notably in POC diagnostics for HIV screening, diagnosis, staging, and infant diagnosis, although viral load testing is still lacking [68]. While the Xpert MTB/RIF test is a promising advance for TB diagnostics, it will likely only be cost-effective in peripheral laboratory and hospital settings, and serological tests for active TB have been found to be neither accurate nor cost-effective [59, 68, 70, 73]. Inexpensive commercial RDTs for malaria have become widely available; however, a poor track record of quality control and quality assurance has led to distrust of test results, substantially limiting the clinical impact of this entire class of diagnostics [6, 72, 74].

Several of the major gaps in current POC diagnostic tests for resource-limited settings are listed in Table 1. It must be recognized that technology development is only one aspect of the complex process of bringing modern diagnostics to low-resource settings; issues of cost, policy guidelines, procurement, market environment, quality control, and training are critically important as well. As pointed out by Palamountain et al., diagnostic tests that are simple and easy to use by less skilled personnel are chronically misunderstood as not requiring training and ongoing monitoring. As a result, even simple POC tests are performed incorrectly and misinterpreted, leading to poor performance in field conditions [72]. Finally, while this chapter focuses on infectious disease, noncommunicable diseases (NCDs) remain the leading cause of death globally and require diagnostics suitable for

Table 1 Technology gaps in current POC diagnostic tests [6, 11, 68, 72]

Technology gap	Explanation
Detection of TB regardless of HIV status	Low-cost POC test to rapidly detect active TB regardless of HIV status; sufficiently cost-effective for implementation in community settings as well as centralized laboratories and hospitals
HIV infant diagnostics and viral load monitoring	Low-cost POC tests for early infant diagnosis and viral load monitoring of HIV; sufficiently cost-effective for implementation in community settings as well as centralized laboratories and hospitals
Quality control for malaria diagnostics	Improved quality control and quality assessment of low-cost RDTs for malaria and elimination of illegitimate and ineffective tests from the market
Sample processing technologies	Low-cost, instrument-free sample processing technologies capable of isolating target analytes (cells, proteins, nucleic acids, etc.) from clinical specimens, for use with various downstream analysis technologies

low-resource settings as well [1]. As POC technologies advance, careful attention to implementation, training, and monitoring procedures will be required in order to realize the potential of modern biomedical diagnostics to improve the health of people throughout the world.

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Lab-on-DVD: Optical Disk Drive-Based Platforms for Point-of-Care Diagnostics

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1 Introduction

Early diagnosis is an important component of infectious disease control and prevention. Although high-quality diagnostic tests are available for most of the infectious diseases, these are often not available to patients in developing countries due to the lack of laboratory infrastructure and expertise. Hence, there is currently an urgent need for accessible quality-assured diagnostics for infectious diseases of poverty and effective implementation of these tests in real-life settings. For instance, the lack of access to laboratory services for tuberculosis diagnosis is so serious in the developing world that only 25% of tuberculosis (TB) cases have a laboratory-confirmed diagnosis [1], which can lead to misdiagnosis. In most countries in Sub-Saharan Africa, malaria cases are diagnosed and reported based on purely clinical grounds without laboratory tests. This leads to overprescription of newer and more expensive drugs, which are now the treatment of choice due to resistance to the older antimalarial drugs. This is not only a waste of resources, but it will speed the development of resistance to therapies. The human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS) epidemic remains a major global public health challenge, having claimed more than 39 million lives so far and more than 34 million people living with HIV [1]. Also, HIV disproportionately affects developing countries with significant resource limitations. For instance, Sub-Saharan Africa accounts for almost 70% of the global total of new HIV infections [2]. Antiretroviral therapy (ART) has proven to substantially reduce HIV and the progression of HIV disease. The diagnostic tools used to decide whether ART should be initiated and to monitor the efficacy of ART are (1) the count of

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CD4-expressing T-cells (CD4 count), which measures the viability of the immune system, and (2) the viral load (VL), which measures the level of HIV infection in the blood. CD4 testing is the most effective and widely used tool used to decide whether and when to start ART, while the first sign that a patient is no longer under optimal treatment is a detectable VL. Hence, HIV treatment should ideally be accompanied by periodic VL monitoring. However, in resource-limited countries, VL tests are not yet available at an affordable cost, thus not part of individualized treatment. In low- to middle-income countries with limited access to specialized laboratories, treatment initiation and modifications are thus guided mainly by clinical disease progression and by CD4 cell counts, when possible. This may result in inefficient treatment as well as the development of drug-resistant viruses. Today, while HIV drug prices have decreased dramatically, thousands of HIV clinics have opened, and more than 10 million people have entered HIV treatment worldwide; the lack of appropriate HIV diagnostic tools has become a critical bottleneck preventing the expansion of efforts to address the AIDS pandemic. To reverse the AIDS pandemic, UNAIDS proposed recently a new set of global targets for scaling-up treatment known as the 90-90-90 targets [3], which call for a scale-up of HIV testing so that 90% of people with HIV are aware of their infection, 90% of people diagnosed with HIV are linked to antiretroviral treatment (ART) and 90% of those on ART adhere and have undetectable levels of HIV in their blood [3]. If all low- and middle-income countries were on track to reach these targets, there would be just over 23 million people on ART by 2018 [3]. To reach these goals, the uptake of CD4 tests and viral load tests will be critical.

For resource-limited settings, it is often suggested that diagnostic tests for use at the point of patient care should meet the *ASSURED* criteria for the ideal rapid test. The ASSURED criteria, coined by WHO, are as follows: *a*ffordable by those at risk of infection, *s*ensitive (few false negatives), *s*pecific (few false positives), *u*ser-friendly (simple to perform and requiring minimal training), *r*apid (to enable treatment at first visit) and *r*obust (does not require refrigerated storage), *e*quipment-free (minimum equipment) and *d*elivered to those who need it. Table 1 shows how a suggested working list of functional requirements for such a device could potentially translate, for instance, into HIV diagnostics. As will be described in the next section, this list of functional requirements can be met in the long term using a technology originally developed for the DVD industry [4].

The challenge of developing ASSURED tests is that POC tests are expected to give laboratory-quality test results but should be simple to perform, requiring minimal training of users. Among new emerging technologies, microfluidic lab-on-a-chip devices have the potential to enable the miniaturization of complex reaction processes into small, self-contained packages. The most attractive features of microscale approaches for blood analysis include the requirement for only microliter blood volumes obtainable without venepuncture and simple operation by minimally trained personnel. Several distinct components are part of any device for blood analysis: sample collection and introduction, sample preparation, analyte separation, analyte detection and signal processing [5, 6]. Practical applications in

Table 1 List of functional requirements for HIV diagnostic in resource-limited settings

Characteristic	Target requirement
Ease of use	Sample in, pos/neg result out
Analytical sensitivity	<1000 copies/mL
Sample size	200uL
Turnaround time	1–2 h
Operator time	Minimal
Sample type	Whole blood
Instrument form factor	Portable
Operating conditions	35C, 80–100% humidity, dusty, transportable on unpaved roads
Electrical requirements	Can run on battery as a backup electrical supply
Controls	Included, positive and negative
Biosafety	Waste contained, no risk to operator outside of biosafety cabinet
Clinical sensitivity	Better than 95%
High-volume test cost	<5 $/sample
High-volume instrument cost	<200 $

point-of-care diagnosis, particularly in global health diagnostics, require that each of these steps be miniaturized and integrated. Traditional paper-based lateral flow assay (LFA) is the most widely used commercially available POC diagnostic assay. However, low sensitivity, high variability and lack of multiplexing ability have narrowed the range of applicability of such POC diagnostic devices. The centrifugal (CD) microfluidic platform on the other hand, with its intrinsic pumping and valving mechanisms, is an ideal choice for POC system [7–9]. CD microfluidics utilizes the platform's intrinsic centrifugal pumping and valving mechanisms, which are ideal for non-contact liquid handling. The advantages of CD microfluidics include external pump-free fluid propulsion, minimal amount of instrumentation and a dynamically variable rotational frequency for manipulating fluidics [10]. These features make them particularly beneficial for biological applications as the biofluids being tested can be processed in an isolated system, thus minimizing their contamination chances [10]. Several fluidic functions have been implemented on the centrifugal microfluidic devices which include capillary valving [11], siphoning [12], capillary priming [7], metering [13], fluid mixing [14] and sedimentation-based methods [15] all of which can be carried out on such disc readers by the addition of an extra fluidic layer. While centrifugal microfluidics systems are adept at integrating sample preparation steps with bioanalyses, integrated detection is not a simple task when dealing with the complexity of a rotating platform. The rotating disc must be very flat, and the detecting mechanism must be capable of self-alignment with a particular spot on the disc surface. Nevertheless, some platforms have reduced this complexity by performing detection while stationary or have reverse engineered a disposable disc to match with the detection components of an existing commercial system. In this book chapter, we will describe the use of optical drives for detection of biomolecules.

Three decades of significant progress in compact disc (CD)- and digital versatile disc (DVD)-based technologies have paved the way for application area in the biomedical field. The utilization of standard optical CD and DVD pickup heads for detection is attractive. Li and coworkers [16] showed that an unmodified CD can be used to read out different binding assays by capturing the error signals produced due to biomolecule/nanoparticle conjugates which blocks the laser beam striking the surface of the CD. The detected error signal determined physical location or spot of the conjugate. Based on a similar principle, Morais et al. [17] had earlier demonstrated an unmodified CD player to read immunoassays by using low reflectivity discs coated on both sides. Our group has taken it one more step further and developed a fully integrated Lab-on-DVD platform by the marriage of optical disc and CD microfluidics for applications in cellular and molecular diagnostics [4].

2 Working Principle of Optical Drive Systems

The function of a DVD player can be understood by looking at the record players of the middle twentieth century. It had a visible needle trying to read the grooves on the vinyl. The CD, DVD and the more recent Blu-ray drives are based on the same principle. In all of these classes of storage media, the information is coded as a series of tiny pits in the disk (Fig. 1). The pits are organized in the form of a spiral track similar to vinyl records. However, as compared to vinyl records, in CD, DVD and Blu-ray players, a laser is used to interpret these pits as binary code. When a smooth surface is read, the machine interprets the data point as a 0. When a pit is encountered, the data point is read as a 1. Standard CD, DVD and Blu-ray substrates have 1.2-mm-thick multilayer structure that contains all the operational information that a standard optical drive requires to read the disc. The structure comprises of a grooved polycarbonate (PC) substrate with encoded digital information covered by a thin metallic film to provide high reflectivity and a protective lacquer of polymer or a hard-coating resin to protect the disc. The total thicknesses of the discs are 1.2 mm. The key difference that makes DVDs superior to CDs and also Blu-ray superior to DVDs for storage is the laser that is used to read and create the pits. DVDs use a laser of 650 nm that can place pits more densely on the surfaces of the disks as compared to CDs (740 nm). Blu-ray discs have five to ten times higher capacity than DVDs as they use a blue laser of shorter wavelength (405 nm) and improved lens specifications leading to a much smaller focus laser beam that allows the storage of much smaller and higher-density pits on the disc. As can be seen in Fig. 1a, the encoded information of a CD is on top (1.1 mm), while for DVD it is at the middle (0.6 mm), and for Blu-ray it is at the bottom (0.1 mm) of the PC substrate. The optics of the drive is optimized to match the position of the encoded information. For DVD, it is possible to use a bottom layer consisting of a 0.6-mm-thick PC with injection-moulded spiral microguide (0.74 mm track pitch) to guide the detector laser (λ = 650 nm) and replace the 0.6-mm-thick top layer of PC substrate with fluidic channels. This would create a multilayer disc with the encoded information on one side and the fluidic channels on the other side, which has the same thickness as a CD (1.2 mm) [4].

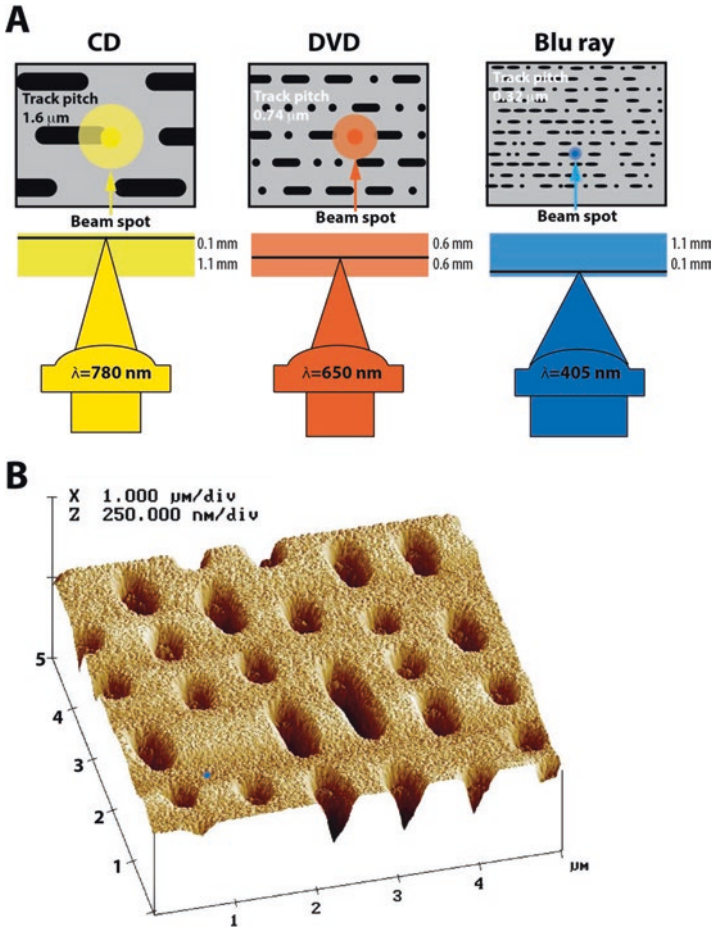

Fig. 1 (**a**) Optical reading principle of CD, DVD and Blu-ray discs. The information is coded as a series of tiny pits in the disks. The pitch track, minimum pit length and hence the storage density are different between the discs. In addition to the different laser wavelength used, the data layer on a Blu-ray disc (at 0.1 mm distance) is placed closer to the laser lens than in DVD (at 0.6 mm distance) and CD (at 1.1 mm distance), which allows high storage densities. (**b**) An AFM image of a DVD surface showing injection-moulded tiny pits

2.1 Detection Principles

Taking advantage of the optical phenomenon occurring on a disc substrate, diagnostic biosensors have been designed based on advanced spectroscopic readout into two main classes: error rate reflective-based detection and modified optical disks for transmission-based detection. The first class, error rate based, does not require any modification of the discs. In this case, a cleverly written algorithm to read the error signals or modifying the DVD disc can do the trick. One of the first efforts in this

direction was capturing streptavidin molecules in selected areas of the disc where biotin target molecules were immobilized. The disc is read in a standard DVD drive, and the error rate pre- and post-incubation is measured and correlated [18]. This was followed by silver staining a CD surface to study biomolecular binding events [19]. The important feature of this technique is the fact that no hardware modification of the optical readout or discs is needed. However, a major bottleneck is sample handling, since the introduction of microfluidic features disrupts the track irradiation and aborts the process using discs and drives for reflection measurements. Furthermore, different drive manufacturer has its own proprietary firmware for error correction, which results in difference in sensitivity across different optical drives. A second method of reading out bioassays on ODD is modifying the discs somehow, to allow transmission of light. Here, semi-transparent disc is used where an elegance balance is achieved between the lights needed for the discs to be read by standard drive while allowing transmission of the incident laser radiation for detection using photodiodes mounted above the drive. Maquieira and coworkers used low reflectivity-based modified CDs along with a planar photodiode to detect pesticides, cancer markers and herbicides in water [17, 20, 21]. Using similar principle, we developed a lab-on-DVD platform by combining a bottom 0.6-mm-semi-transparent PC (for tracking) and 0.6 mm fluidic PC and demonstrated sub-micrometre image resolution using a modified commercial DVD drive [4].

3 Lab-on-DVD Platform

Towards fulfilling the ASSURED criteria, we have developed a truly integrated lab-on-DVD system that combines low-cost optics of a DVD drive with inherent well-controlled liquid handling in CD microfluidics [4]. Our novel approach has two technology cornerstones: a modified consumer DVD reader for molecular "imaging", equipped with integrated functionality to enable control of liquid handling, and optical disks with integrated microfluidics enabling integrated sample processing and on-board molecular capture reagents. The detection principle is based on absorbance change and/or scattering of captured biomolecules onto the DVD surface detected by an external photodiode (Fig. 2). The key difference compared to a standard DVD drive is the use of a second photodiode (D2) that is distal to the laser source with reference to the disc. This approach enables monitoring of reactions both in the microfluidic channel structure placed above the reflector and of objects placed within the focal plane. The fundamental principle of data generation using the prototype system is based on end user instructing (via the graphical user interface) the DVD drive to read from a known start position and following the spiral track (groove) of the disc, while the D2 captures the amount of light transmitted through the disc. Signal wave-based processing of captured data generates a two-dimensional image that is subsequently analysed. Two different detection modes can be used: absorbance mode which involves measuring the change in colour of the sample over time and scattering mode based on imaging captured biomolecules on the surface of DVD (Fig. 3).

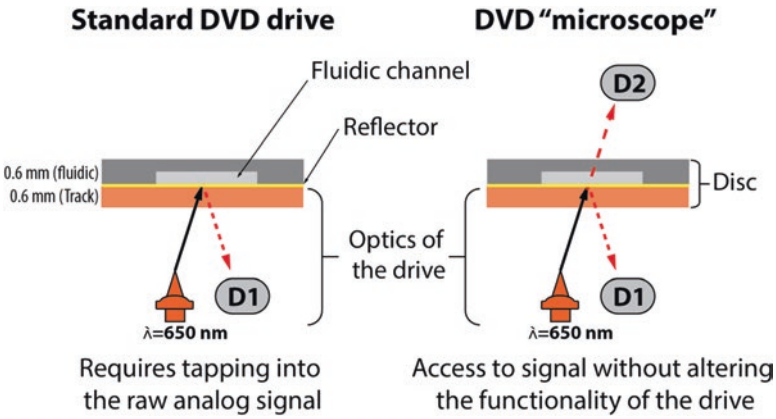

Fig. 2 Comparison of readout principles of standard DVD drive (*left*) and the prototype system (*right*). D1 refers to the normal detector of the optical pickup unit of the DVD drive, while D2 represents the series of photodetectors added to the DVD microscope prototype drive

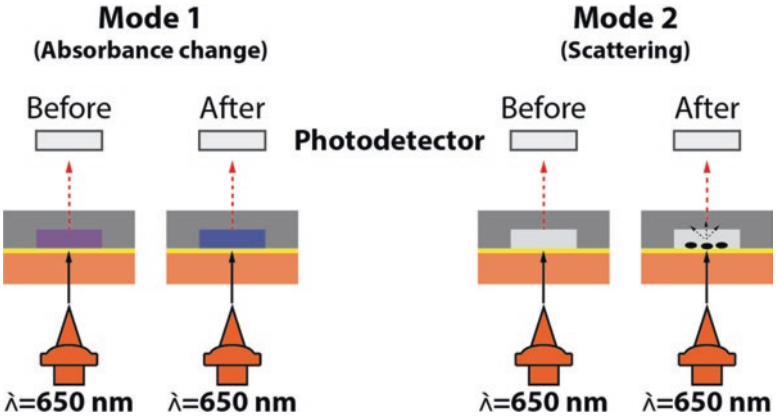

Fig. 3 Two modes of image generation by the DVD microscope: the *left-hand side* showing the principle of changed absorbance while the *right-hand side* showing the image generation process by the microscope using scattered light

3.1 Absorbance Mode

The lab-on-DVD platform enables a unique absorbance measurement option, which allows detecting the absorbance change in the fluid in a channel in real time. The disc channels contain different sample that may have variable absorbance. With disc rotation, the channels pass in turn under the detector, which detects the reduction in

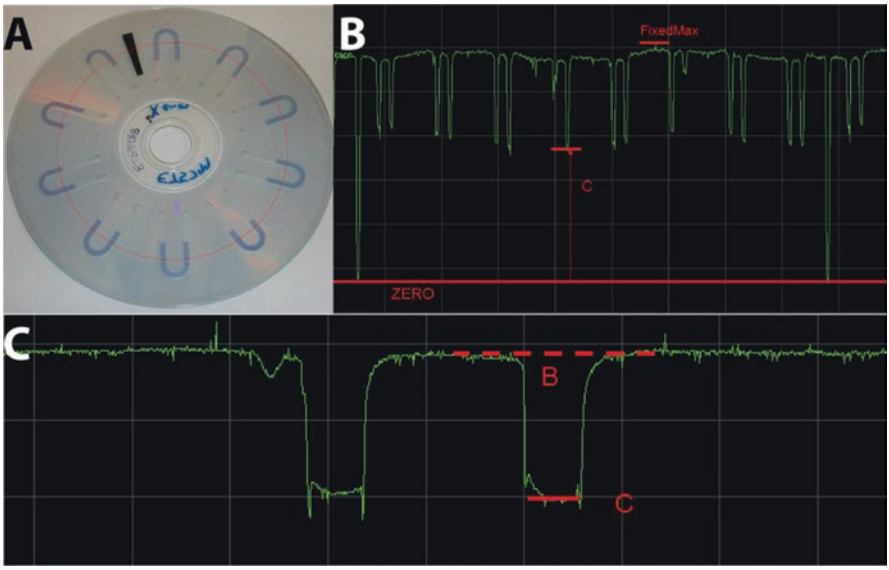

Fig. 4 Absorbance mode: example of the absorbance waveforms generated with the peaks indicating the legs on the disc. (**a**) DVD with U-shaped channels. (**b**, **c**) Waveform is generated, and the absorbance is calculated inside each channel leg

light passing through the absorbent fluid. The result is a waveform, as shown in Fig. 4. A tape is attached on top of the disc to trigger the software to start counting the channels. There are three methods of defining the absorbance function that is plotted: relative, baseline and absolute methods. Relative absorbance is a time-based change in absorbance starting from time at 0 s. Baseline methods differentiate between darker and lighter channels as a function of the maximum signal at $t = 0$. Absolute absorbance uses the background absorbance on either side of the U-shaped channel as reference.

3.2 Imaging Mode

The imaging mode uses the absorbance function to generate a bitmap image or a series of bitmap images by plotting each track of the DVD spiral as a row. A black tape on the surface of the disc beside the microfluidic chamber acts as a trigger to initiate the data stream capture. Usually some jitter correction feature is also implemented in which the rows of the bitmap image are moved laterally relative to each other to give a clearer image. Figure 5 shows an example of the scattered images generated in the imaging mode.

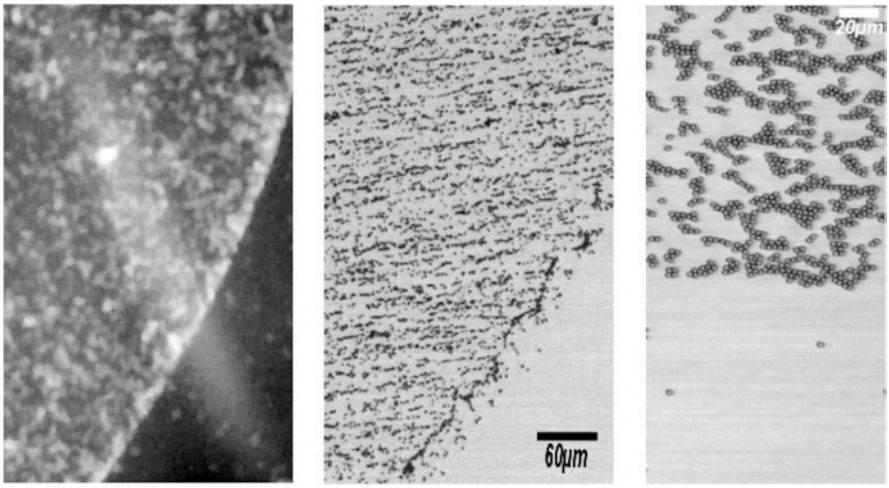

Fig. 5 Scattering mode of the DVD microscope: example of imaging of scattering objects on disc surface with the DVD drive reading track by track to image of microspheres. *Left* shows USB microscope image, while the *middle* (low-resolution) and *right* (high-resolution) images are obtained using the DVD drive

3.3 Additional Hardware Modifications Converting a Drive into a Bioanalytical Prototype

A standard low-cost (~$20–40) DVD drive is used as the core of the system. The key modifications and functionalities that have been added to the system include a series of photodiodes and a bespoke controlling board to collect the transmitted light; temperature detectors, heaters and fans to control the temperature according to user instructions; an extra inbuilt motor to provide a fine level of control of the rotation; a PCB and associated software that integrate and control all functionalities of the drive; and a custom software to process and analyse the data, including a graphical user interface for user presentation (Fig. 6). The PCB is used to provide integration and control of the add-on functionalities of the drive. The key functionalities of the PCB include analogue to digital conversion (ADC), connectivity to the photodetector (PD) board, control of PID and other temperature-regulating components and connectivity to PC and drive. The system provides essential functionalities of a core laboratory: liquid handling, centrifugation, temperature control, real-time monitoring of reactions and high-resolution, microscope-type imaging.

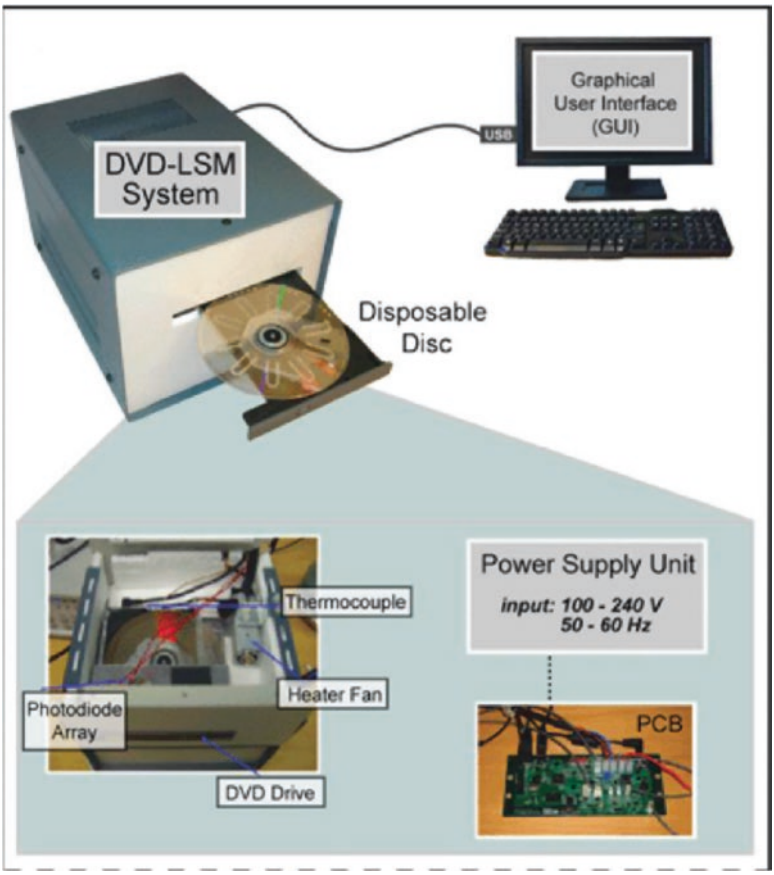

Fig. 6 Integrated lab-on-DVD system: it comprises of analogue to digital conversion (ADC), additional photodiode array to capture scattered image, modified PCB unit to power up the additional components and connectivity to a graphical user interface (GUI) on the PC (Reproduced with permission from Ref. [4])

4 Bioapplications

The driving force for the lab-on-DVD platform stems from a need for low-cost and all-integrated solutions for advanced molecular and cellular diagnostics. The DVD platform has an in-built advantage where rotational forces provide the energy needed for liquid transfer. Unique advantages differentiating it from similar technical solutions include (i) DVD readers having the capability to read nanometre-sized features at a speed of >8 m/s, a property that can be utilized to generate high-quality images with extremely low-cost optics, and (ii) utilizing DVD production lines to manufacture the disks at competitive prices. Importantly, the low system cost

enables decentralization of bioassays that normally are performed at central labs. The applications include cellular assay based on either direct visualization or enhanced visualization using secondary labels such as microspheres, immunoassays including competitive assays and ELISA-based assays and nucleic acid assays based on hybridization and/or continuous real-time monitoring of isothermal amplification protocols. Here below, we will describe cellular and molecular assays.

4.1 Cellular Diagnostics

Enumeration of CD4-positive cells (CD4+) provides a measure of the condition of the immune system. This is especially important for HIV-infected patients for whom CD4 counting is an essential tool for diagnosis and monitoring of the development of the infection. We applied the DVD platform for CD4 counting. Prior to bonding, the DVD half discs are coated with a thin layer of SiO2 for surface functionalization based on proven silane chemistry. The DVD half discs were first surface modified with an epoxy-silane chemistry prior to UV bonding as a batch consisting of 25 discs at a time, which enables scale-up production. After bonding, the channels were functionalized by filling NeutrAvidin solution followed by biotinylated anti-CD4 antibodies. Figure 7 shows specific cell capture on the DVD system. To enhance the amount of scattered light, a staining procedure was used. Small volumes of blood (<10 μL) can be rapidly processed using this platform. In addition, integrated "imaging" of captured cells literally takes minutes using the DVD platform, keeping the total analysis time to less than 10 min [4]. Future direction in this work will include optimization of the surface geometry and integration of the sample processing and washing steps.

4.2 Molecular Diagnostics

DNA microarrays are widely used for gene expression analysis and genotyping for diagnostics of infectious diseases. Conventional microarrays have several disadvantages such as long incubation time, slow diffusion kinetics and sample volume. In addition, expensive and bulky scanners are typically used to image the spots. This has limited the widespread of the technology towards resource-limited settings. Merging microarray technology and microfluidics holds great promise in the development of POC diagnostics. In particular, the lab-on-DVD platform, with its intrinsic pumping and valving mechanisms and integrated detection, is an ideal choice for such an integrated point-of-care system. In this study, we apply the integrated DVD platform for the development of microarray-based DNA analysis (Fig. 8). DNA arrays were printed on the silane-epoxy-modified bottom DVD ROM, and the semi-transparent DVD substrate was bonded to the top substrate consisting of pressure-sensitive adhesive with fluidic structures. Next, we performed DNA hybridization

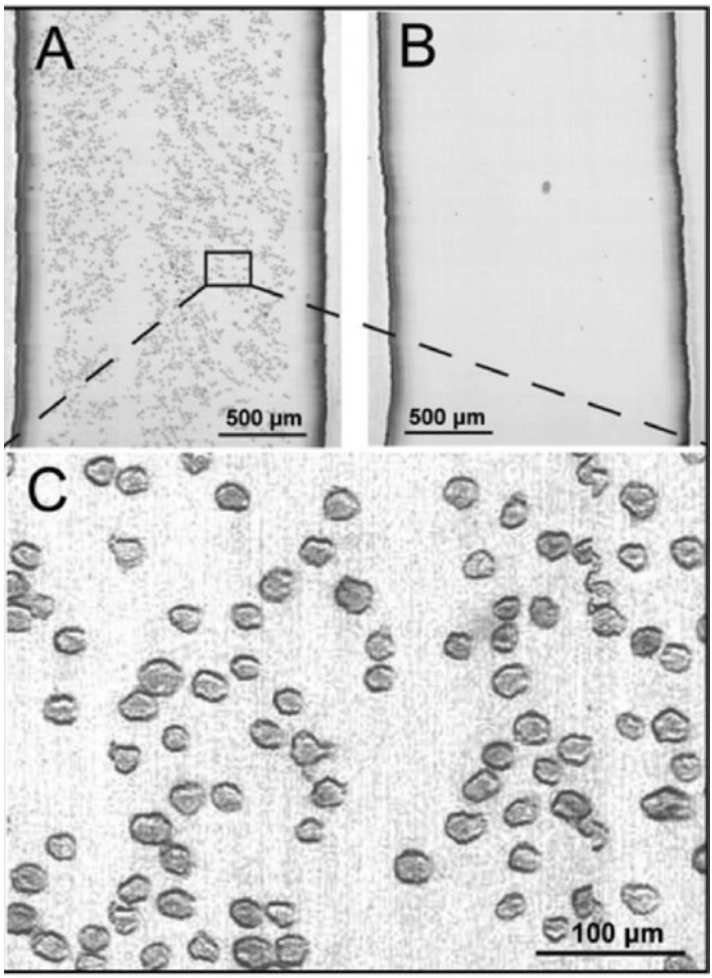

Fig. 7 Specific cell capture on DVD surface: (**a**) U channel on the DVD substrate filled with anti-CD4 bodies. (**b**) U channel on the DVD substrate filled with anti-CD8 substrate. Almost no cells are captured here which shows that the nonspecific binding is very low (**c**) (zoomed in image of **a**) (Reproduced with permission from Ref. [4])

followed by silver-gold enhancement chemistry for visualization of the microarray spots. High-quality, low-background "images" of microarray spots were achieved inside the channels of DVD surface, available for automated analysis (Fig. 8).

Most nucleic acid-based POC tests are not amenable to resource-limited settings because they do not meet the WHO's ASSURED requirements. For instance, the gold standard amplification, PCR, has the desired high sensitivity and target specificity, providing accurate quantification, but the method is costly and time-consuming and often requires skilled technicians and laboratory settings. Alternatively, DNA and RNA analysis can be performed by isothermal methods. Of these methods,

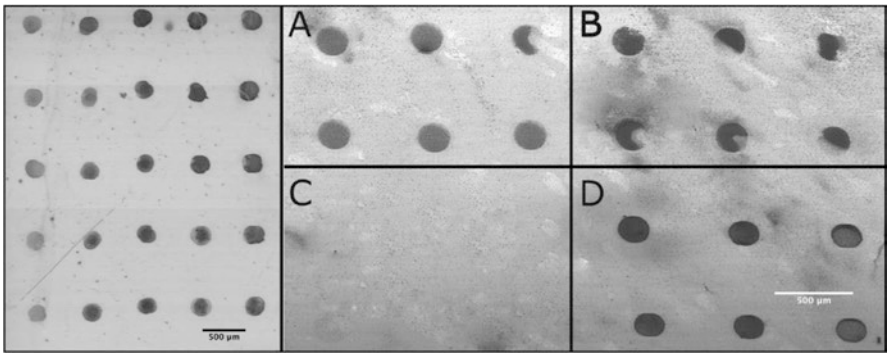

Fig. 8 DVD image of microarray spots. (*Left*) streptavidin-gold spotted on DVD at different dilutions and developed with gold-silver enhancement. The strep-gold dilution series spotted on DVD at different dilutions were (*left* to *right*) 1/200, 1/100, 1/50, 1/20 and 1/10, respectively. (*Right*) DNA hybridization inside the channel structures. (**a**) The sample, (**b**) positive control, (**c**) negative control (strep-HRP) and (**d**) streptavidin-gold spots. All the protocol steps (DNA hybridization, washing, silver enhancement) were performed in a plug-flow microfluidic system

loop-mediated isothermal amplification (LAMP) is an ideal technique for use in miniaturized microfluidic systems [23, 24]. While LAMP assays are often detected using absorbance or fluorescence, we demonstrate an integrated system for LAMP assays through the detection of precipitate formation using the DVD platform (Fig. 9). Pyrophosphate (PPi) is released during polymerase-mediated nucleotide incorporation into the DNA template during extension reaction. A metal, such as magnesium, can then precipitate the released PPi. This system was initially verified using a commercial LAMP kit, to both validate the integrated heating and imaging processes and also explore the use of precipitate formation as a novel indicator of DNA amplification, as shown in Fig. 9 [22]. This low-cost integrated system automates the sedimentation process and scattering-based detection of accumulated precipitate, all while maintaining the constant temperatures needed for LAMP analysis. Continued work is being performed to further optimize the assay for HIV-1 and tuberculosis-specific assays to enable semi-quantitative measurements.

5 Conclusions

Taking advantage of more than 30 years of development in the optical disk industry, we describe here the combination of low-cost optics of a DVD drive with inherent well-controlled liquid handling in CD microfluidics. We have developed an integrated DVD platform, with proven sub-μ image resolution, and applied it to isolate CD4 cells from the blood for HIV diagnostics. The DVD platform is a versatile sample preparation and imaging device for resource-limited settings. A number of assays, which are typically carried out in laboratory settings, can be outsourced with

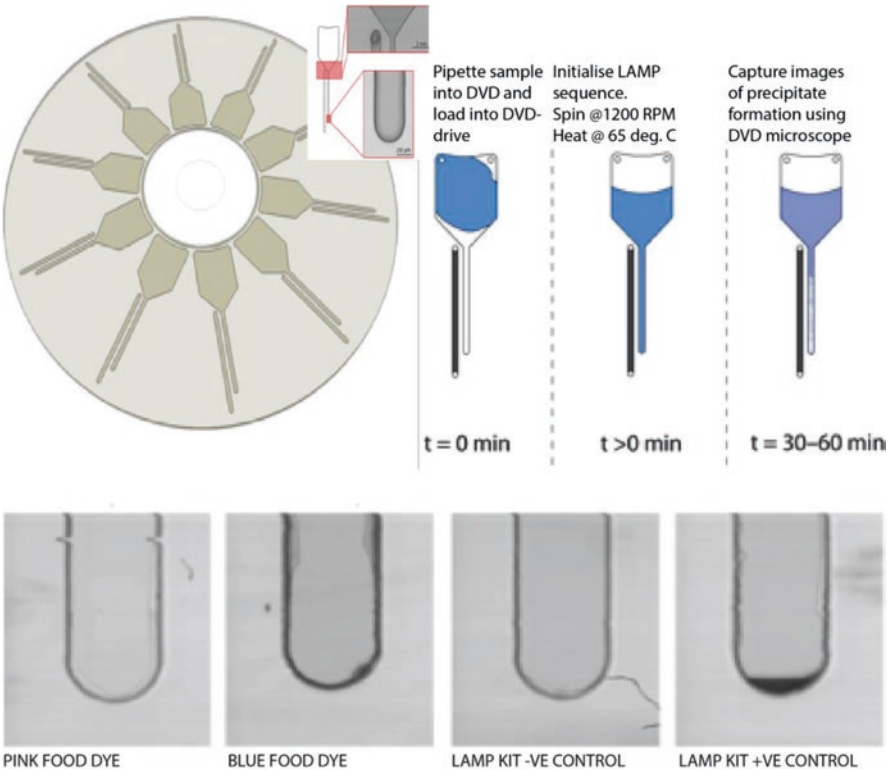

Fig. 9 Salt precipitate-based LAMP detection: the upper section shows the principle and the disc design. Inset is DVD "image". The three basic steps in LAMP precipitate formation and capture are shown on the right of the upper section. Lower section shows the captured DVD images as proof of principle for LAMP detection using salt precipitate with the LAMP positive control showing distinct formation of precipitate (Reproduced with permission from Ref. [22])

this platform, adding a whole new dimension to microfluidics. The DVD platform meets most of the requirements according to the ASSURED criteria as an ideal detection unit for resource-limited settings.

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High-Performance Paper Microfluidic Malaria Test for Low-Resource Settings

Tinny Liang and Elain Fu

1 Introduction and Motivation

Malaria ranks in the top three for infectious disease burden worldwide [1]. Approximately two million deaths per year are attributed to malaria infection, with about 60% of these deaths in children under the age of 5 years [2, 3]. A disproportionate number of these deaths occur in the developing world [2, 3].

Many of the deaths due to malaria would likely be preventable if an appropriate tool for accurate diagnosis was available for use. Currently, clinical treatment in the developing world is initiated based on patient symptoms and regional prevalence, a practice referred to as syndrome-based management [3]. Though syndrome-based management can be effective for treating diseases with distinct symptoms, multiple diseases may present with similar symptoms. For example, malaria, pneumonia, influenza, and dengue have the common symptom of high fever. Using only syndrome-based management, a child presenting with early-onset fever in malaria-endemic regions might be automatically treated with antimalarial drugs. This child could have a fever from another infection, which if not treated appropriately, could result in disability or death [3]. In addition, the misuse of antimalarial drugs is one of the main culprits behind the increase in antimalarial drug resistance and the transition to expensive combination therapies [4]. Timely, accurate diagnosis at the point of care (POC) has the potential to reduce the global disease burden, by improving health outcomes and decreasing healthcare costs on the population and individual levels.

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Current gold standard diagnostic tests for malaria require laboratory facilities that are often not accessible to patients in the developing world. These laboratory-based tests require a high level of resources that typically include expensive instrumentation, trained personnel to perform or maintain that instrumentation, facilities with electricity and clean water, and local infrastructure for maintaining cold chain and supporting communication between the patient, the caregiver, and the laboratory [3]. The high level of resources required to support laboratory-based testing is not commonly available in the developing world [3]. Thus, for any POC device to have functional value, it must be compatible with the constraints presented by the existing healthcare infrastructure [3]. In 2006, Peeling et al. developed general guidelines for the development of appropriate POC diagnostic tests. These guidelines state that appropriate diagnostics should be affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, and deliverable (ASSURED) [5].

Designing devices to be appropriate for use in the lowest-resource settings translates to directly addressing the ASSURED criteria in a process of frugal innovation in bioengineering. In this chapter, we describe an excellent example of frugal innovation in bioengineering, the use of paper microfluidic tools for the high-performance detection of a malaria analyte in a disposable device.

2 Current Methods and Limitations

2.1 Laboratory-Based Tests: Microscopy and ELISA

The gold standard for malaria diagnosis is laboratory-based, high-magnification transmission light microscopy. In this diagnostic method, a stained blood smear is used to identify malaria parasite levels [6]. Though capable of detecting low levels of the parasite, the cost of the equipment and the requirement for trained personnel to perform the assessment limit applicability in the developing world [6]. More recently, laboratory-based enzyme-linked immunosorbent assay (ELISA) has been developed for malaria diagnosis in high-resource settings [7–9]. The method requires equipment and also trained personnel, in this case to perform multiple sample processing steps that include a lengthy incubation and numerous rinses and labeling steps. Though these laboratory-based diagnostic methods are not accessible to many in low-resource settings, these established methods provide useful performance metrics to work toward as new devices are developed and will be revisited later in the chapter.

2.2 Lateral Flow Tests

Lateral flow tests (LFT) are simple bioassays that have been used in low-resource settings for decades [10]. In the well-known pregnancy LFT, the user adds a urine sample to the device, and fluid transport occurs due to capillary flow in the porous,

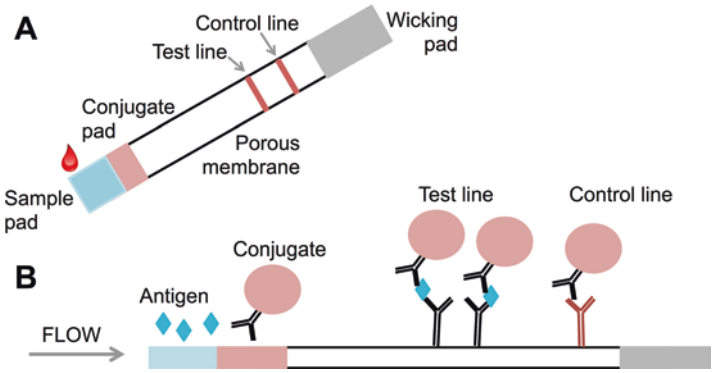

Fig. 1 Schematic of a lateral flow test from the *top* (**a**) and *side* (**b**). Sample is added to the sample pad. Upon flow through the conjugate pad, the sample rehydrates conjugate and continues to flow through the porous membrane. Signal is generated at the test and control lines in the event that analyte-conjugate is captured at the test line and conjugate is captured at the control line, respectively. The wicking pad continues to drive flow after wet out of the porous membrane

paperlike material, without the need for instrumentation. Analyte in the sample first mixes with a detection label stored dry in the device. The analyte and detection label form complexes that flow further downstream and are bound at a test line by capture molecules immobilized in the membrane. The binding of analyte-label complexes to immobilized capture molecules in the test line produces a visible signal that the user can read by eye within 20 min. Binding of detection label to a downstream control line indicates that detection label was delivered to the test line and the device ran properly. The schematic of Fig. 1 illustrates the main components of a LFT.

In recent years, several commercial LFTs have been developed for malaria detection in the developing world. Although LFTs are straightforward to perform for untrained users, the tests often cannot achieve the performance metrics required for a number of clinical applications. Specifically, LFTs can suffer from poor sensitivity or limits of detection [11]. Note that sensitivity is defined here as the slope of the response curve of the system, while the related metric, concentration limit of detection, depends both on the sensitivity and the noise of the system [12]. The reported limits of detection of malaria LFTs performed in the field are ~90 ng/mL [6, 13], while the performance of similar tests under laboratory conditions fall between 7 and 28 ng/mL [14]. However, even the laboratory performance of LFTs is not as sensitive as detection by laboratory-based ELISA, which ranges from 0.1 to 4 ng/mL.

Although LFTs meet a number of the ASSURED criteria, there is still a clear need for improved limits of detection when diagnosing diseases, such as malaria, at the POC. A POC diagnostic device that combines the ease of use of LFTs with the sample processing capabilities of laboratory-based tests would enable lower limits of detection in low-resource POC settings.

3 Paper Microfluidics as a Solution

In the past 10 years, there has been a surge in interest in paper microfluidics. Paper microfluidics is a subfield of microfluidics that generally utilizes microporous, paperlike materials to create analytical devices. Common materials used in paper microfluidics include porous materials such as nitrocellulose [15] and glass fiber, in addition to cellulose.

Paper microfluidic systems are often characterized by low Reynold's number (e.g., Re ~ 10^{-3} for the system described here) laminar flow and low capillary number (e.g., $Ca \sim 10^{-6}$ for the system described here). Paper microfluidic devices have the shared advantage with LFTs of capillary action for fluid transport, eliminating the necessity for the external pumps characteristic of conventional microfluidic systems. In addition, materials costs for these devices are typically lower than materials costs in conventional microfluidic systems based on glass and silicon. Table 1 contains a comparison of device characteristics for conventional LFTs, lab-based tests, and paper microfluidic tests.

One area of focus in paper microfluidics has been enabling automated multistep sample processing [16–18]. This ability enables operations such as analyte concentration and signal amplification (as in laboratory-based ELISA) to achieve detection of low levels of analyte not currently enabled by LFTs [19]. The key to the automation of paper-based devices is the development of non-instrumented tools to mimic the pump controls and valves of conventional microfluidics. These tools are used to manipulate fluids and reagents within paper-based systems. In the remainder of this chapter, we focus on the discussion of several tools that have been developed and demonstrated in the context of paper microfluidic devices for improved malaria diagnosis in low-resource settings.

Table 1 Comparison of device characteristics for conventional lateral flow tests (LFTs), lab-based tests, and paper microfluidic tests

	Conventional LFTs	Lab-based tests	Paper microfluidic tests
Cost	Low cost	Compatible with low cost for high-throughput tests	Low cost
Assay time	Less than 20 min	Hours to days including sample transit time	Less than 20 min
Assay sensitivity	Low sensitivity	High sensitivity	High sensitivity
Ease of use	Minimal training	Requires training	Minimal training
Shelf life/storage requirements	2 years/ambient temperature	2 years/4 °C	2 years/ambient temperature

4 Experimental Methods

4.1 Malaria Immunoassay System

The tests most widely used for diagnosis of malaria infection target the parasite protein histidine-rich protein 2 (HRP2). This makes *Plasmodium falciparum* histidine-rich protein 2 (*Pf*HRP2) a medically relevant model analyte. A murine *Pf*HRP2 antibody (Immunology Consultants Laboratory, Portland, OR) was patterned as the capture molecule within the test region of the nitrocellulose membrane. An anti-mouse antibody was patterned on the membrane as the process control capture molecule. Varying concentrations of recombinant *Pf*HRP2 (CTK Biotech, San Diego, CA), containing 18 epitopes, were spiked into fetal bovine serum (Invitrogen, Carlsbad, CA) to serve as mock samples. A second *Pf*HRP2 murine antibody (Immunology Consultants Laboratory, Portland, OR) was custom conjugated to a 40 nm gold nanoparticle label (BBInternational, Cardiff, UK), forming the "conjugate." Each gold nanoparticle label was conjugated to an average of 12 antibodies for a total of 24 possible binding sites. Tris-buffered saline with Tween® 20 (TBST) was used as the rinse buffer. The signals from the bound labels were subsequently amplified using a commercially available gold enhancement solution (Nanoprobes, Yaphank, NY) that contained a gold salt and a reducing agent. The reducing agent facilitated additional gold deposition onto the surface of the gold nanoparticle label via a metal catalytic reaction [20–22]. The increase in nanoparticle size shifted the particle absorption spectrum and produced a change in color from pink to dark purple [18, 19, 23]. At low concentrations of analyte, and correspondingly low levels of captured label, this change was appreciable, as described below. Figure 2 is a schematic of the complete malaria immunoassay stack.

4.2 Device Materials and Fabrication

The CO_2 laser system is a powerful tool that enables rapid prototyping of paper- and plastic-based devices. A commercial CO_2 laser system (Universal Laser Systems, Scottsdale, AZ) was used to cut the porous materials nitrocellulose (for the porous channel, Millipore, Billerica, MA), glass fiber (for sample and reagent pads, Ahlstrom, Helsinki, Finland), and cellulose (for the wicking pad, Millipore, Billerica, MA). Supporting material composed of Mylar® and adhesive layers was also cut with the CO_2 laser (Fralock, San Carlos, CA) and used to create folding cards. By varying the power and speed of the laser, materials can be cut through to create distinct boundaries or etched, as shown in Fig. 3.

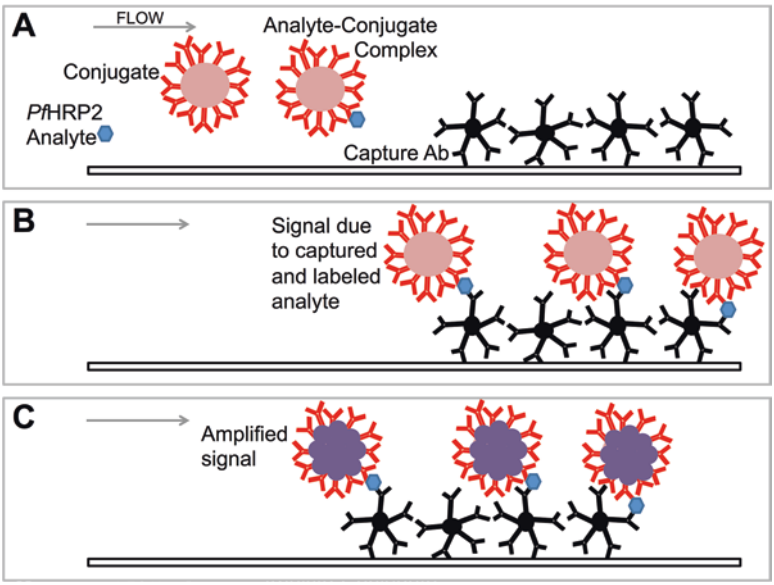

Fig. 2 Schematic of the relevant species in the amplified immunoassay for malaria analyte detection within the nitrocellulose membrane. (**a**) *Pf*HRP2 analyte in the sample mixes with conjugate (*Pf*HRP2 IgG antibody conjugated to gold nanoparticle labels) and forms analyte-conjugate complexes. (**b**) The analyte-conjugate complexes bind to *Pf*HRP2 IgM antibody immobilized to the nitrocellulose. (**c**) Signal amplification is accomplished using gold enhancement, in which gold salt and a reducing agent in solution act to increase the size of the gold nanoparticles. The result is a significantly darkened signal. For simplicity, no higher-order complexes are shown

4.3 Reagents on Membranes

Test region analyte capture molecules and control region anti-mouse antibody were patterned onto nitrocellulose with either a piezoelectric noncontact printer (SciFLEXARRAYER S3, Scienion AG, Berlin, Germany) or by hand spotting at concentrations of 1 mg/mL and 0.1 mg/mL, respectively. Patterned membranes were dried in a desiccator overnight.

To minimize nonspecific adsorption, the nitrocellulose for the staggered three-inlet device was soaked in a solution of 0.25% BSA, 0.25% PVP, and 5% sucrose in phosphate-buffered saline for 30 min and dried in a desiccator overnight. For dry reagent storage in the staggered three-inlet device, reagents were added to glass fiber pads, dried at 37 °C overnight, and stored in a desiccator until use. For dry storage of the conjugate, the solution (at optical density (OD) 10) was mixed with 1% BSA in TBS, 5% sucrose in water, and 5% trehalose in water in volumetric ratios of 7:1:1:1, respectively, and dried in a glass fiber pad. For rinse buffer dry storage, TBST was dried down in a glass fiber pad. For gold enhancement reagent dry storage, equal volumes of each of the components was added to individual glass

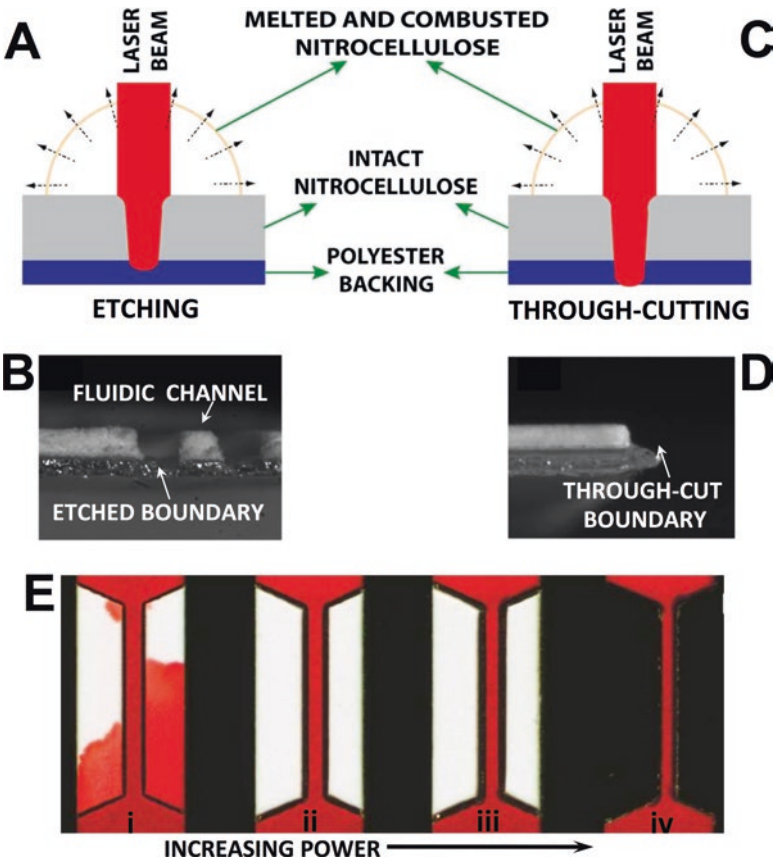

Fig. 3 Ablative etching and through cutting of a multilayer substrate composed of nitrocellulose and polyester. (**a**) Schematic of ablative etching. The laser operating parameters are chosen to completely remove the nitrocellulose in select regions, leaving only the polyester backing. (**b**) Side view stereomicroscope image of an etched substrate. (**c**) Schematic of through cutting. The laser operating parameters are chosen to completely cut through the multilayer substrate (both the nitrocellulose and polyester layers). (**d**) Side view stereomicroscope image of a through cut substrate. (**e**) The series of channels (*i–iv*) were fabricated using increasing laser power. The drawn channel width is 3 mm. For device *i*, insufficient power (and a resulting insufficient removal of nitrocellulose material) leads to leakage. For devices *ii* and *iii*, the power used was above the threshold for creating robust fluidic boundaries, but did not fully cut through the polyester layer. For device *iv*, the power was above the threshold for cutting through the entire depth of the multilayer substrate. (Reprinted from Spicar-Mihalic et al. [24]. Copyright IOP Publishing. Reproduced with permission. All rights reserved. doi:10.1088/0960–1317/23/6/067003)

fiber pads and stored in the desiccator to dry. Components were then assembled into complete devices.

For the devices relying on dissolvable barriers and absorbent pad shunts, all reagent solutions were pipetted directly into appropriate source pads at the time of device use.

4.4 Data Acquisition and Analysis

A web camera (Logitech, Fremont, CA) and a high-resolution scanner (Epson Perfection V700, Nagano, Japan) were used to acquire image data. Images were processed using a custom analysis program (Matlab, Natick, MA) and Image J (NIH, Bethesda, MD). Details of signal intensity extraction, analysis, and limit of detection calculations have been published elsewhere [23, 25, 26].

5 Automated Multistep Sample Processing in Paper

The potential for paper-based devices to achieve higher performance over conventional LFTs lies in their ability to carry out automated multistep chemical processes. Several paper microfluidic tools that control the timing of fluid delivery have been developed and demonstrated. The focus of this section is the implementation of three tools to create automated paper-based devices for malaria analyte detection: device geometry with varying path lengths, dissolvable fluidic barriers, and absorbent fluidic shunts. First, the development of a paper-based device using varying path lengths to perform the sequential delivery of the multiple reagents is discussed. Next, an alternate device design utilizing dissolvable fluidic barriers is described. And finally, a third device design that relies on absorbent fluidic shunts for the sequential delivery of reagents is explained. In all cases, automated sequential delivery was used to perform signal amplification in the context of the malaria immunoassay.

5.1 Device Geometry

Device geometry can be used to automatically deliver multiple reagents to a common downstream location. The time when a reagent reaches a desired location, e.g., such as the analyte detection region, depends on the path length from its inlet. Multiple reagents can be sequenced to arrive at the detection region in order of increasing path length, as shown schematically in Fig. 4a. Figure 4b shows an image series demonstrating the sequential delivery of colored fluids. Synchronized contact between the source pads of varying fluid capacities and the respective inlets of the device produced the simultaneous initiation of multiple fluid flows. In the example shown, the fluids arrived at the downstream detection region in the order of yellow, red, and blue with limited co-flows of multiple colors.

The staggered three-inlet device design was then used to carry out the chemical steps in an amplified immunoassay in a paper-based device. First, analyte and conjugate were delivered for binding to the capture antibody in the detection region. Second, a rinse fluid was delivered to remove nonspecifically bound conjugate. And third, signal amplification reagents were delivered to increase the signal per bound

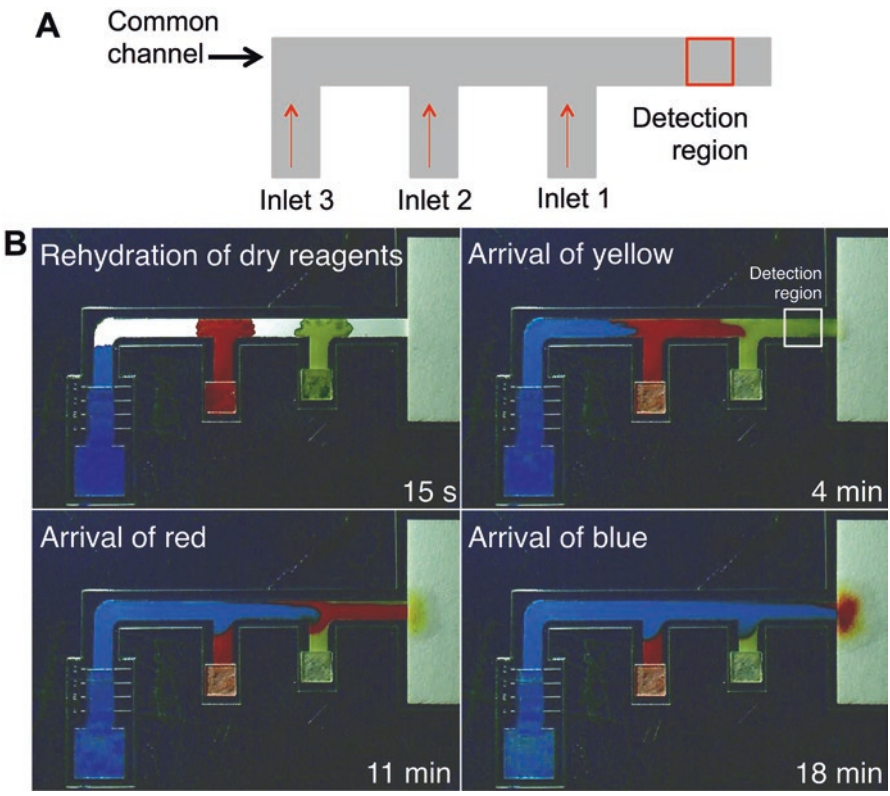

Fig. 4 Paper-based device for the automated multistep chemical processing of a sample. (**a**) In the design, fluid from Inlets 1, 2, and 3 are staged in the common channel of the device and then delivered to the detection region. (**b**) Visualization of the sequential delivery of three fluids in a paper-based device. The image series show the flow of three fluids in the device after simultaneous activation. First, the *yellow* fluid was delivered to the detection region, followed by the *red* fluid, and finally, the *blue* fluid. (Adapted with permission from Fu et al. [23]. Copyright 2012, American Chemical Society)

original label. An image of the paper-based device is shown in Fig. 5. This device, by utilizing dry reagents, only required the user to add sample (10 μL) and water (10 and 40 μL) to the appropriate source pads. After fluid addition, the device was folded closed in a single activation step. The detection region of the device was then inspected after 40 min.

Although the device is intended for simple visual readout in the field, during development, acquisition of image data and quantification of the signal intensity using instrumentation are critical to impartial characterization of device performance. Each of the concentrations (40, 20, 10, 5, and 0 ng/mL for the device with signal amplification and 40, 20, 10, and 0 ng/mL for the device without signal amplification) was run in replicates of $N = 4$ for a total of 36 devices. A device was considered to have run properly if the control region turned pink (for devices without

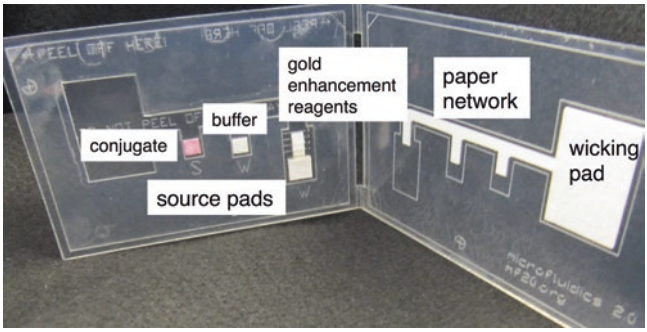

Fig. 5 Paper-based device for an amplified immunoassay using varying path lengths to achieve sequential reagent delivery. Conjugate, rinse buffer, and gold enhancement reagents were stored dry on the device. Water is added by the user to the pads labeled "W" to rehydrate the dried reagents. Sample is also added by the user to the pad labeled "S." After fluid addition, the card is folded to activate reagent flow in the paper-based device. (Reprinted with permission from Fu et al. [23]. Copyright 2012, American Chemical Society)

signal amplification) or purple (for devices with signal amplification), indicating that the conjugate or the conjugate and the gold enhancement reagents arrived at the detection region. Based on this criterion, 35 of 36 devices performed properly. Images of a concentration series of the devices with signal amplification are shown in Fig. 6a, and dose-response curves for the amplified and the unamplified immunoassays are shown in Fig. 6b.

The results from devices with and without signal amplification can be directly compared. The limit of detection (LOD) of the paper-based device with signal amplification was estimated to be 2.9 ± 1.2 ng/mL, while the LOD of the device without signal amplification was estimated as 10.4 ± 4.4 ng/mL [23]. Thus, the device with signal amplification produced a lower LOD compared to the unamplified device. The LOD for the amplified case is comparable to the LOD reported for laboratory-based *Pf*HRP2 ELISAs [7–9]. Moreover, this system has the potential for greater improvements in LOD by using more sensitive signal amplification chemistries [20, 27, 28].

5.2 Dissolvable Fluidic Barriers

Automated sequential delivery can also be accomplished using methods such as dissolvable barriers, shown schematically in Fig. 7a. Fluid transit through an initially dry membrane can be slowed by pre-drying a sugar solution into the porous channel, termed "sugar barrier" and shown in Fig. 7b. The rehydration of the sugar creates a higher viscosity solution that significantly slows flow [25]. Varying the concentration of sugar dried into paper membranes produced time delays of a magnitude that are appropriate for POC diagnostic assays, from a few minutes to nearly

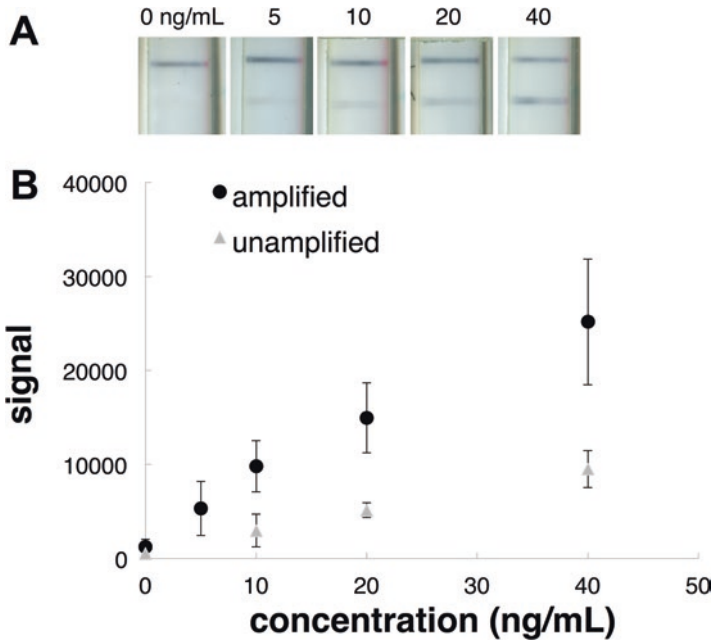

Fig. 6 Improved limit of detection in a paper-based immunoassay with integrated signal amplification. (**a**) Image series of the detection region after signal amplification for different concentrations of *Pf*HRP2 analyte. (**b**) Response curves for the amplified and unamplified immunoassays show the average signal intensity for each analyte concentration ($N = 3$ or 4). For the unamplified case, additional water, rather than gold enhancement reagent, was delivered to the detection region. Error bars represent ± one standard deviation. The amplified case has a higher sensitivity (*slope*) than the unamplified case and a lower limit of detection. (Reprinted with permission from Fu et al. [23]. Copyright 2012, American Chemical Society)

an hour (Fig. 7c) [25]. Although dissolution of a sugar barrier is complex and the wet-out path can be highly variable, the time delays achieved with this method are reasonably reproducible, with errors in delivery time of less than 20%.

Using the sugar barrier tool, a paper-based device designed to sequentially deliver four fluids of equal volume to a common detection region was demonstrated (Fig. 8a (*top*)). The fluid from each leg of the device reached the main channel at a time determined by the concentration of the sugar solution pre-dried in that leg. Higher concentration sugar barriers translated to longer times between fluid activation and the fluid reaching the detection region. Sequential delivery of colored fluids is demonstrated in Fig. 8a (*bottom*). The same device (Fig. 8b (*left*)) was then used to perform automated signal amplification in the malaria reagent system. For this demonstration, wet reagent applications of a mock sample (20 ng/mL *Pf*HRP2 analyte) premixed with conjugate (OD 5), TBST as a rinse buffer, and gold enhancement solution were applied to the inlets of the device. The result was a clearly darkened signal after signal amplification, at 30 min, compared to a device run without any signal amplification at 13 min (Fig. 8b (*right*)). Based on these data, a signal enhancement of almost threefold was achieved.

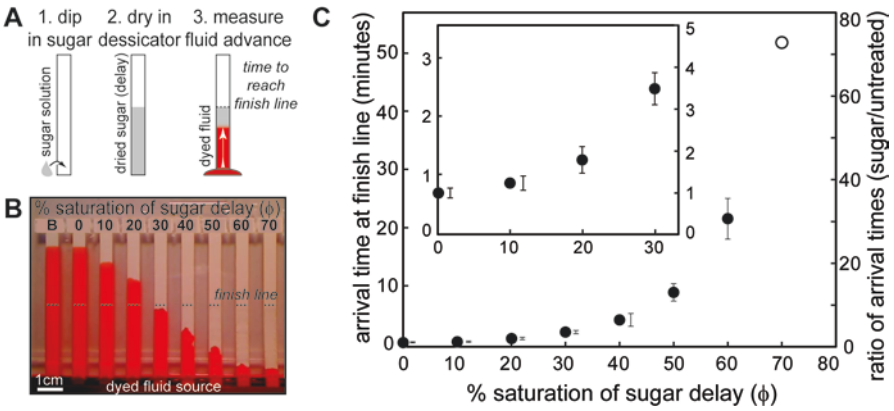

Fig. 7 Dissolvable sugar barriers as time delays for fluid progression in a porous channel. (**a**) Schematic of sugar barrier manufacture and delay in fluid progression. (**b**) Sugar solutions of varying concentrations were dried into porous membranes to create delays of varying magnitude in the arrival time of fluid to a downstream location. (**c**) Dissolvable sugar barriers were demonstrated to produce delays from a few minutes to almost an hour. (There was only one measurement for the 70% saturation of sugar, denoted as an open circle). (Reproduced from Lutz et al. [25] with permission from the Royal Society of Chemistry)

5.3 Absorbent Fluidic Shunts

Sequential delivery of reagents can also be achieved by diverting fluid into an absorbent pad shunt placed in contact with the main membrane channel. Figure 9 outlines fluid flow through a channel of porous membrane in the absence of a shunt (left) and in the presence of a shunt (right). Fluidic time delays using this shunt system are influenced by the relative capillary forces of the two materials, the relative fluidic resistances of the materials, and the relative fluidic capacities of the materials. The magnitude of the fluidic time delays can be controlled by altering the length, thickness, or material of the shunt. The method allows for the creation of reproducible time delays between 3 and 20 min with coefficients of variation of less than 10% [26].

Figure 10a shows the sequential delivery of colored fluids in a paper-based device using absorbent pad shunts to divert fluid flow. A time series of images show the flow across the detection region of a device with (Fig. 10b) and without (Fig. 10c) shunts. The results indicate that the device with the shunts enables the delivery of larger volumes of fluid from the first two inlets to the detection region compared to the device with the same geometry and footprint with no shunts. Devices with paper shunts were then used to detect the malaria antigen, *Pf*HRP2. For this demonstration, wet reagent applications of a mock sample (20 ng/mL *Pf*HRP2 analyte premixed with conjugate at OD 5), TBST as a rinse buffer, and gold enhancement solution were used. The results showed a clearly darkened signal after amplification, at 40 min (Fig. 10d) [26].

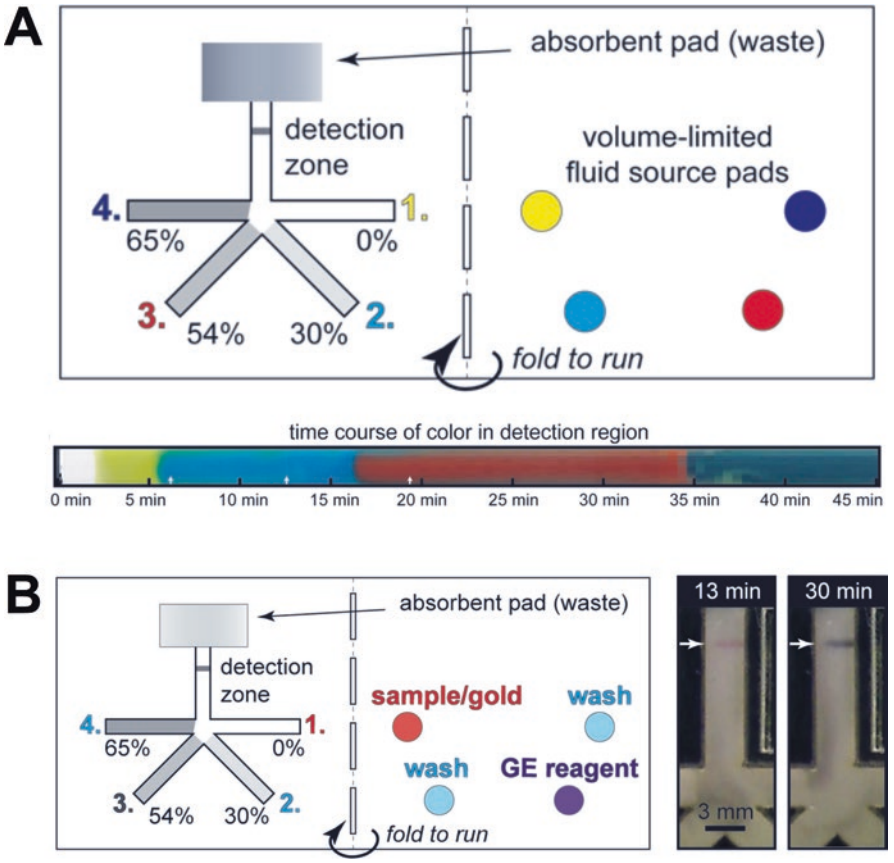

Fig. 8 Paper-based device utilizing dissolvable sugar barriers for the sequential delivery of reagents. (**a**) Schematic of a paper-based device in which sugar solutions of varying concentrations were dried into the legs of the device (*top*). Time series of images of colored fluid flow across the detection region of the paper-based device (*bottom*). First, the *yellow* fluid arrived in the detection region, followed by the *blue* fluid, followed by the *red* fluid, and finally, the *green* fluid. (**b**) Schematic of a paper-based device utilizing dissolvable sugar barriers to perform an immunoassay with integrated signal amplification (*left*). Images of the detection region at 13 min, after the delivery of conjugate and the first rinse, and at 30 min, after the delivery of gold enhancement solution (*right*). *White arrows* mark the location of the detection region. The signal after amplification was significantly darker than the unamplified signal. (Reproduced from Lutz et al. [25] with permission from the Royal Society of Chemistry)

The use of device geometry is a simple and inexpensive method for controlling the sequencing of multiple fluid flows within a paper-based device. A drawback of this method is that to achieve sequential delivery of larger reagent volumes, the path lengths and the total time for delivery can rapidly increase and become inappropriately large for a field assay. Introduction of dissolvable barriers or shunts enables more efficient manipulation of multiple fluid volumes. This comes at the expense of additional fabrication processes to integrate the barriers or shunts into final devices.

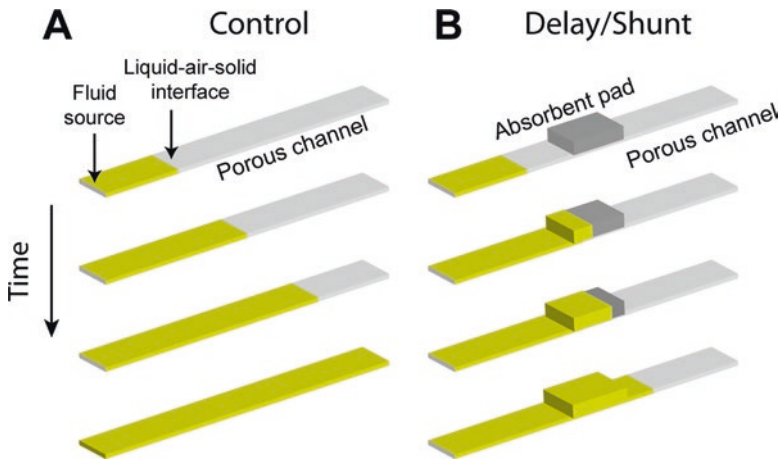

Fig. 9 Absorbent pad shunts as paper microfluidic fluid delays. Schematic of fluid flow (**a**) in a porous channel and (**b**) in a porous channel with an absorbent pad shunt. The absorbent pad shunt creates a delay in fluid flow to downstream locations of the main channel by diverting fluid into the absorbent material. (Reprinted with permission from Toley et al. [26]. Copyright 2013, American Chemical Society)

The choice of paper microfluidic tool for use in a device can also be motivated by other factors. For example, the level of reproducibility of the tool may be critical for a specific application. Alternatively, assay chemistry compatibility can also exclude the use of a particular tool, e.g., assay chemistries that are sensitive to high concentrations of sugar would be adversely affected by the dissolvable sugar barriers. Thus, the choice of which tool(s) to use in a device depends on the requirements of the specific application, and the ideal solution for a particular device could be utilization of a combination of methods.

6 Summary and Future

A main challenge in point-of-care diagnostics development continues to be the development of high-performance assays that are appropriate for use in even the lowest-resource settings. For these settings, the requirement to satisfy the ASSURED criteria brings about specific design and implementation challenges. Paper microfluidics is especially well suited to addressing these challenges. In this chapter, we have described an example of frugal bioengineering: the application of paper microfluidics to develop a high-performance malaria test appropriate for use in low-resource settings. Paper microfluidic tools have been used to achieve assays with higher sensitivity and lower LODs compared to conventional LFTs. Although this chapter focused on a malaria assay, the multiple tools and devices described here have the potential for application to a number of other diseases. The main challenges in the

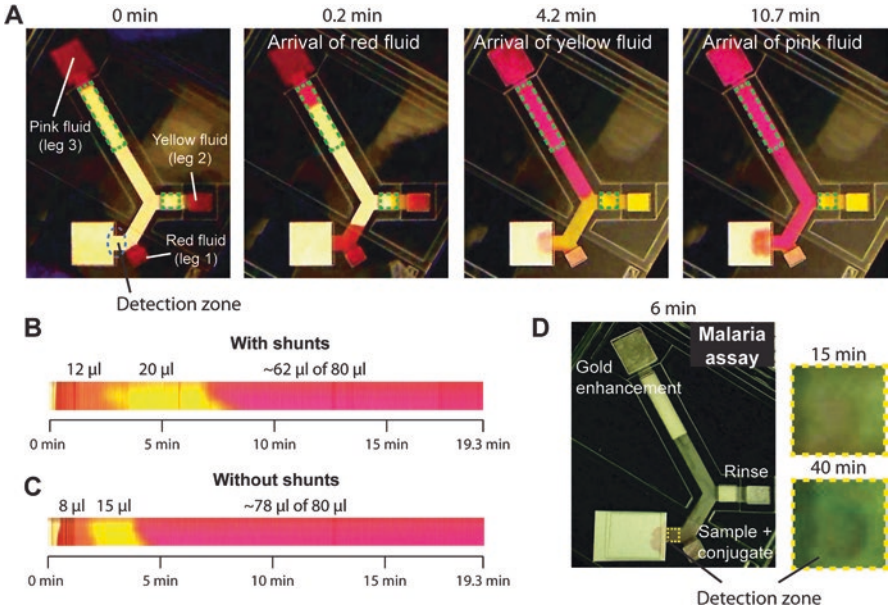

Fig. 10 Paper-based device utilizing absorbent pad shunts for the sequential delivery of reagents. (**a**) Image series shows the flow of three fluids in the device after simultaneous activation. First, the *red* fluid was delivered to the detection region, followed by the *yellow* and *pink* fluids. Absorbent pad shunts, outlined by the *green dotted lines*, were located in legs 2 and 3 of the device. The detection region is outlined by *blue dotted lines*. (**b**) Time series of images of colored fluid flow across the detection region of the paper-based device of (**a**). (**c**) Time series of images of colored fluid flow across the detection region of a paper-based device of design similar to that in (**a**) without shunts. Use of the shunts allowed for the delivery of larger volumes of fluid from the first two legs of the device. (**d**) Paper-based device utilizing shunts enabled an automated immunoassay with integrated signal amplification. Images of the detection region at 15 min (without amplification) and 40 min (with amplification) show that the signal after amplification was significantly darkened. (Reprinted with permission from Toley et al. [26]. Copyright 2013, American Chemical Society)

field moving forward are to continue to develop robust and precise paper microfluidic tools for the automation of devices, to develop and characterize high-performance reagents and materials, and to implement these in integrated systems that include sample transfer and pretreatment with minimal user steps.

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Microfluidics for Fast and Frugal Diagnosis of Malaria, Sepsis, and HIV/AIDS

Andy Kah Ping Tay, Bee Luan Khoo, and Majid Ebrahimi Warkiani

1 Introduction

Although elements of public health including sanitation and clean water access have improved in the world, these developments have been disproportionately concentrated in developed societies [1]. Figure 1 shows that infectious diseases such as malaria and HIV/AIDS are major contributors to disability-adjusted life years surveyed over 21 developing nations. In the poorest developing nations, infectious diseases account for more than half of the total deaths [2]. This is in stark contrast to the 5% contribution of infectious diseases to total deaths among the richest developed nations [2]. Arguably, the management of infectious diseases depends on the state of economic developments, as citizens in poor developing nations often face monetary issues that limit their access to proper healthcare.

Diagnostics play an important role in the management of infectious diseases. With better diagnosis, infected patients can be treated earlier, hence increasing the likelihood of a favorable prognosis. The ability to target diseases at an earlier stage may also reduce overall healthcare spending. Nonetheless, current diagnostic tests for infectious diseases are unfortunately not suitable to be widely adopted in

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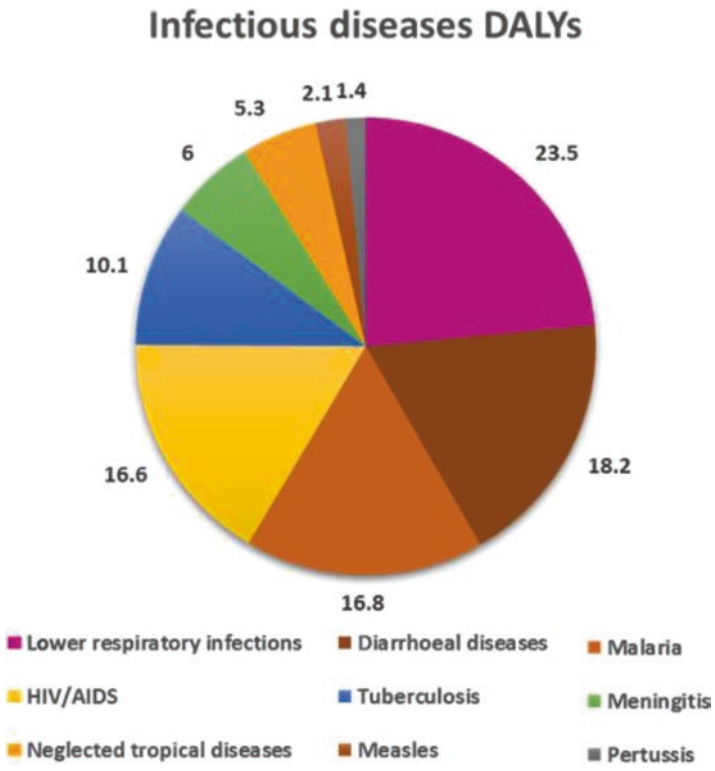

Fig. 1 Disability-adjusted life years (DALYs) for infectious diseases in 2010. DALYs (%) are calculated based on findings by Murray et al. [9] The data shows that infectious diseases such as malaria and HIV infection/AIDS over the 21 epidemiological regions surveyed in the study are important contributors to DALYs. Improvements in their diagnostic can therefore help to reduce deaths due to these diseases

developing nations. The current tests for sepsis, for instance, rely on bacterial culture isolated from fluid samples such as blood and urine, which are time-consuming [3]. With sepsis mortality rising 7.6% for every hour delay of administering the appropriate antibodies [4], culture-based diagnostic is definitely insufficient for providing timely medical information. To cater to the needs of infectious diseases diagnostic in resource-scarce regions, the World Health Organization (WHO) therefore recommends the "ASSURED" (affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, deliverable to user) criteria to guide designs of diagnostic kits [5].

Microfluidic technologies can overcome limitations, such as high cost and portability (for use in the field), which conventional methods of infectious diseases diagnostic suffer from. Microfluidic systems enable the manipulation of small sample and reagent volumes (μL–mL range). This translates into shorter processing time and also less cost incurred from expensive, shelf-life limited reagents. Despite their

miniaturized size, complex reactions can be integrated into a single microfluidic system to form an automated micro total analysis system (μTAS) that is more precise and reproducible, as the assay results are not dependent on the training levels and interpretations of users [6]. Importantly, microfluidic platforms are usually made with light, low-cost materials such as paper or polydimethylsiloxane (PDMS), making it easily transported and suitable as an affordable diagnostic tools in resource-scarce regions. Lastly, most microfluidic platforms only require an external pump or some form of power such as a magnet [7] to operate, in contrast to bulky equipment for conventional processing such as centrifuge machines. Paper microfluidics even circumvent the use of pumps by capitalizing on inherent capillary action forces in paper channels [8]. All in all, microfluidic technologies have proven to be suitable for use in infectious diseases diagnostic especially in developing nations as they fulfill the ASSURED criteria. In this chapter, we focus on the use of microfluidic platforms for the diagnosis/treatment of sepsis, malaria, and human immunodeficiency virus (HIV) infection/acquired immune deficiency syndrome (AIDS), concluding with a brief review of commercial successes for microfluidic systems.

2 Sepsis

Sepsis is a life-threatening illness characterized by whole-body inflammatory state [10]. It is the consequence of complex interactions between the infectious agents, host immune cells, and immune mediators [11]. Sepsis affects both developing and developed nations. It is estimated that USD 14.6 billion is spent on hospitalization for septicemia alone in the United States annually. However, due to poorer healthcare system, septic patients in resource-scarce societies often suffer from deleterious consequences of sepsis such as permanent organ failure and death [10]. Current diagnostic and therapeutic approaches such as physical examination, analysis of blood, urine and molecular diagnostic techniques, unless integrated, can be suboptimal. Besides, due to drawbacks like high sample volume required, and being time and labor-intensive, these methods are not compliant with the ASSURED criteria [3].

2.1 Microfluidics for Diagnosis of Sepsis

Due to the prevalence of *Escherichia coli* (*E. coli*) in early-onset sepsis [12], many different approaches have been developed for *E. coli* detection. For instance, methods such as fluorescence staining, electrical detection [13, 14], and microfluidic immunoassays were used to capture *E. coli* from blood. A multiwavelength microflow cytometer using grooved microfluidic channels that can differentiate different types of functionalized microspheres was developed to detect *E. coli* using fluorescence signals [15]. Dielectrophoretic (DEP) microfluidic device was also created to

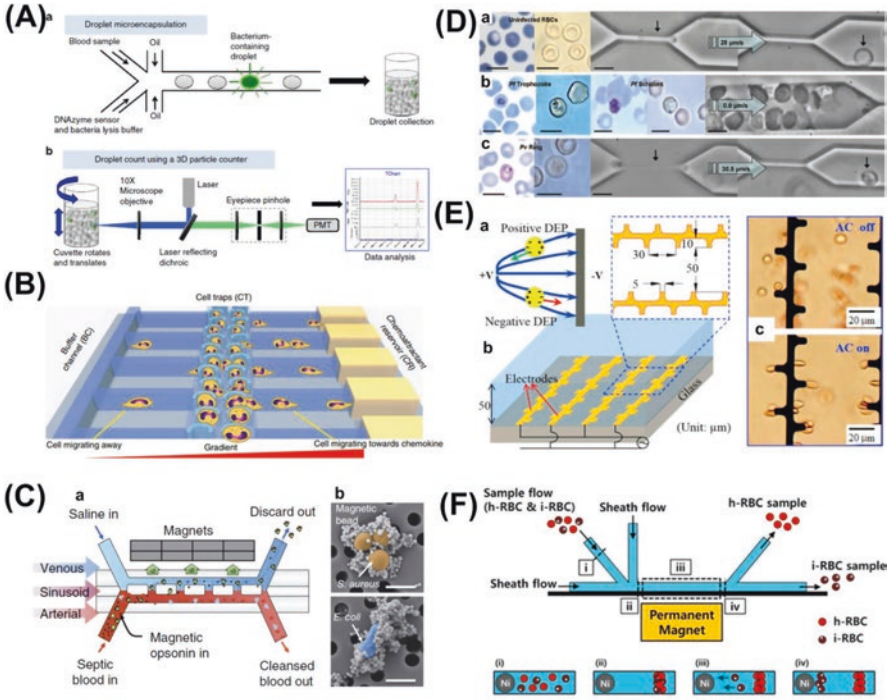

Fig. 2 Microfluidic technologies for sepsis and malaria diagnosis. (**a**) The Integrated Comprehensive Droplet Digital Detection (IC 3D) platform can detect 1–10,000 clinically isolated *E. coli* per mL of blood in a one-step, culture- and amplification-free process in 1.5–4 h. [18]. (**b**) Using this microfluidic device, gradients of chemokines could be established and the migratory patterns (speed, directionality, persistence) of leukocytes could be characterized [40]. The authors found that T lymphocytes migrated toward complement component 5a and IL-8 but away from stromal cell-derived factor 1. (**c**) The artificial biospleen made use of magnetic beads coated with MBL to capture multiple strains of Gram-positive, Gram-negative, fungi, and endotoxins from whole blood at a flow rate of 1.25 L/h. [41]. More than 90% of spiked bacteria could be removed with the device which helped to reduce immune cell infiltration into organs and inflammatory mediators in the body. (**d**) *P. vivax*-infected RBCs were passed through a 2 μm constriction channel, and it was found that they possessed higher deformability than healthy RBCs and *P. falciparum*-infected red blood cells (iRBCs) [45]. Even after passing through the narrow constrictions, 67% of *P. vivax* iRBCs regained normal appearance, suggesting that this might attribute to their ability to avoid spleen clearance. (**e**) DEP was used to stretch healthy and *P. falciparum* iRBCs with higher throughput than conventional methods such as micropipette aspiration [46]. The results were consistent with reported literature that iRBCs are less deformable. (**f**) iRBCs have greater content of paramagnetic heme in the cell bodies. By using a high magnetic field gradient, 98% of late-stage iRBCs and 73% of ring-stage iRBCs could be isolated from 40× diluted blood [47]

isolate 97% of spiked *E. coli* [16]. In general, DEP cell separation method still has to overcome its sensitivity to clogging, use of highly diluted samples, and possible cell death due to Joule heating [17].

Expertise in deoxyribozymes (DNAzymes)-based sensor, droplet encapsulation, and particle counting system were integrated to create the comprehensive droplet digital detection (IC 3D) system (Fig. 2a) [18]. This platform could detect as low as

a single *E. coli* bacterium in diluted blood sample in a culture−/amplification-free process within 1.5–4 h. The technique of isotachophoresis was also demonstrated to concentrate fluorescently labeled antimicrobial peptides in a stationary zone to continuously label *E.coli* suspended in water for over an hour [19]. This technology has also been used previously to identify bacteria from urinary tract infections [20].

Different strains of sepsis-causing bacteria/fungi may require different treatments for effective therapies for right antibiotic administration. Recently, a novel microfluidic chip that could discriminate microbial pathogens, particularly for *Salmonella enterica* (*S. enterica*) serovars derived from whole blood of septic mice, was reported [21]. This microfluidic device supported isothermal amplification of *S. enterica* serovars' genomic material. A microfluidic-based detection system for cell lysis and DNA extraction of Gram-positive and Gram-negative bacteria was also developed where the steps for DNA extraction, amplification, and even mixing are integrated into a single chip capable of detecting as low as ten colony-forming unit (CFU) of bacteria [22]. This is an improvement from a similar work where the limit of quantification was 100 CFU of bacteria [23]. Commercially available lab-on-chip system for DNA-based detection of ten sepsis-causing bacteria as well as methicillin-resistant strains of *Staphylococcus aureus* (*S. aureus*) from positive blood culture samples was also developed by ST Electronics [24].

Making use of mannose-binding lectin (MBL)-coated magnetic beads and subsequent magnetic flux concentrator, *Candida albicans* (*C. albicans*) fungi (99%) were captured in a microfluidic device to optimize optical imaging [25]. This strategy was also used to image *E. coli* using the shadow-based lens-free imaging platform [26]. The incorporation of magnetic markers with magnetoresistive sensors in microfluidic device for diagnosis of sepsis was also reported to detect for *C. albicans* [27].

Several groups have also capitalized on the biochemical composition in septic patients for diagnostics and monitoring. Evidence shows that excessive nitric oxide (NO) production plays a key role in the cardiovascular manifestations of severe sepsis [28]. An amperometric NO microfluidic sensor was fabricated to monitor changes in blood NO levels rapidly in small sample volumes [29]. Embedded magnetoresistive sensors were also created using magnetic coils and functionalized surfaces to detect four sepsis-related cytokines from 5 μL of whole blood quantification [30]. Another reported technique was using chemiluminiscent enzyme-linked immunosorbent assays (ELISAs) to detect for IL-8 [31].

As conventional antibiotic susceptibility tests usually require a few days, single-bacteria time-lapse imaging in a microfluidic channel was employed to determine the minimal inhibitory concentrations for each strain of *S. aureus* [32]. A gradient microfluidic that tested the inhibitory effects of antibiotics on bacterial growth was also developed to relate bacteria cell morphologies with their antibiotic response [33]. A size-exclusion microfilter to separate blood cells from bacteria that preserved 100% cell viability was also reported [34]. As it can be important to differentiate live from dead bacteria to prevent unnecessary administration of antibiotics, one group designed a microfluidic system based on ethidium monoazide-based assay and polymerase chain reaction (PCR) to probe for live bacteria from fluid

isolated from periprosthetic joint infection with reported sensitivity of 10^4 CF/mL of joint fluid [35].

Sepsis can be associated with the systemic intravascular activation of coagulation [36]. Hence, it is crucial to understand the spatial distribution and location of tissue factor (TF), as well as the geometry of the vasculature that regulates coagulation. These factors can be useful means to determine the severity of sepsis. Shen et al. developed microfluidic systems with surfaces of phospholipid bilayers patterned with TF to demonstrate experimentally the threshold responses of initiation of coagulation to the size and shape of surfaces presenting TF [37]. Thermal injury can trigger an inflammatory cascade that heralds shock, systemic inflammatory response syndrome (SIRS), and even death [38]. Butler and colleagues designed a microfluidic device to measure neutrophil directional migration speed in healthy and burn patients in response to chemoattractant gradients that can be established in response to infection [39]. Boneschansker et al. also quantified leukocyte migration patterns (chemoattraction, repulsion, kinesis, and inhibition) using their microfluidic device in response to diverse chemokines [40]. The group has extensively quantified the persistence and speed of migration with different chemokine dosage and receptor expression (Fig. 2b).

Developing microfluidic technologies can be extremely useful for timely, sensitive, and frugal approaches for sepsis diagnosis. However, one big challenge now will be to integrate all these capabilities to create a platform that can provide bacterial/fungi strain identification and aspartate aminotransferase (AST) assay with high sensitivity of <10 bacteria/mL and in a timely (< 30 min) manner.

2.2 Microfluidic for Treatment of Sepsis

Extracorporeal blood purification was proposed as a means to remove septic-causing microorganisms from patients. A microfluidic device that removed *E. coli* from bloodlike particles using magnetic beads conjugated to antigens targeting *E. coli* was created. An improved version of the device that could remove up to 90% of *E. coli* and *S. aureus* using magnetic beads coated with mannose-binding lectin (MBL) was subsequently designed (Fig. 2c) [41]. Highly parallelized microfluidic device utilizing the margination phenomenon has also shown success in isolating ~80% and ~90%, for *E. coli* and *S. cerevisiae* pathogens, respectively, at a high flow rate of 90–150 mL/h. [42]. The same team also reported successful removal of pathogens from a murine model of sepsis at flow rate of ~90–150 mL/h using a 32-channel parallelized platform, demonstrating the feasibility of their systems as a blood cleanser in clinical settings [43]. Another device making use of similar concept of inertial microfluidics in straight microchannels was also created by Di Carlo and his team to passively separate pathogenic bacteria cells from diluted blood (1% hematocrit) with flow rate of around 240 mL/h and over 80% removal efficiency [44].

For these microfluidic platforms to be clinically useful, it is necessary to demonstrate the potential for blood cleansing at higher flow rates in the range of mL/min

as human patients have a much larger volume of blood than murine models. The pursuit of higher bacterial isolation efficiency is also critical to minimize the risk of propagating the spread of bacteria in the patients' bodies. Furthermore, comprehensive long-term characterization of immune response to potential debris from microfluidic substrates and other materials used such as magnetic beads is also warranted before clinical use.

3 Malaria

Malaria infection by *Plasmodium* protozoa through the intermediate host, *Anopheles* mosquito, is currently the most common [48]. The infected red blood cells (iRBCs) can disrupt microcirculation [49], manifesting into anemia and organ [50] failure in severe cases. Despite improvements in malaria management, 600,000 people continue to fall victim to the disease annually [51].

The gold standard test for malaria is the microscopic method (thin and thick Giemsa blood smear) that allows trained technicians to detect parasitemia level up to one iRBC in 10^6 cells [52]. Although this test is one of the most affordable means for malaria diagnosis, its specificity relies critically on the quality of the microscope and skills of technicians. There can be misinterpretation of microorganisms like bacteria and difficulty in differentiating different *Plasmodium* strains [53].

Another important malaria diagnostic tool is the rapid diagnostic test (RDT) which detects for malaria antigen, usually in 5–15 μL of blood with monoclonal antibodies specific to the target parasite antigen. RDTs produce results obtainable in 5–20 min via simple self-testing and interpretation [54]. Nonetheless, humid and warm climate can degrade chemicals adsorbed onto RDTs [55]. The price of RDTs which stands at about US $0.55–1.50 (depending on the manufacturers and order quantities) can also be prohibitive for resource-scarce communities [56].

Laboratory alternatives such as PCR are capable of detecting low parasitemia levels or mixed infections [57]. Unfortunately, the accuracy of PCR result is subject to the suitability of primers, storage, transport procedures, and nucleic acid extraction protocols. Furthermore, the susceptibility of sample and reagent to contamination and the logistics of transporting samples to distant laboratories discourage routine use of PCR as a malaria diagnosis tool as it is incompatible for immediate patient care [58].

One other technique for malaria diagnosis is through hemozin detection. Hemozin is converted by the malaria parasite from the degraded product of hemoglobin (heme). However, the sensitivity of heme detection depends strongly on the parasitemia level and the amount of heme present [59].

There are various microfluidic-based techniques that have been developed for malaria diagnosis. In this book chapter, we classify them into (i) cell deformability, (ii) electrical signatures, (iii) molecular analysis, and (iv) optical and (v) magnetic methods. Table 1 presents a comparison for the techniques across different parameters.

Table 1 Comparisons of different malaria diagnosis methods

Performance	Cell deformability	Electrical signatures	Molecular analysis	Optical	Magnetic
Affordable	G	F	F	F	F
Sensitive	F	F	G	F	F
Specific	F	F	G	F	F
User-friendly	G	F	P	G	G
Rapid & Robust	G	G	F	G	F
Equipment-free	G	F	P	P	P
Deliverable to end users	F	F	G	F	F
Detect all strains and stages	P	P	G	P	P
Adaptable to µTAS	G	G	G	G	G

G: Good, F: Fair, P: Poor, µTAS: micro-total analysis system. Note that affordability was calculated not taking account into the cost of equipment such as syringe pumps and microscope. The different parameters in the performance column is adapted from [43].

Cell Deformability This method makes use of differences in the deformability between healthy and iRBCs for malaria diagnosis. iRBCs become progressively stiffer as the parasites mature. Consequently, iRBCs are less deformable than healthy RBCs [60]. Therefore, iRBCs are blocked more easily by narrow constrictions, and it has been found that iRBCs in the schizont stages were typically blocked by the 3 µm constriction [61]. Making use of this margination phenomenon where less deformable particles are displaced to the microchannel sidewalls, less deformable iRBCs can thus be isolated through the peripheral channels of their designed microfluidic devices [62]. A similar strategy that capitalizes on differences in cell deformability was also exploited to isolate leukocytes from a mixture of leukocytes and malaria parasites and enrich malaria parasites for diagnosis [63] (Fig. 3). Compared to the other methods, the malaria diagnosis technique capitalizing on cell deformability is inexpensive as it only requires an external pump. It is thus highly useful in a frugal setting. However, in light of emerging data, the use of cell deformability as a definite biomarker may face challenges. Results from microfluidic devices with a narrow constriction of 2 µm found that in contrast to *Plasmodium falciparum* (*P. falciparum*) iRBCs, *Plasmodium vivax* iRBCs at all developmental stages were able to transverse the gap smoothly (Fig. 2d) [45]. Additionally, there is a likelihood that the rigidities of ring stage *P. falciparum* iRBCs might overlap with older, healthy RBCs [64].

Electrical Signatures Dielectrophoretic (DEP) forces are generated when dielectric particles, i.e., polarizable electrical insulators such as cells, are subject to alternating electric field. When the cells move in the direction of increasing electric field, the behavior is called positive DEP, and the converse is termed negative DEP [65]. Readers can refer to Pethig for mathematical equations governing the generation of DEP forces on spherical and nonspherical particles [65]. As the DEP responses of cells depend on the composition and conformation of their cell membranes, cells of

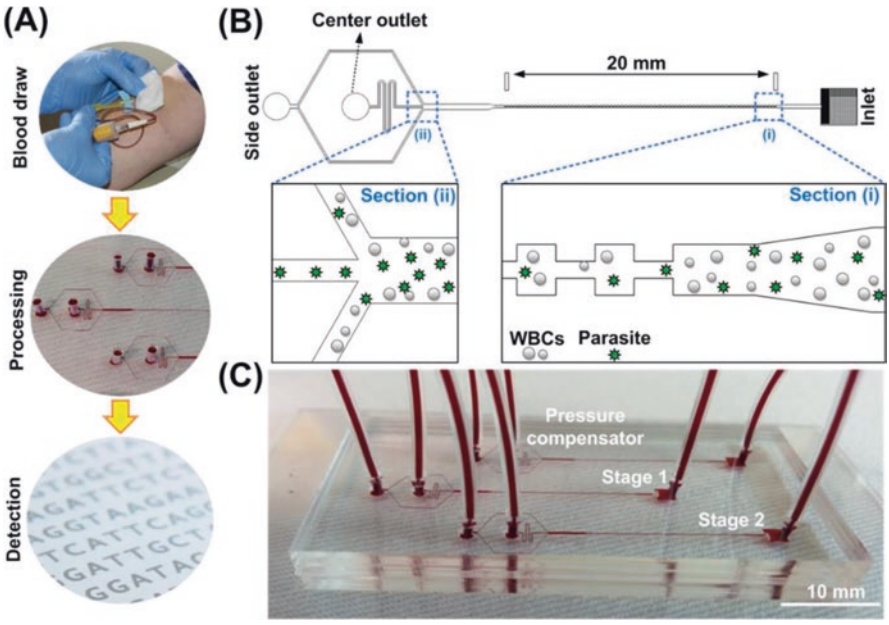

Fig. 3 Microfluidic chip for frugal malaria diagnosis. The chip exploits differences in cell deformability between leukocytes and malaria parasites and makes use of inertial focusing to enrich the latter for downstream applications such as PCR [63]. Whole blood is drawn from the patient followed by brief RBC lysis to release malaria parasites from iRBC. The lysed blood (diluted with PBS to 0.25x) is then processed through the chip, and due to the larger size of the leukocytes, they are displaced to the sidewalls by the larger inertial lift forces. Using this technology, up to 99.9% of the leukocytes can be depleted, and minimal channel clogging was observed due to the large channel dimensions (30 μm by 90 μm). This platform offers a sensitivity ~100 times greater than conventional Giemsa smear on microscope slides and has also shown potential for integration with qPCR for more sensitive malaria diagnosis

different types and physiological states can be differentially isolated using different media and frequency of the electric field [66]. DEP was utilized to isolate iRBCs [67] as the electrical conductivity of iRBC was significantly higher than uninfected RBCs attributing to membrane permeation pathways induced by malaria parasites [66]. Using DEP field-flow fractionation (FFF), microfluidic device nucleated blood cells remained in the DEP-FFF chamber and emerged only after the electric field was turn off, preventing leukocyte contamination. Next, by applying a certain frequency of electrical signal (40–250 kHz), iRBCs were levitated more strongly than healthy RBCs and emerged more rapidly from the DEP-FFF chambers. DEP stretching also revealed that iRBCs had more rigid membranes than healthy RBCs (Fig. 2e) [46]. Although DEP may offer higher diagnosis sensitivity than other methods, one of the critical challenges of DEP is the strong size dependence as most cells exhibit variations up to 10% which may interfere with cell type-specific differences in DEP particle separation. The conductivity of the media or extracellular solution could

also lead to Joule heating near electrodes, bubble generation, and heat-related cell death [68]. This device is also incompatible for diagnosis in geographical remote areas with no access to stable electricity.

Molecular Analysis PCR is an extremely useful molecular biological tool to replicate DNA. Through the steps of denaturation, annealing, and extension each at specific temperatures, millions of copies of DNA can be generated from minute quantities of DNA fragments [58]. A microfluidic platform that purified DNA released from malaria parasites residing in iRBCs generated isolated DNA compatible to quantitative PCR with a sensitivity of 0.5 parasites/nL [69]. Microfluidic PCR with its adaptability to μTAS and portability of microfluidic PCR can be useful in resource-scarce regions where malaria is endemic [70]. In addition, microfluidic devices which only require small sample volume and have high surface area to volume ratio offer higher temperature transition speed, more uniform heat distribution, and reaction efficiency. While there have been several reported innovations in microfluidic PCR, to the best of our knowledge, they usually suffer from limitations in certain sample processing steps. Therefore, to create a truly functional prototype, it is necessary to combine innovations in each step of PCR such as heating, mixing, fluid delivery, storage of reagents, and quantification.

Optical Optical means have been used traditionally for the diagnosis of diseases, including malaria (Giemsa smears). The ubiquity of optical instruments and their wide range of resolution and lens angles make them valuable tools for the study and of monitoring disease progression [71]. Nonetheless, conventional optical microscopy is bulky and expensive. Recently, however, this field has evolved with the emergence of cheaper optic alternatives enhanced with add-ons such as wave guides to create portable diagnostics [72]. DNA cleavage-litigation event was combined with droplet microfluidic for malaria parasite enzyme activity detection [73]. This microfluidic platform could operate with a detection limit of less than 1 parasite/μL of unprocessed blood. Mobile phones have also been employed in imaging thick and thin Giemsa-stained smears of *P. falciparum* iRBCs for diagnosis [74]. Recently, a paper cartridge coupled with an automated image processing software to detect for malaria parasites was described [75]. The cartridge consisted of both thin and thick smear regions for species identification and limit of detection (~100 parasites/μL) enhancement, respectively. Optical means of malaria diagnosis can be highly affordable due to advances in optic technologies. Cheap optics can also help further develop the secondary speckle sensing method that Cojoc et al. proposed for malaria diagnosis [76].

Magnetic Paul et al. reported that iRBCs behave like paramagnetic particles in a magnetic field [77]. This has motivated research in using magnetic means to identify iRBCs [78]. The idea of using magnets to concentrate iRBCs to enhance the sensitivity of microscopic method for detection of malaria was also proposed. A microfluidic device that capitalized on the magnetic properties of accumulated hemozin in iRBCs can achieve >70% in ring stage iRBC recovery rate (Fig. 2f) [47]. Nonetheless, this device works at an extremely low flow rate of below 1.6 μL/min. Unfortunately, the concentration of hemozin may be skewed by the number of

parasites, i.e., singlet, doublet, and triplet [79] in iRBCs, thus adversely affecting the specificity of the microfluidic device. Peng et al. more recently made use of magnetic resonance relaxometry to detect for hemozin in iRBCs [80]. This new approach can detect for <10 parasites/μL of whole blood and may be adapted into a microfluidic device by using portable magnets and radio-frequency detection probe. In general, the magnetic methods for malaria diagnosis are still underdeveloped. While microfluidic devices have been created, their performance suffers from poor sensitivity and specificity. However, with better knowledge on the paramagnetic properties of heme in RBCs, it may be possible to integrate magnets with microfluidic to create a portable device suitable for use in resource-scarce settings.

4 HIV Infection and AIDS

HIV infection and AIDS are broad terms that describe a range of symptoms caused by a retrovirus [81]. The retrovirus is primarily transmitted to the host via fluid exchanges during sexual contact or contaminated needles (horizontal epidemic) but can also spread from an infected mother to her child (vertical epidemic). Despite the lack of any obvious signs, the infected individuals actually undergo continuous loss of CD4+ T cells. This can cripple the immune system and render the patients susceptible to infections, such as pneumonia. The later stage is termed as AIDS [82].

There is currently no established cure for HIV infection, but several antiretroviral drugs have shown efficacy in suppressing late-stage symptom onset [83]. These drugs are more effective when the disease is detected in the earlier stages [84]. Current strategies for disease detection include measurement of viral load [85] and determination of CD4+ cell counts [86] from blood or oral fluids. Conventional techniques such as PCR runs or Western blots are rapid but are limited by the inability to detect low viral load or gradual fluctuations in CD4+ cell counts, especially in the early stages of symptomless HIV infection. For infants within 1 1/2 years old, rapid antibody assays are also not sufficient to validate the presence of HIV infection due to persisting maternal antibodies [87]. Although clinical staging of HIV infection/AIDS does not require laboratory testing (positive predictive value, 64%; negative predictive value, 81% [88]), accurate early-stage detection will be a profound problem in resource-scarce regions.

To solve these problems, researchers are developing immunoassays [89] (detection of protein markers) that can generate rapid screening for a wide range of diseases. The simplest POC tests for HIV-1 detection involve basic lateral flow procedures requiring only the introduction of a sample [90]. To allow quantitative readings, lateral flow assays are often coupled to detection optics. For example, HIV bound to protein-coated microbeads could be gathered into a microchip for imaging with capacitance spectroscopy [91] (Fig. 4a). Alternatively, HIV could be directly bonded to gp120 (HIV antigen)-coated channels, as described in the mChip protocol [92] (Fig. 4b). The microbeads were further bonded to gold nanoparticle-conjugated antibodies before exposure to an inflow of silver reagents, which formed a cloud of

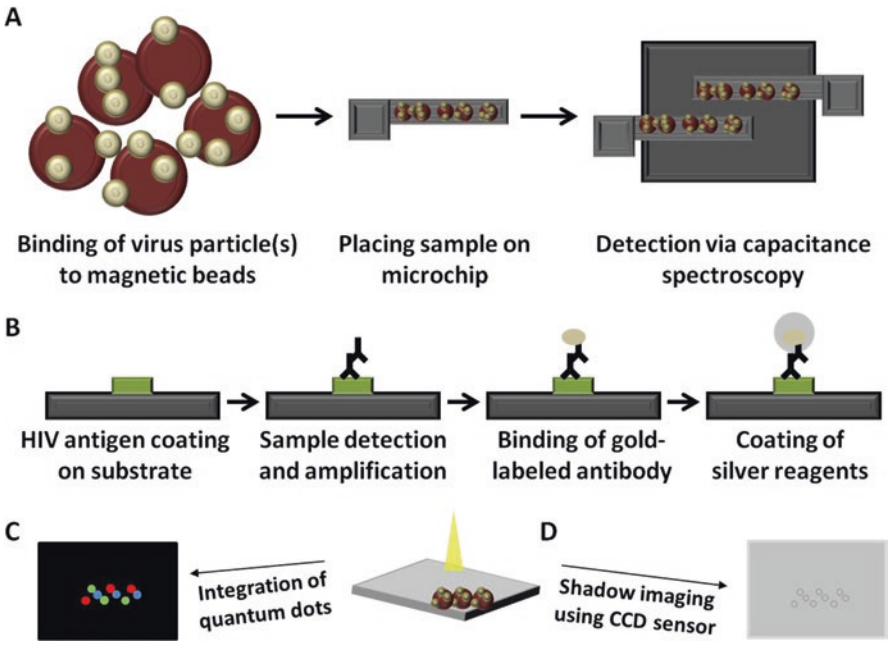

Fig. 4 Microfluidic technologies for HIV diagnostics (**a**) Antibody-coated magnetic beads were incubated with the sample to capture HIV. Bound HIV-bead complexes were then seeded into a microchip for imaging via capacitance spectroscopy [91]. (**b**) Microchannels were coated with HIV antigen before being passed over to the coated substrate passively to allow binding. Signals were amplified and further bonded to gold nanoparticle-conjugated antibodies. Silver reagents were subsequently flown in to create a cloud of reduced silver ions to enable imaging [92]. (**c**) Integration of quantum dot (Qdot) barcode (QdotB), microfluidics, photon-counting detection system, and signal processing allowed sensitive detection of viral particles [93]. (**d**) Combination of a charge-coupled device (CCD) sensor and lensless shadow imaging techniques allowed the rapid generation of a gray-scale image that performed at higher performance as compared to conventional flow cytometry techniques [94]

reduced silver ions to amplify the signals and enable visibility for imaging. In cases where higher sensitivity is desired, the assays may be further integrated with quantum dots, microfluidics, photon-counting detection system, and signal processing, allowing sensitive detection of serum markers of any blood-borne pathogen, including HIV [93] (Fig. 4c). Charge-coupled device (CCD) sensor and lensless shadow imaging techniques can also be used in conjunction to obtain higher detection rates [94] (Fig. 4d).

5 Commercial Developments

Commercial developments help to deliver point-of-care technologies to resource-scarce communities [95]. Table 2 shows the list of some microfluidic devices for diagnosis of various infectious diseases. Unfortunately, most of these commercially

Table 2 List of microfluidic-based diagnostic companies

Infectious diseases	Technology	Technical features	Name of device	Company	Website	Portable?
Multiple pathogens	Primer or antibody	Integrated platform, small sample volume, 1 h test time	PanNAT®	Micronics	micronics.Net	Yes
Urologic pathogens	Antibody	Disposable, compact, small sample volume, 10 min test time	Na	OPKO diagnostics	opko.Com	Yes
Multiple pathogens	Antibody	Self-contained waste reservoir, small sample volume, multiplexed detection	Asklepios	Genefluidics	genefluididcs.Com	Yes
Multiple pathogens	Primer	Lab in a tube platform, 30 min test time	Liat™	IQuum (part of Roche)	roche.Com	Yes
HIV infection/AIDS	Antibody	Differentiate between HIV 1 and 2, 100% sensitivity, 99.75% specificity, 15 min test time	Alere determine™	Alere	alere.Com	Yes
Sepsis, HIV infection/AIDS	Antibody	Disposable, integrated platform with positive and negative controls	Spinit® CRP/BC	Biosurfit	biosurfit.Com	Yes
HIV infection/AIDS	Electrical impedance	Count cells by analyzing intracellular content electrically	CD4 system	Daktari diagnostics	daktaridx.Com	Yes
Sepsis, HIV infection/AIDS	Antibody	CD4+ counting, CD64 sepsis marker monitoring, small sample volume	Accellix	LeukoDx	leukodx.Com	Yes
Sepsis	Primer	Integrated PCR platform, detect multiple bacteria strains	Jaguar™	HandyLab (part of BD)	Bd.Com	Yes

Information was obtained from the website of the various companies. There may be further developments since the time of sourcing these information, and readers are encouraged to refer to the original websites of the companies to learn about the various technologies. The cost of the devices could not be determined as prices were not stated explicitly on the webpages. Also, the cost per test is also likely to differ depending on the number of users and regions. Generally, the costs of devices using antibodies are expected to be higher due to the shelf life of the materials

available platforms are usually antibody/DNA primer based despite the presence of other biophysical means of diagnostic (deformability, electrical, and magnetic signature). This phenomenon is possibly due to the greater adaptability of analyte-based platforms for diagnosing a wider range of diseases. For instance, the platform developed by Micronics was used for malaria initially but has since expanded also to sepsis diagnosis.

Another noteworthy observation is that many of these commercial microfluidic kits are targeted at infectious diseases that affect developed and developing nations alike. Many of these point-of-care devices are used for HIV infection/AIDS and sepsis diagnosis, but there is a neglect of diarrheal diseases and severe acute respiratory syndrome that affect developing societies more severely. This could be explained by the lack of profitable market for diagnosing infectious diseases prevalent in poor nations.

6 Conclusions and Outlook

The importance of developing reliable point-of-care assays for infectious diseases diagnostics is critical. However, conventional diagnostic tools for infectious diseases are limited in their long processing time, high costs, and poor sensitivity and specificity. Microfluidic-based assays are attractive alternatives as they operate with small sample volume and can offer high diagnosis accuracy at a cost and portability compatible with the income of resource-scarce communities. They also often provide a means for more rapid results due to their higher surface to volume ratio, promoting reagent interaction. Other than being a μTAS, different techniques (e.g., measuring cell deformability) can be incorporated, making microfluidic devices an extremely flexible tool to diagnose and study diseases.

Despite the burst of technological advancement, there are still many challenges that have to be overcome to enable routine clinical usage. In practical settings, the microfluidic assays must perform reliability under a vast range in conditions (e.g., temperature) and should be easily operated (fully automated or simplistic). Also, analysis of the results from the microfluidic device often requires more sophisticated equipment or training, which is undesirable in resource-scarce communities. Nevertheless, the obvious benefits of microfluidics for development of point-of-care assays are significant and will continue to boost the use of miniaturized and affordable assays in all regions.

At this point of time, miniaturizing techniques have enabled the development of assays for diagnosis and treatment of malaria, sepsis, and HIV infection/AIDS. Future challenges to translate these technologies for use in resource-scarce settings will involve the reliable and sensitive quantification of viral/bacteria load from small samples. The devices (per microfluidic chip) and the equipment needed for detecting the diseases must also be affordable to benefit the patients in developing countries. More complex techniques, such as PCR, may have to be incorporated into the assay as a single unit to create a μTAS for enhanced detection limit and

specificity. Portable devices which can identify proteins or nucleic acid could provide fingerprinting services at convenience.

As mobile technology continues to develop for remote health monitoring, electrochemical and colorimetric readouts in microfluidic devices begin to gain importance. These analyses procedures could be coupled to mobile devices and carried out using detection of optical signals or other electronic or electrochemical approaches. Also, the current detection principles using microscale assays can also be expanded to other fields, such as food and environment issues. We believe continued efforts by scientists and the biomedical industry in developing microfluidic technology can benefit the millions of people who are disproportionately affected by infectious diseases.

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Perspective from Industry: AROMICS

Carmen Plasencia

1 Overview

*A*pplied *r*esearch using *OMIC S*ciences S.L. (AROMICS) (www.aromics.es) is a privately owned development-stage pharmaceutical company founded in 2005 and focused on developing novel first-in-class therapies for the treatment of cancer and viral infectious diseases. The business model is to move validated inventive findings up to early clinical stages and licensing out to pharma companies that will drive the product up to the market. Revenues will come from deal making after clinical efficacy proof.

AROMICS conducts a number of internal and collaborative R&D projects across a range of therapeutic areas, focused on the identification and validation of molecular biomarkers and their applicability to:

- Therapeutics: promoting new approaches to identify therapeutic targets and developing a proprietary pipeline of novel drugs
- Diagnostics: developing new testing tools for accurate diagnosis and drug response monitoring

Human diseases such as cancer and infectious diseases represent the core of our investigation activity. Staffed by an experienced scientific and management team, AROMICS offers its partners a fully translational research program focused on:

- Target-oriented drug discovery programs: identifying genes and proteins related to human diseases in order to develop specific therapies and to evaluate drugs' mechanism of action
- Accurate diagnostics: developing technologies for the diagnosis and prognosis of complex diseases based on molecular biology

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- Omic sciences: incorporating new technologies to increase our pipeline of relevant techniques for characterizing the mechanism of action of candidate drugs

As a result, the company is evolving the following product portfolio:

1.1 Therapeutic Pipeline

Berberine derivatives

SERPIN platform

COMPOUND	INDICATION	IN VITRO PoC	IN VIVO Poc	PRECLINICAL	CLINICAL	DOSAGE FORM	MARKET VALUE
NAX035	Malignant Mesothelioma					Oral/ i.v.	$218 Mn
	Small cell lung cancer					Oral/ i.v.	$367 Mn
	Non-small cell lung cancer					Oral/ i.v.	$ 6,9 Bn
	CDDP-resistant Ovarian Cancer					i.p. / i.v.	$1,6 Bn
AB200	Hard-to treat HCV					S.C. injection	$17,7 Bn
	Immunol. Non-responders HIV					S.C. injection	$3,02 Bn
	Co-infected HIV/HCV non-candidates to DAA* treatment					S.C. injection	$3 Bn
	HBV/HIV Non-stable HIV					S.C. injection	$1,5 Bn
AB100	HSV					Gel cream	$3 Bn
AB	H1N1 (influenza)					Inhaler	$2 Bn

Oncology Inf. Diseases *DAA: Directantiviral agent

*All productsin ourpipeline are beingdevelopedunderspecificcooperationagreements. To checkvistitus@AROMICS website

1.2 Diagnostic Pipeline

PATHOGEN	DESIGN & DEVELOPMENT	PROTOTYPE	PROOF-OF-CONCEPT	CLINICAL	COMMERCIALIZATION	MARKER TYPE	TECHNOLOGY
Salmonella E. Coli	BUGCHECK					Direct pathogen detection	Capacitive immunosensor
Legionella	IMMUNOLEGIO					Direct pathogen detection	Magnetic Immunosensor
S. Aureus MRSA	PATHFINDER®					Direct pathogen detection	SPR biosensor
N. Cerana N. Apis	CLEANHIVE					Direct pathogen detection	LFD
Coxiella Brucella	PATHOGEN OMICS					Host-response genes	qPCR
Borrelia	HILYSENS					Host serological response	Lab-on-chip immunoassay

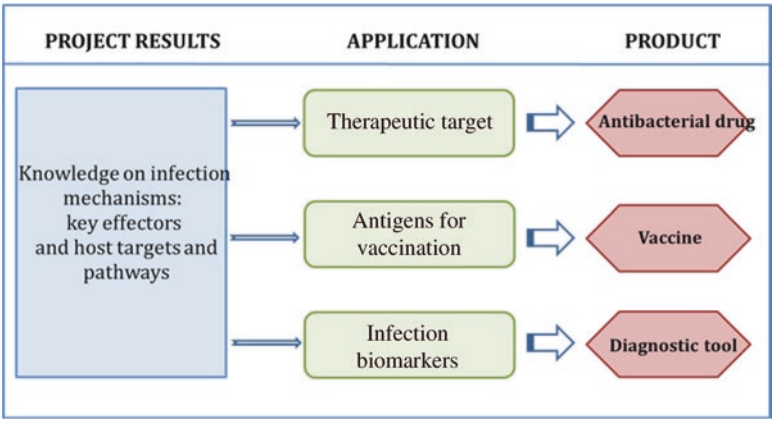
Proprietary Co-developments Non-proprietary Co-developments

As a growing company, AROMICS has relevant number of projects in pipeline at different stages of development. The highly innovative component, technological capacities, and business models strongly based in the establishment of strategic alliances allow AROMICS to be positioned at an international level as well as contribute to the development of novel products with a great potential for the pharmaceutical sector.

This chapter is more focused on the area of biosensors and devices, which are mainly devoted, in our case, to *infectious diseases*.

2 Current Need and Approach

To develop and improve the management of infectious diseases, it is essential to increase our knowledge about pathogen's virulence factors, pathogen cell cycle of life, and host cell signaling pathways necessary for the multiplication of the pathogens. As exemplified by recent studies, this can lead to the identification of selective and potent inhibitors targeting directly virulence proteins or those which interfere with host cell signaling pathways required for the pathogen survival. Expanding our knowledge to learn about virulence factors and their function will help us develop new therapeutics and improve diagnosis to combat and manage infections.



The need for developing molecular diagnostic tests in tandem with targeted therapeutics has important impacts as mentioned below:

- From the medical and patients' view: earlier detection and effective treatment of the disease will thus greatly reduce the associated costs of treatment and management of illness as well as increase the possibility of the patient making a full recovery and going on to live an active life as a contributor to society and the economy.

- From the business perspective: the anticipated success of personalized medicine and nowadays "precision medicine" will in part depend on a molecular-targeted drug having a linked, or "companion," diagnostic test designed to determine precisely whether a patient will benefit from the specific treatment or to monitor therapy in "real time" as a way to determine ongoing efficacy. This offers an important new financial model to drive the development of biosensors, since the principal customer is not the patient but the pharmaceutical company seeking to deliver an efficacious treatment.

Over these years, AROMICS has capitalized on its technical capabilities working in collaboration with for-profit companies and nonprofit institutions and research centers, identifying and validating biomarkers. This could be used as potential novel targets or for diagnostic and prognostic purposes, developing new approaches for individualized disease management and personalized drug treatment.

2.1 Our Approach in Infectious Diseases Area

AROMICS specializes in using different technologies (particularly genomics, transcriptomics, and proteomics) and applying them on developing novel treatments and diagnostic methods through gaining insights on the interplay between pathogen and host and how they impact upon clinical outcomes.

The company will then go through the specific necessary steps to comply with regulation: EMA, FDA, and IVD directives and marking required for commercialization.

Over the last 10 years (see pipeline above), the company has focused on several infections caused by different agents:

- Bacterial infections: particularly focused on bacterial infections caused by *Borrelia*, *Rickettsia*, *Ehrlichia*, Chlamydia, *Brucella abortus*, *Coxiella*, *E. coli*, *Salmonella*, and *Listeria*, among others
- Viral infections: particularly HCV, HBV, HIV, and RSV infections

And approaching them according to the two major strategies for product development (diagnostics and therapeutics) and taking also in consideration market requirements from three perspectives:

(a) *Scientific and technical needs*: Establishing a rapid and accurate cause of the infection, as a fundamental point for patient management, therefore impacting its quality of life.

  – Development of new diagnostic tests, based on generated knowledge on bacterial pathogenesis mechanisms aimed at successfully maintaining the infection in the host.
  – To improve current diagnostics by better detecting early and chronic disease stages as well as infections caused by more than one bacterial type or strain, where current tests fail.

- Inclusion of new antigens to detect specific bacterial forms, to distinguish between bacterial and viral infections, and/or to provide information on susceptibility to antimicrobial treatments that will improve the ability to directly detect the pathogens in late-stage infections and reduce chronic patient's suffering due to years of unfruitful seeking of right diagnosis and effective treatment.
- Discovery of new antigens or bacterial genes and host responses could also lead to the implementation of new therapeutic options offered to patients suffering from acute and chronic harmful infestations.

(b) *Economic impact*

- Global needs can be approached with improved test, resulting in public health preventive policies together with educational measures for frontline physicians in areas where the incidence of a specific disease is highly increasing, ultimately leading to successful control of the disease before it becomes epidemic and benefiting all patients with independence of their age and origin. Special emphasis on children and lower socioeconomic groups should be given.
- Production of the test in a standard format based on techniques routinely applied in all clinical laboratories (as opposed to currently used research "home-brewed" testing) will allow testing standardization and harmonization, hopefully leading to improve disease surveillance. That should pave the path for effective and standardized tests that would dramatically reduce the economic burden of these diseases to society and health systems due to both misdiagnosis and overdiagnosis [1].
- In limited resource or nonexistent healthcare settings or where it is very hard to physically access relevant facilities, POC approaches can save hundreds of thousands of lives every year [2].

(c) *Business orientation and market perspectives*

- The clinical laboratories and the health services are under the influence of a society that increasingly demands a personalized and more qualified healthcare procedure. Centralized testing seems not representing the most convenient process for many patients since usually diagnostic process is disconnected from the doctors' office. Recent market studies appoint as one of the most relevant aspects for patients and doctors the need for affordable test, shortening the long periods for a diagnostic result, or just a simple test to be used for those patients that require regular testing of specific parameters at home (such as chronic disease patients). A growing point-of-care diagnostic market that is stimulating the development of new, inexpensive over-the-counter sensor platforms that can compete effectively to meet consumers' needs.
- Pharmaceutical companies become a relevant stakeholder, as they may seek for a complete set of treatment tools, not being any longer a diagnostic device and a separate requirement for drug treatment but instead a part of the personalized therapy.

  - Novel sensing technologies, integrative approaches, incorporation of new materials, cost-effective manufacturing techniques and integration with telecommunication technologies, and potential utility of some of these materials for novel therapeutic and drug release strategies are currently economic growing areas representing an opportunity for small and innovative companies.

To provide an overview of the future prospects and advances of the infectious disease global market, one can take a look on the figures of the:

- Molecular diagnostics market: The immense business potential of this market is based initially on one side, on the identification of infectious diseases more accurately at an earlier stage than currently possible, as well as, on an increasing need for monitoring treatment responses to avoid infection chronicity. Only the global market for point-of-care diagnostics is expected to reach $27.5 billion by 2018 at an estimated compound annual growth rate (CAGR) of 9.3% from 2013 to 2018 [3]. It represents then, an attractive business opportunity as diagnostic businesses generally receive higher returns of investment than pharmaceutical and other biotech businesses. Molecular diagnostics represents then an attractive business opportunity for small knowledge-generation companies as AROMICS, as diagnostics businesses generally receive higher returns of investment than pharmaceutical and other biotech businesses.
- *Therapeutic market*: in this case, AROMICS intends to carry out the preclinical development of the potential drug and seek for external investment or a pharma partnership for the later clinical stages. However, big pharma often becomes involved later through in-licensing later-stage pipeline drugs, as a strong sales force is a key factor to success in the anti-infectives' market.
  - *Antibacterial market*: although the market size is significant, with estimated total sales of US$25.5 billion in 2005, 10 out of the 15 largest pharmaceutical companies have fully abandoned, or cut down significantly, their discovery efforts in this field since 1999 to focus on more profitable areas. Smaller firms have been taking over the drug development role by identifying innovative drugs or formulations. Currently, much R&D effort is focused on solving antibiotic resistance and identifying potential new targets and molecules.
  - *Antiviral market*: in 2010, the global antivirals market was estimated to be worth $22.1 billion and is expected to reach revenues of approximately $30.1 billion by 2017 at a CAGR of 4.5% between 2010 and 2017. Overall antiviral market is driven by human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) therapeutic sales which accounted for 61% of the market in 2014 and new hepatitis C drugs which are growing more rapidly around 10% CAGR, with impacting sales of newer drugs like Sovaldi (with $10.3 billion sales in 2014, close to being the best-selling drug in the world in only its first year on the market), Harvoni (which recorded $2.1 billion in sales since its approval in October 2014), Olysio ($796 million in 2014), and Viekira Pak (that was expected to reach $3 billion sales by the end of 2015). Other markets like herpes therapeutics (herpes simplex and herpes zoster infections market) is forecast to grow at a CAGR of 11.2% during the forecast period 2010–2018.

3 Technologies

As mentioned, AROMICS specializes in using different technologies (particularly genomics, transcriptomics, and proteomics) and applying them to identify biomarkers. AROMICS' facilities are located in the Barcelona Science Park (PCB), a 20,000 m^2 area which hosts companies and research centers of excellence and owns modern platforms supporting R&D activities in emerging research areas such as chemistry, pharmacy, biotechnology, and nanobioengineering. AROMICS facilities inside PCB include a laboratory fully equipped for conducting experimental research studies using conventional cell culture techniques, genomics (microarrays), proteomics, and other common molecular biology (qPCR, ELISA, Western blot, dot blot, etc.) and biochemical procedures and with access to the different platforms available at PCB.

The company started different research and development activities devoted to the development of novel biosensor capable of detecting serveral biomarkers identified (nucleic acids, antigens, pathogens). As a first objective, the company conducted different investigative activities to understand the relevant aspects involved in biosensor functioning that may impact in our areas of interest.

3.1 Fundamental Aspects of a Biosensor

A biosensor is an analytical device capable of providing selective quantitative or semiquantitative analytical information due to the specific biological recognition among the element/analyte of interest and the recognizing element. It consists then of three main components: a biological detection system, a transducer, and an output system. In fact, biosensors are classified according to the nature of the three elements. Figure 1 shows a schematic diagram of a typical biosensor.

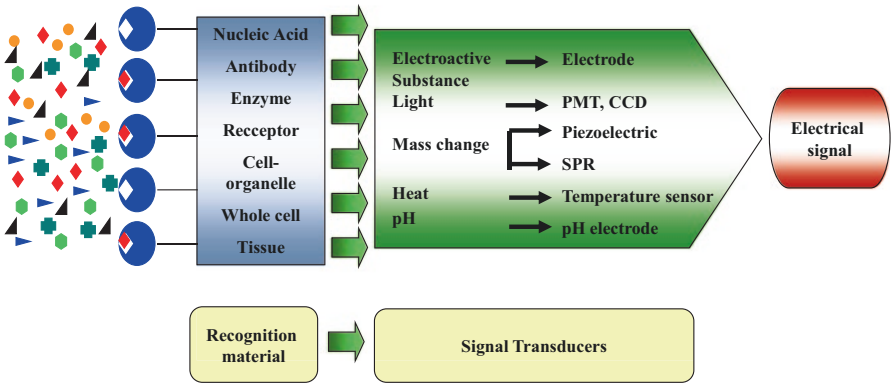


Fig. 1 A schematic diagram of a typical biosensor

3.2 Integration of the Different Parts of Biosensor and Miniaturization

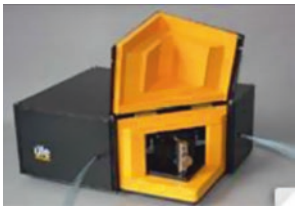
The application of microfluidics allows miniaturization and integration of complex functions that facilitate their usage in limited resource settings. The advantages offered by such systems, including low cost, ruggedness, and the capacity to generate accurate and reliable results rapidly, are well suited to the clinical and social settings of the developing world.

However, fully integrated systems that bring together the components of sample preparation and analyte detection remain a *critical challenge* for technology transfer from laboratories to the clinical market. Recent system-oriented microfluidic strategies that facilitate system integration include multilayer soft lithography, multiphase microfluidics, electrowetting on dielectric, electrokinetics, and centrifugal microfluidics among others [4]. Microfluidics incorporates some of the previous steps mentioned above: sample preparation and concentration, mixing, pumping, and separation. Another crucial element for system integration is the detection module. For example, many of the optical detection strategies require a bulky microscope and laser source, which are not practical in clinical settings. Recently, research has focused on portable detection system based on optical, electrical, and magnetic sensing. AROMICS does not have capabilities for large scale manufacturing as company and thus, it necessarily brought us to a couple of different research and technological centers, as well as collaborations with SMEs (small medium enterprises) in the field, capable of manufacturing and integrating cartridges and devices.

Below are some of the examples of current knowledge and technology incorporated in our product pipeline. The company has incorporated these technologies by working with relevant stakeholders (research and technology centers and SMEs) with expertise required in the area. The technology achievements have been possible due to the incorporation of AROMICS in specific European Union R&D projects (under Framework Program 6 and 7), focused on developing diagnostic tools detecting pathogen for such diverse industries such as health, honey, or milk that has allowed the company to grow and mature different technologies.

FP6. COOP-CP-2005-017969

Bugcheck. A rapid handheld analyzer for control of microorganisms in the complete meat supply chain.
A new label-free biosensor device for gram-positive pathogen detection and monitoring in complex samples such as meet, water, etc. to be used mainly in food industry. The sensor represents an advanced molecular diagnostic product enabling simple, affordable in situ controls to certify pathogen control of the meat before reaching the market, thus safeguarding the health and safety of European consumers

 FP6–2004-SME-COOP-032169	*IMMUNOLEGIO. Rapid biotechnic based on an immunosensor for in situ detection of Legionella industrial and environmental water samples.* Development of a new bioanalytical instrument, based on magnetoresistive biosensors, to detect *Legionella* by a rapid and low-cost assay
 FP6–2004-SME-COLL-030392-2	*Pathfinder®. A rapid and cost-effective tool for multi-pathogen detection in milk.* Being able to detect specific biological molecules at very low concentrations, this optical sensor represents an advanced molecular diagnostic product enabling simple, affordable on-farm controls to produce safe milk and certify its quality, keeping disease outbreaks under control by early detection of their origin, thus safeguarding the health and safety of European consumers
 *Research for the Benefit of SME Associations-218,416.*	*CLEANHIVE.* (Detecting the pathogen that threatens European honey bees). Development of a diagnostic kit (lateral flow device) that will allow the detection of the pathogen *Nosema ceranae* and *Nosema apis* in honeybee colonies. The project aims to develop a portable, cost-effective, easy-to-use, and sensitive tool, able to detect *Nosema* pathogens involved in the depopulation syndrome and to be used in field conditions by nontechnical staff in the beekeeping sector in order to stop the spread of *Nosema*
 *FP7-SME-2010-1-262411*	*HILYSENS.* The project aims to develop a novel lab-on-chip diagnostic tool to improve clinical diagnostic, disease monitoring, and treatment of Lyme disease by enabling specific and sensitive detection of the human serological response against its causative agent *Borrelia burgdorferi* infections

4 Technologies as They Relate to Frugal Innovation

All the systems generated so far in European collaborative projects where AROMICS has been part of are based on the market needs particularly related to in-field applications that facilitate their usage in limited resource settings. All of the addressed required features clearly identified:

- The tools developed should be robust and easy-to-use and enable unambiguous interpretation of test results as they try to replace the current molecular biology techniques which are in most cases labor-intensive, time-consuming, and difficult to standardize.

- The solutions developed have to permit simple, robust, and automatic (whenever possible) sample processing and detection in a single step. To achieve that goal, it is a requirement the marriage of technologies combining specific recognizing elements and detection systems with enhanced signal-to-background detection and performance in multiplexed assays to provide outstanding features in terms of sensitivity and assay robustness.
- To improve accuracy, specific markers are included and effectively transferred onto a new and innovative product, achieving the accuracy required. The new markers (proteins, antigens, antibodies, etc.) are validated markers that have to allow accuracy, sensitivity, and specificity of the device, as mainly the detection relays on the specific reaction.
- Reagents should be used in low quantities, avoiding a high cost per analysis, and easy to store and transport.
- Whenever possible technologies for microfluidics are required to integrate the steps of a complex chemical process into a monolithic disposable (diagnostic card), an assay performed in such a way can be more precise, more accurate, and more reproducible than the same assay performed by hand.
- The reagents and cartridges developed are required to have a low cost of the production, low cost of maintenance (i.e., at room temperature), and handheld devices to be used infield (i.e., CLEANHIVE for the beekeepers, Pathfinder for veterinarians or farmers in farm, Bugcheck in slaughterhouses, or IMMUNOLEGIO in refrigeration towers).
- The devices should be smaller, less complex and inexpensive instruments with no specific requirement of laboratory installations, more user-friendly and that can be used by non-trained healthcare personnel.

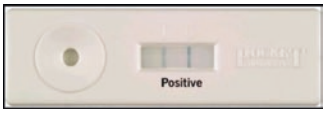
In general, their intrinsic advantages such as small size, manipulation of small volumes of liquids, high capability of integration, rapid reactions offered by microfluidic technology, and analytical steps such as sample pretreatment, assay, and detection, on a single easy-to-use, accurate, and automatic test system together with a low cost, ruggedness, and the capacity to generate accurate and reliable results rapidly make them promising candidates as POC diagnostic platforms in low-resource and laboratory-free settings.

5 Technical Aspects

From the simplest lateral flow to most complex system (lab-on-chip), developed in market, we have tried to include most of the methodologies currently available in developed and commercial sensors to be applied in biotechnology area.

Simple tests. Lateral flow devices These tests are often used as a frontline screening test and include the measurement of protein and metabolites in samples. Nowadays many of the tests use immunological reactions to make a measurement in just a few minutes where the traditional laboratory-based test may take several hours. A new generation of “dipstick”-type tests relies on immunological interactions

between an antibody and the target molecule being measured. These tests use the sample to rehydrate dried reagents which then flow through a porous membrane. The sample and the reagents are pulled through the membrane by capillary action, and when they reach a line of immobilized antibody on the membrane, a colored line becomes visible to the user. A second line acts as a control telling the user when the reaction is complete and that the test can be read. There are now a number of devices on the market that can simultaneously measure multiple targets, but the greatest growth in lateral flow technology is for the rapid detection of infectious agents.



In our case, inside the CLEANHIVE project, we used to detect two different pathogens (*Nosema ceranae* and *Nosema apis*) in a complex sample like honey, relying on the following technical aspects:

- Production of *specific antibodies* for *N. apis* or *N. ceranae*, suitable for use in lateral flow assays.
- A *sample treatment protocol adapted to field conditions* to ensure maximum sensibility of the test and minimize cross-reactivity risks.
- Development of a *highly sensitive detection system* by using fluorescence labeling. This type of label improves sensitivity and permits to use lower amounts of antibodies therefore lowering the lateral flow costs but requires an optical reader to detect and distinguish different signals.
- Development of a *portable reader*. Although lateral flow assays are effective diagnostic tools, they can lead to interpretation errors when several signals are present, as in this case, where two signals could be obtained: one for *N. apis* and the other for *N. ceranae*. Current optic technologies allow the production of such a system at a very reasonable cost.
- Development of simple *data handling software* to allow communication of the reader with a PC to record and store the results.

The system was developed with the collaboration of research centers (FERA Science Limited and the private research center CRIC, now part of Ateknea Solutions, for the antibodies and the optical reader, respectively) and the SME Forsite Diagnostics Limited, now trading as Abingdon Health, a leading lateral flow device (LFD) manufacturer with a strong and diverse customer base, which includes UK and European regulatory authorities and multinational diagnostic and food companies. The company has invested in creating a state-of-the-art manufacturing facility, which has the capacity to produce many millions of lateral flow tests per annum.

All these events have contributed to the generation of novel bio- and nano-sensors that can be coupled to analytical techniques such as electrochemical, chemiluminescence, electrochemistry, and electrical or optical methods. All of them though have

their own advantages and disadvantages in terms of sensitivity, simplicity, and cost-effectiveness.

Electrochemical sensors While the "dipstick"-type tests described above are rapid and robust, they are used where a yes-no answer is required. In the case where it is important to know the concentration of the measured compound, technology has allowed the traditional laboratory test to be brought into the POCT domain. Instrumentation that is based on electrochemical measurement enables the use of inexpensive, mass-produced disposable electrodes and allows miniaturization and packaging of small volumes of reagents. These technologies can be miniaturized and offer great promise to the POC market in the future as they do not require reagents such as a label.

These devices are mainly based on the observation of current or potential changes due to interactions occurring at the sensor-sample matrix interface. These changes of charges could be measured using spotted electrodes on thin matrices and transferred into measurable signals. Techniques are generally classified according to the observed parameter: current (amperometric), potential (potentiometric), or impedance (impedimetric). Compared to optical methods, electrochemistry allows the analyst to work with turbid samples, and the capital cost of equipment is much lower. On the other hand, electrochemical methods present slightly more limited selectivity and sensitivity than their optical counterparts.

Among the available electrochemical technologies, Bugcheck was a European project led by a group of SME partners, including AROMICS, and focused on an immunosensing system to detect *E. coli* and *Salmonella* based on electrochemical impedance spectroscopy (EIS) at interdigitated electrode structures. EIS encompasses a powerful set of electrochemical techniques. It has been successfully used in biosensors to monitor processes of biological interest. In this technique, a cyclic function of small amplitude and variable frequency is applied to a transducer, and the resulting current is used to calculate the impedance at each of the frequencies probed [5]. One of the features making EIS more attractive is that unlike amperometric biosensors, it does not require the use of electroactive labels for detecting the biorecognition event.

Bugcheck was relying in:

(a) *Interdigitated microelectrodes* on silicon and glass, fabricated according regular manufacturing using standard photolithographic techniques [6]. Microelectrodes are characterized by scanning electron microscopy, profilometry, conductivity measurements, and also electrochemically cyclic voltammetry. They have moderate electron transfer properties, but the effect of this in the performance of the Bugcheck immunosensors was not significant, since the final measurements was not based in faradaic impedance but capacitance measurements.
(b) *Microelectrode functionalization*: The main drawback of EIS, as with most electrochemical techniques, is that it does not afford selectivity on its own. This fact antagonizes with the concept of biosensors, where selectivity is a must. This is particularly important when dealing with complex samples where other processes may interfere with the measurement and become sources of error.

In our case, selectivity was introduced by immobilizing polyclonal antibodies onto the gold interdigitated structures treated with NeutrAvidin.

(c) *Disposable plug and play cartridge system* including this interdigitate based on a printed circuit board of 75 mm long and 0.8 mm thick was designed with 6 mm in his narrow side to be housed on a 1.5 ml Eppendorf tube and with 14.5 mm in his large side to be connected to a standard SIM card reader connector.

(d) *A compact antimicrobial analyzer based on electrochemical impedance spectroscopy* consisting of an aluminum base where in the rotational thermostatized sample holder and a stainless steel prismatic rail are screwed. The signal transduction circuitry is fixed to a linear sliding stage to allow the linear displacement through the prismatic rail. The system is provided with a digital control to allow the data transfer to a PC.

(e) Finally a *software* for analysis of data.

Development was made in collaboration with the miniaturized microelectronics group at the Centre of Institute of Microelectronics of Barcelona IMB-CNM (part of The Spanish National Research Council (CSIC), one of the largest public institution dedicated to research in Spain and the third largest in Europe) and the private research center Ateknea Solutions, together with the collaboration of different SMEs in the field of biosensing, pathogen detection, and food processing. Among the technological SMEs are BVT Technologies, a private company based in the Czech Republic that develops and manufactures a wide range of electrochemical sensors, enzyme biosensors (AChE and GOx), and related laboratory equipment specialized in thick film technology for the production of screen-printed electrochemical sensors, and Biosensor Technology Gmbh, a German company and a market leader company in multi-way biosensors.

Although screen-printed electrodes have already been widely adopted in the mass production of low-cost disposable biosensing, further research on surface modification, incorporation of new materials, and elaborate geometries will make the application of electrochemical sensors even broader.

Introduction of new materials like paper or flexible material technologies has created an exciting avenue in this type of sensors, as they are thin, light, flexible, inexpensive, easy to fabricate, mass producible, easy to use, and disposable [7, 8]. Shaffie et al. provide in their article some examples of this type of sensors employing polyester film or paper for the detection of different pathogens using impedance spectroscopy.

Porous silicon (Psi) has also emerged as promising material for manufacturing biosensors, because of the nanostructured nanopores that provide large surface areas enabling large amount of interactions between the biorecognition elements in a small working area, facilitating the miniaturization of the sensors and a range of electrical properties for optical and electrochemical transducing mechanisms.

More recently the Belgium Research Centre on semiconductor IMEC in collaboration with Ghent University has developed ultrathin chip packages, allowing the integration of electronic circuits inside elastic and flexible materials without affecting the microchip functionality.

Optical sensors Increasing sensitivity may often require labels or optical detection methods. The latter are well established in practice. In the case of these technologies, one reacting agent is immobilized on a transducer surface, while the binding event of the second reacting agent is detected via an optical change. These measurable optical changes can be different signals such as intensity, phase polarization, wavelength, fluorescence/phosphorescence, reflectance, refractive index, or the spectral composition of the light.

Some of the advantages offered by an optical biosensor are selectivity and specificity, remote sensing, isolation from electromagnetic interference, fast and real-time measurements, multiple channels/multiparameter detection, compact design, minimally invasive for in vivo measurements, choice of optical components for biocompatibility, and detailed chemical information on analytes.

Optical biosensors can be broadly classified based on the different parameters:

- Signal transduction: most used includes absorption, fluorescence, and luminescence changes.
- Based in the optical geometries: the choice of the different geometries available is dependent on the nature of the analyte and the optical probing method used. Here, the major consideration is to enhance the sensitivity and specificity. Most used includes optrodes, SPR, and fiber grating biosensors.
- Based on evanescence wave.

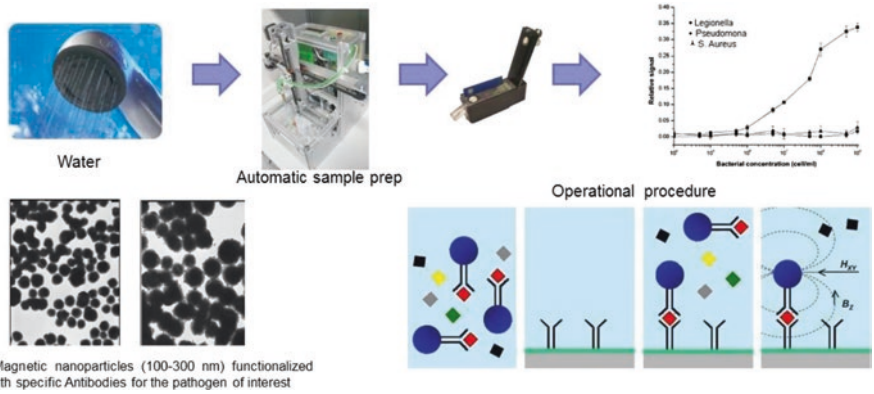
Magnetic sensors Sensitivity and versatility of magnetic biosensors provide unique platform for high-performance diagnostics in clinical settings. Magnetic biosensors required well-tailored magnetic particles as probes for detection that generate large and specific biological signal with minimum possible nonspecific binding. Magnetic nanoparticles are a class of nanoparticles which can be manipulated using magnetic field. Such particles usually consist of magnetic elements such as iron, nickel, and cobalt and their chemical compounds. While nanoparticles are smaller than 1 micrometer in diameter (typically 5–500 nanometers), the larger microbeads are 0.5–500 micrometer in diameter. These particles offer advantages in terms of enhanced sensitivity, low-limit detection, high signal-to-noise ratio, and shorter time of analysis. Moreover, they offer some advantages as bio-recognition elements, allowing whenever appropriately functionalized the capture and concentration of the analyte even in difficult or dirty samples.

Among the available magnetic sensors technologies, IMMUNOLEGIO was a European project led by a group of SME partners, including AROMICS, and focused on an immunosensing system for in situ detection of *Legionella pneumophila* spp. by a rapid and low-cost assay in water coming from industrial cooling towers, hospital showers, or drinking fountains among other sources.

IMMUNOLEGIO was relying on:

- Selection of *specific antibodies* for *Legionella pneumophila* ssp., allowing the detection of different serogroups and suitable for use in capture beads.
- An automatized method for *sample treatment and biorecognition adapted to in-field conditions* to ensure maximum sensibility of the test and minimize cross-reactivity risks.

- Development of a *highly sensitive detection system* by using magnetic properties. Functionalized magnetic nanoparticles allowed sample pre-concentration, improve sensitivity, and permit to use lower amounts of antibodies but require a magnetic sensor to detect and distinguish different signals (distortion of magnetic field).
- Development of a *portable reader*. Current technologies allow the production of such a system at a very reasonable cost.
- Development of simple *data handling software* to allow communication of the reader with a PC to record and store the results.



The selectivity of the sample and the target represents a rapid, sensitive detection strategy with the integration of a magnetic detector. This integration is facilitated by the development of solid-state magnetic sensors (i.e., microelectromechanical (MEMS) devices or more sophisticated giant magnetoresistance (GMR) sensors) that can be used as the magnetic detectors in these applications. These sensors enjoy the advantage of being compatible with silicon IC fabrication technology, resulting in the placement of a single detector – or even multiple detectors – on a chip with the required electrical circuitry.

However, there are visible knowledge gaps in our understanding of the strategies to overcome existing challenges related to the synthesis and sensing principles of magnetic nanoparticles as well as surface functionalization and modification. The manufacture of clean and uniformed in size and shape particles, a solution for clustering issues and agregation and the manufacture of robust functionalized particles are main requisites for the success of this type of sensors that are more sensitive.

Technologies for microfluidics lab-on-a-chip devices Lab-on-a-chip technology has been long envisaged to have tremendous commercial potential, owing to the ability of such devices to encapsulate a full range of laboratory processes in a single instrument and operate in a portable manner, rapidly, and at low cost. Conventional approaches for producing microfluidic devices are based on Si planar technology.

The production of micro- and nano-fluidic devices requires formation of comparatively large channels (μm), which is usually based on etching. This means special process steps, or even establishment of, which makes the devices expensive to produce compared to Si-microcircuit production where highly standardized processes are used [9].

In recent years, a form of MeV ion beam lithography proton beam writing (PBW) has drawn considerable attention because it is a direct-write technique capable of writing high spatial density patterns in thick resist (30–60 μm) to produce 3D structures [10]. Producing biomedical lab-on-a-chip devices requires small features such as nano-channels to be combined with large-area features such as reservoirs. The ability of MeV ion beam lithography using the PBW techniques can be used for direct writing of prototype devices as well as manufacturing master stamps for mass production by hot embossing process[33] or molds for casting[35, 36].

HILYSENS project includes a *lab-on-a-chip device* that utilizes the capabilities of MeV ion beam lithography, for making an entire lab-on-a-chip device. MeV ion beam lithographic methods write directly in thick polymers without requiring a premade mask or separate process steps. Conventional lithographic fabrication approaches, based on thin-layer lithography combined with etching of silicon or glass substrates (e.g., electron-beam lithography), and deep lithography of polymers using synchrotron light (e.g., X-ray lithography) are both time-consuming and too expensive to produce. The high cost comes mainly from the requirement of expensive lithographic masks combined with etching techniques. The new approach addressed this issue by using direct mask-less technology and polymeric materials and allowing production by low-cost manufacturing technology such as molding used for rubber and plastic components and hot embossing used for credit cards or CD and DVD production and laying the ground for subsequent flexible low-cost mass production.

HILYSENS project is devoted to the generation of a serological assay on a lab-on-a-chip device for the diagnosis of Lyme disease. In brief, sample serum from the patient flows into the analysis chamber where antibodies bind to the immobilized antigens. Afterward, the anti-Ig-coated QDs flow into the chamber binding to the sample antibodies. After a washing-up step, the QD signal is detected. The device relies on:

- A novel *lab-on-chip diagnostic test cartridge*, with all functions integrated in the chip without external connections (inlet reservoirs, mixing valves, detection area, and fluids pumping), to permit robust and automatic sample processing and detection in a single step. The chip contains (a) capillarity pumping structures dimensioned to achieve a flow rate (up to 0.4 μl/min) for the transport of sample and reagents to the detection chamber and (b) a detection chamber, where the human immunoglobulins against *Borrelia* antigens are trapped by the immobilized antigens.
- Production of *specific antigenic proteins and peptides* suitable for being immobilized in lab-on-a-chip surface. The proteins were previously validated as biomarkers of Lyme in small experimental clinical studies to differentiate between acute, chronic infections and autoimmune response associated with Lyme disease.

- *Optimized procedures for the immobilization of the antigens* into the detection chamber.
- Specific *quantum dot (QD) nanoparticles coated with antibodies* against human sera to be incorporated as reagents in the chip. QDs were used because of the excellent properties in terms of enhanced signal-to-background detection and performance in multiplexed assays.
- A *sample treatment protocol adapted to field conditions* to ensure maximum sensibility of the test and minimize cross-reactivity risks.
- A *highly sensitive fluorescence readout device compatible with the proposed lab-on-chip cartridge*, including the required waveguide structures able to detect and quantify the QD signals from the *different antigen spots in the detection chamber***.**
- Development of simple *data handling software* to allow communication of the reader with a PC to record and store the results.

Lab-on-chip will continue to be the most likely technological driver to transform the POC diagnostic industry. The potential to generate newer applications in the market are enormous. However, and despite the tremendous advances made in the lab-on-chip area, still, there are many challenges to solve [11]:

1. Development of components and procedures that integrate sample collection and processing, long-term stability of reagents, and materials that allow working with complex sample (blood, serum, urine, etc.).
2. Manufacturing of the different components that will contain liquid reagents and samples, sealing, and in many cases connections with electrical and microelectronic components. Improving of scale-up manufacture with rapid and low-cost techniques for prototyping. Despite numerous techniques and materials have been used for fabricating and sealing lab-on-a-chip devices (PDMS, PMMA, SU-8 silicon base, among others), many of them could not be cost-effective once scalable to be finally applied in commercial products.
3. Improvements in system integration allowing the connection of these miniature labs remain with record systems to be used in remote settings.

6 Lessons Learned with a Biotech Start-Up

Science is evolving rapidly, particularly in nanotechnology field. Advances in new recognition and sensing elements allow improvements in sensitivity, selectivity, limit of detection, signal-to-noise ratio; advances in new materials (polymer, paper, flexible or biodegradable polymers) that will achieve wearable and implantable biosensors for monitoring specific analytes; and new and improved manufacturing techniques, inexpensive and capable of producing thousands of chips integrating the different microelectronic components. Positive results encourage the future of point-of-care market, allowing sensitive, robust, portable, and inexpensive platforms that are being validated for clinical applications.

These are some of the lessons learnt while applying some of the possible solutions:

(a) Market-oriented approach: the sooner the target market is approach, the better to develop a successful solution. Developing a test from the background up to commercialization is a long path. It is of paramount importance to take a look on current players in the area (who can acquire the technology, what are their interests) and clarify the role that small companies want to assume on advancing over the different market requirements (product development, manufacturing, commercialization, etc.). That will also delineate R&D collaborations and joint ventures to key players in order to advance over product development and finally target the market.

(b) Establishing collaboration and partnership agreement: businesses, even small businesses, are increasingly integrating innovation elements in their overall strategy. In this context, R&D and technology transfer (TT) are progressively becoming core activities for companies or at least are directly linked to core activities. That is a fundamental aspect for all companies, especially for start-ups, as the path of development is long and includes multiple technical and economic aspects. In the biotech sector in particular, R&D collaborations and technology transfer are critical to SMEs in order to:

   – *Expand their capacity***.** There are many small, very specialized biotech companies. Sooner or later they need to collaborate with public entities, bigger companies, or each other in order to become part of a critical mass that they cannot generate themselves.
   – *Obtain public funding***.** Research funds are normally granted to consortia. Thus, collaboration with other entities (often established in other countries) is necessary to access public research grants.
   – *Gain access to venture capital***.** Strategic collaborations and TT agreements are common elements of a business plan that seeks to make the company more attractive to investors.
   – *Trade long-term risk for short-term benefit***.** Research in the biotech and medical areas is a long-term investment. Smaller companies are often willing to provide their knowledge and capacity for a smaller price and let others assume the financial risk of further development and commercialization.

   Thus, R&D and TT negotiation is highly relevant of the strategic plan and decisions of a business. In the biotech industry, it is almost impossible to separate one from the other; therefore, it is crucial for SMEs to define their strategy and be able to establish their position before negotiations. Moreover, both R&D and TT (licensing) agreements create long-term relationships and collaborations. Thus, their correct negotiation becomes even more crucial, since its outcome is likely to considerably affect the overall strategy of a business over an extended period of time.

(c) Financial aspects:

   – *Business risks are high*. Revenues, if any, are usually not expected in the short term. In the biotech sector, not only R&D collaborations but also licenses and

other business deals are often negotiated well before commercial products are expected to emerge. This makes particularly hard to calculate future revenues, percentages of ownership, royalty rates, etc.
- A good financial plan is mandatory to cover all parts of a long development up to market. Behind most of the start-ups, there are scientists, usually more focused on the technical aspects rather than economic or market orientation. Incorporation of skilled and trained financial person is mandatory.
- Despite possibilities on achieving public funding, that can also be disturbing from focused strategies up to market, since often R&D projects under public funding have specific constraints (specific collaborations, job creation, specific indication, etc.). Other financing strategies (loans and/or attraction of investors) may be more convenient and efficacious. Undoubtedly, the financial plan requires to be aligned up with the development strategy.
- *Commercialization costs and benefits are unpredictable***.** Therefore, it is also harder to establish objective criteria when negotiating the financial clauses of a deal or sharing exploitation rights and revenue shares. IP valuation is still volatile and knowledge is hard to assess in negotiations. The tangible parts of the deal (mainly biotech material, samples, or patients for testing, often provided by larger companies) may end up having disproportionally big weight in negotiations.

(d) Legal barriers. To reach market, tight regulatory requirements are required. Moreover, for commercialization, each country may have different rules for implementing technologies. Identifying regulatory requirements requires to be addressed during the project life as not to hinder or delay the commercialization.

(e) Intellectual property rights: The effective exploitation of the research relies on the proper management of the intellectual property, and so the early-stage protection of IPR is essential. As sooner, the analysis of the exploitation of our results shows the market opportunity, proceed and ensure the adequate protection form. Afterwards, it can be disseminated for publication in a scientific journal looking for a wider audience.

7 Concluding Remarks

The aim of biotechnology start-ups is to develop a novel diagnostic tool to improve clinical diagnostic, disease monitoring, and treatment of diseases by enabling specific and sensitive detection of the causative agent or the host response. Current laboratory diagnostic methods lack sensitivity and specificity to detect early cases as well as late manifestations of the disease such as chronic or autoimmune-related infections. For these reasons, disease incidence is underestimated as many cases go mis- or undiagnosed. Late, delayed, or inadequate treatment can lead to serious symptoms which can be disabling and difficult to treat. Sensitive and reliable patient diagnosis provided by novel devices will suppose newer resources available to medical practitioners, heavily reducing the current costs of the disease and increasing profitability and, most importantly, patients' quality of life.

The interdisciplinary of the scientific disciplines (biology, chemistry, nanotechnology, engineering, physics, mathematics, informatics, etc.) behind the teams of these start-ups is another aspect to take into consideration. These interdisciplinarity and cross-collaboration are required in order to cover the gap between research and market.

It is undoubtable that biosensors may play an important role in disease monitoring. Although many challenges still lie between the laboratory and the market, there is some evidence that commercial sensors are emerging in the market place. In fact, bio- and nano-sensor technologies if successfully commercialized into viable products or processes can revolutionize entire industries and significantly improve the quality of life for millions of people.

In this sense, innovative start-ups and SMEs are contributing enormously to face technical challenges related to POCT and biosensor area, by incorporating newer and sensitive technologies to raise accuracy and sensitivity while minimizing the costs. These companies represent a translational point from research to market; they are able to assume the risk to perform focused applied research. They are flexible companies with small overheads and full of highly qualified personnel opening vast new opportunities for scientists, engineers, and entrepreneurs coming from universities and top-level research centers.

But the road from idea to commercially viable product or process is long and challenging, especially when innovation level is high and research and development phases become lengthy and risky as they involve not only a substantial investment of time and effort but also require a relatively large amount of capital investment. This time from initial idea to successful market introduction is often called the "valley of death." It is in this phase that most new ventures fail. Nonetheless, the importance and impact of these technologies in real life are making large companies invest in potential solutions and, therefore, offer a wide range of opportunities for entrepreneurs to establish successful new businesses.

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Funding Frugal Innovation in Global Health: Philanthropy, Aid, and Industry

Rebekah Neal

1 Introduction

Fail early, fail often, and fail forward. Reduce complexity. Do more with less. Frugal innovation. Frugal engineering. These phrases, and the ideas they encapsulate, are hot topics that may have begun life as mantras in Silicon Valley but now span multiple arenas, including engineering, biomedicine, and global health and development. The term frugal innovation was coined by Carlos Ghosn, chairman and CEO of Renault and Nissan, in 2006, more than ten years prior to the publication of this edited book. It seems he intended the term to mean how to do more with less: produce products that are good enough at a fraction of the cost, presumably through less expensive components and less costly research and development (R&D) infrastructure. The cost saving in R&D then allows for the products to sell to end consumers for less, expanding potential markets. This concept plays particularly well in the developing world. For example, in India, where the concept of frugal innovation is called *jugaad*, we have illustrations of the concept from Ghosn's Renault-Nissan in the newly released Renault Kwid [1], a base-model sedan developed and made in India that sells for less than $5000, as well as General Electrics (GE) portable electrocardiogram (ECG) [2] and Manu Prakash's origami microscope, the foldscope [3], all cheaper, more portable versions of the original products. These examples of doing more with less in the auto, medtech, and instrumentation industries are just a few; many companies have embraced the idea of frugal engineering to try to attract consumers and provide products and services in markets not previously considered profitable. But to save and improve lives in the Global South, doing more with less does not just mean making simpler, cheaper versions of existing solutions. It must mean doing what Renault-Nissan is attempting with the Kwid and Prakash with the

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foldscope: developing solutions with direct relevance to consumers in a particular country with mostly local materials, as well as providing the ability to maintain and repair the technology with local expertise and parts. The jury is still out on how the Renault Kwid will do in India, but many of the right ingredients went into the design, development, and manufacture to give hope for success.

As we move more consumer goods into these markets, we should ensure we are not just creating new consumers, but are also helping to develop healthy, productive consumers. It is in our best interest as global citizens to enable all people to live healthier, more productive lives. Healthier populations tend to live longer, are more productive economically, and have more financial resources to buy consumer goods. Improved global health and economic status, particularly for those currently at the bottom of the health and socioeconomic pyramids, means more children survive, thrive, and contribute to society, and more parents can raise families with good nutrition, send their kids to school, and break the cycle of poverty.

Historically, frugal engineering has meant developing simpler, cheaper versions of technologies and tools that already exist and marketing them to populations who were not traditionally considered consumers. This model works relatively well for direct-to-consumer goods, such as cars, cell phones, and even some medical technologies. For example, the Renault Kwid may improve lives in India, making cars accessible, available, and safer for more people, and the Nokia 1100, a base-model cell phone marketed to consumers in India and on the African continent [4], may provide better connectivity and opportunities for improving health and financial stability through SMS health system reminders and mobile banking, respectively. But neither product begins to address the underlying health burdens faced by India and many other countries in the Global South, particularly the burden of infectious disease. The cost of infectious diseases in the Global South is high, both in terms of lost lives and productivity and of actual financial cost to discover, develop, and deliver interventions. And unlike direct-to-consumer goods, the cost of these interventions is often borne not directly by the consumer but by local governments, philanthropic organizations, and foreign governments' development aid organizations and programs. As a result, frugal innovation and engineering, at least by their standard definitions, struggle to produce impactful solutions for infectious diseases.

This chapter will examine how frugal engineering and innovation apply in the global health arena, with a particular focus on infectious diseases, the burden of which is borne disproportionately by the poor living in the Global South, and will offer suggestions for how philanthropy, the development and foreign aid communities, and industry might better work together to bridge the gap between innovation, frugal or otherwise, and impact.

2 Frugal Innovation and Engineering in Global Health

Frugal engineering cannot be straightforwardly applied to global health, particularly infectious diseases. The consumer population in the Global South typically has limited ability to pay compared to their counterparts in the Global North. Certainly

low-cost but "good enough" solutions would help in some cases; however, developing cheaper and lower cost versions of existing products or interventions fails where limited or no current products exist. When we consider the infectious diseases that generate the biggest burden in the developing world, including malaria, dengue, and neglected tropical diseases [5], we find a broad set of parasites, diseases, and conditions that do not similarly affect populations in the developed world. For example, malaria was eradicated in the USA in 1950s [6], and while the recent Zika outbreak has illustrated the possibility of the return of some of these diseases to the USA and other nontropical locations, the likelihood of significant disease burden is relatively low. However, no matter where we live and what we do, most of us in the developed world have a cell phone, and many own or have access to a vehicle or other transportation. But how many of us living in the developed world have malaria or dengue fever (carried by *Aedes aegypti*, the same mosquito that carries the *Zika virus*) or suffer from elephantiasis, a painful and stigmatized swelling brought on by infection with filarial worms? Instead of deciding which car or cell phone to buy, a family in rural Tanzania, in contrast, may actually have to choose between malaria treatment and primary school tuition for their children. With the imbalance between the funds available from consumers for treatments and the cost of pharmaceutical development, it is no surprise that the pharmaceutical industry focuses its efforts on noncommunicable diseases (NCDs) such as diabetes and cancers. For infectious diseases, often the market forces do not match the demand for intervention, and the budget for development of new interventions may come from government aid programs, philanthropists, and occasionally impact investors rather than from multinational corporations such as Renault-Nissan and Nokia.

Drug Donations for Neglected Infectious Diseases

As the exception that proves the rule, pharma does provide interventions for a handful of neglected tropical diseases. For example, in 2015, Merck KGaA donated over 100 million tablets of praziquantel to combat schistosomiasis [7], Bayer donates treatments for Chagas disease and human African trypanosomiasis [8], and a number of other companies donate similarly [9]. These donations are typically a part of the companies' corporate social responsibility programs and commitments and consist of offering existing drugs, rather than a new innovation or a commitment to new research and development matched to the needs of the developing world. While Merck, Bayer, and others deserve recognition and accolades for their contribution to the fight against these infectious diseases, currently available drugs only treat the infection but do not prevent reinfection, requiring regular administration to entire communities year after year. True innovation in this space requires new interventions – effective vaccines or curative therapeutics, likely combined with creative sanitation solutions – large donations of drugs or even improved in-country manufacturing will likely not be sufficient to solve these health problems.

Infectious diseases cause a significant portion of the world's global burden of disease. In 2012, the most recent year for which WHO reports causes of death, none of the top five causes of death in high-income countries were infectious diseases (ischemic heart disease, stroke, trachea bronchus and lung cancers, Alzheimer's disease and other dementias, and COPD), whereas the top three causes of death in low-income countries are infectious diseases (lower respiratory infections, HIV/AIDS, and diarrheal diseases) [10]. In high- and middle-income countries, people die predominantly from NCDs, while those living in low-income countries die predominantly of infectious diseases. Unfortunately, solutions for infectious diseases often do not already exist and must be discovered, tested, and developed from scratch. In addition, the most cost-effective interventions in the long run are often vaccines or therapeutics, which are also among the costliest solutions to develop [11]. New diagnostics can be cheaper to develop and face fewer regulatory hurdles but tend to be less useful when not paired with effective therapies or preventive strategies. For example, understanding and diagnosing which patients infected with *Mycobacterium tuberculosis* (the causative agent of tuberculosis) will go on to develop active disease and be at risk for transmission to others would be a tremendous leap forward in the field of TB diagnostics. However, until a drug regimen exists that works faster than four to six months of daily doses, the applicability and ultimate impact on TB infection, duration, transmission, and outcomes in the developing world may be limited.

In order to discover and develop new interventions for infectious diseases in a productive and cost-effective way, let us consider a shift from the traditional definition of *frugal engineering* to the broader concept of *frugal innovation*.

Navi Radjou, an innovation and leadership expert focused particularly on frugal innovation or *jugaad* in India, and his colleague Jaideep Prabhu at Cambridge Judge Business School outline six principles for frugal innovation in the developed world [12]. These ideas grew from three original principles Navi Radjou popularized in his TED talk in 2014 [13]:

1. Keep it simple. Develop products and interventions that are easy to use and make use of widely accessible materials, skills, and information.
2. Do not reinvent the wheel. Understand the current landscape and what has already been tried, and leverage existing resources.
3. Think and act horizontally. Rather than building larger and larger manufacturing capacity in a location in the traditional sense of scaling up, develop a distributed supply chain of small-scale manufacturers or providers in various geographic contexts.

These principles fit well in the context of refrigeration or sanitation solutions, such as those discussed in the TED talk. However, these principles of frugal innovation are insufficient to drive real change in burden of infectious diseases in the developing world.

The challenges for frugal innovation in standard pharmaceutical development include mismatches in terms of (1) budget, (2) timeline, and (3) pipeline for drugs, vaccines, and diagnostics for infectious diseases. Let us consider these shortcomings and elucidate the types of questions we must ask in order to modify and apply

the concept of frugal innovation to infectious diseases of the developing world. First, considering budget, it is a significant challenge to be frugal in an expensive and highly regulated industry. Although the final product may be relatively cheap to administer (e.g., oral polio vaccine costs less than $0.20 per dose [14]; TB diagnosis via GeneXpert costs about $10 per test [15]; ivermectin is free through the Mectizan Donation Program [16]), the development and regulatory process far exceeds those price points. The costs of development, including clinical trials for regulatory approval, can run over a billion dollars for vaccines and therapeutics [17, 18], not including the additional development costs for drugs and vaccines that fail at the preclinical or Phase I trial stage. Second, considering timeline, new products in the pharmaceutical industry may take years for development and licensing. Vaccines, in particular, face a high burden for safety and efficacy, since the target population is healthy, sometimes requiring larger and longer clinical trials. From a profit perspective, we can understand why pharma companies focus their efforts on drugs for hypertension, diabetes, cancers, and other NCDs: the developed world market is large, and reasonable uptake of an effective drug, vaccine, or diagnostic can help to recoup the costs of research, testing, and development. In addition, the pharmaceutical industry is not set up for horizontal scaling. Big pharmaceutical companies have big manufacturing facilities and need them to achieve economies of scale on more expensive products. While some progress is being made with vaccine manufacture in India [19, 20] and China [21], illustrating the potential for scaling locally while developing local infrastructure, talent, and resources, these examples remain relatively few and far between. Third, filling the pipeline with new ideas and development, such as new hits or leads in the drug discovery space, is particularly fraught with challenges. A few key questions to consider include:

- If free market economics will not drive the development of vaccines and therapeutics for the global poor, how do we engineer or innovate a solution?
- How do we balance the goal of bottom-line returns with the need to develop solutions for those who can't afford to pay?
- If industry is unlikely to develop new drugs and vaccines for the developing world based on market forces, what role do development aid and philanthropy have to play?

The concept of frugal innovation remains silent about these questions. Thus, switching paradigms from frugal engineering to frugal innovation is not sufficient; even the more expansive concept of frugal innovation fails to capture what's needed in the medical context of infectious disease. For this purpose, we may need to further expand and modify the standard definition of frugal innovation.

History shows that government aid and philanthropy alike have struggled with sourcing and developing innovative ideas for drugs and vaccines for global health. This mismatch between both frugal innovation and frugal engineering and the standard drug and vaccine development timeline, budget, and pipeline are the reasons the development/aid sector has not been consistently successful with discovering, developing, and delivering these solutions.

Sourcing Innovation

As outlined in "The Re-emerging Art of Funding Innovation" by Gabriel Kasper and Justin Marcoux [22], the strategic philanthropy movement has pushed many funders away from innovative, high-risk projects and instead toward relatively sure bets that will advance their strategic agenda. While this may lead to progress, it doesn't leave space for those "crazy" ideas that may change the world. As the pharma industry [23, 24] and government aid agencies (e.g., USAID Development Innovation Ventures, Global Innovation Fund) have tried to do, some grant-making organizations have begun to intentionally develop funding mechanisms and portfolios that allow for greater risk-taking in the hopes of finding those ideas that will result in transformative change. Kasper and Marcoux discuss a focus on experimentation – the notion that we have to try, fail, and try again. An example of an ongoing program for sourcing innovation is the Bill & Melinda Gates Foundation Grand Challenges Explorations (GCE) program. Launched in 2007 as a companion program to the Grand Challenges for Global Health, the GCE program seeks to engage more innovators around the world more quickly. Applications are two pages, and the review process is champion based (i.e., a handful of "innovation experts" have the opportunity to champion a proposal for funding) rather than consensus based. Awards are $100,000, with minimal restrictions on the funding and light oversight to allow grantees to explore their idea in their own way. While this represents one method for sourcing innovation that has shown success in bringing in new ideas and new people to focus on challenges in global health and development, it is not and should not be the only method. Agile, small grant programs of this sort enable the engagement of many new people; however, they do not allow for the try, fail, and try again opportunities that bigger, longer-term grants do.

3 Innovating on Frugal Innovation

Standard definitions of frugal engineering and frugal innovation have not worked well for the development of new interventions for infectious diseases, particularly drugs, vaccines, and diagnostics for the developing world. What do we need to do differently as philanthropy, development aid, or industry interested in addressing the infectious disease burden in the developing world? Specifically, how might we modify or extend the current principles of frugal innovation and effectively apply these ideas to development processes that are currently anything but frugal and innovative?

Expanding upon the key components of frugal innovation outlined above, I suggest supplementing with two principles of critical importance for the success of frugal engineering and innovation in interventions for infectious diseases of the developing world.

- Evaluate the impact of potential solutions. Impact in this context means the health impact of an intervention such as a drug or vaccine once it enters the global market and reaches the end users [25].
- Build better bridges between grant funding and sustainable business. More consistent coordination efforts among philanthropy, aid, and industry, whether in the form of public-private partnerships, mentoring relationships, or other alternatives, would push us toward sustainable, marketable interventions that won't rely on grant funding or subsidies in the long term.

3.1 Evaluate Impact

To be frugal and efficient in the interventions we choose to fund, develop, and transition to scale, we must *better understand and comparatively evaluate the impact of the solutions we seek to develop.* Understanding the impact of a particular intervention includes clearly understanding the problem and the multiple possible solutions, not just from the point of view of the funder, discoverer, and developer but also of the buyer and the end user. Unlike consumer goods where the buyer and the end user are often the same (e.g., if I buy a car, I typically purchase it for my own or my family use), drugs, vaccines, and diagnostics for infectious diseases, particularly those affecting the world's poor, are often purchased by the Ministry of Health or paid for by philanthropic funds (e.g., GAVI funding for vaccines in GAVI-eligible low- and middle-income countries [26]), while the end users may be individuals in communities receiving the diagnostic test, drug, or vaccine.

In understanding the infectious disease burden, we often turn to the Global Burden of Diseases, Injuries, and Risk Factors Study (GBD), a relatively comprehensive set of metrics and modeling developed by the Institute for Health Metrics and Evaluation (IHME) at the University of Washington. Unfortunately, any model is only as good as the baseline data, and when it comes to infectious diseases of the developing world, data is often scarce and difficult to access. So while we have estimates of the global burden of various diseases from the GBD, it can be quite difficult to use this data to evaluate the potential impact of a variety of solutions, particularly in a comparative way. For example, if we eradicate malaria, do we actually remove the roughly 9.5% of under five deaths caused by malaria, or will those children still die before their fifth birthday from lower respiratory infections (~14.5% of total deaths under five in the developing world), diarrheal diseases (~8% of total deaths), measles, whooping cough, or other infectious or noninfectious causes?

The metric of choice in the development community tends to be disability-adjusted life years (DALYs), a calculation that sums the years of life lost (YLL)

and years lived with disability (YLD) for a specific disease, condition, or injury, taking into account the average life expectancy in that country and the level and length of disability caused by the disease or condition. DALYs are a useful way to compare conditions, but again we run into difficulties when comparing DALYs from multiple diseases or conditions. Many in the developing world, particularly children under five, have multiple coinfections, as well as a lack of access to good sanitation and clean water. With the complex interplay of living conditions, diseases, and other risk factors, we must ask ourselves if DALYs are really the best metric to decide whether to put our dollars behind a vaccine for malaria or therapeutics for bacterial pneumonia. Until we have better data at the country, regional, and even village level, it will remain very hard to establish the potential impact, particularly in terms of lives saved or deaths prevented, to allow for comparison across multiple solutions and effective decision-making on which to develop with a limited budget and timeline.

With the existing incomplete data and knowledge about infectious diseases, how can we make effective impact assessments? The answer unfortunately is that we cannot. We need better data to build better models and make better decisions on how to spend the limited development budget. There are efforts underway to improve the baseline data, including the Child Health and Mortality Prevention Surveillance Network (CHAMPS) [27], and the GBD is continually seeking newer, better, and more granular data to build out better estimates of the burden of disease. All of us with a vested interest in developing the best, most effective, and efficient products for the reduction of the infectious disease burden in the developing world should work together *to develop clearer evaluation plans and success metrics for the projects we pursue and to share the data we collect on the problem* (e.g., disease burden) *and the solution* (e.g., development and delivery costs, uptake and community engagement challenges, lives improved, lives saved, etc.) *rapidly and publicly*. Diligent data collection and, more importantly, data sharing will help ensure the development community can support solutions that work and help bring them to scale while avoiding repeated investments in ideas or interventions that have failed to deliver on their promise. If we can all commit to a better data collection and analytics, as well as consistent sharing of results and the underlying data, by the time this book issues a second edition, we may find ourselves able to provide suggestions on how best to use the wealth of data available on drugs, vaccines, diagnostics, and other solutions for infectious diseases, rather than recommending that we do a better job of collecting and reporting data.

3.2 Better Bridge Between Philanthropy/Aid and Industry

To truly deliver transformational and sustainable solutions for infectious diseases, we need to find a way to bridge two big gaps: (1) the pathway between discovery/innovation and impact and (2) the space between grant funding and sustainable,

marketable solutions. A key component to understand and ultimately overcome these obstacles may lie in *better coordinating and collaborating between philanthropy and industry*.

The approach to innovation and strategic focus tends to differ widely between philanthropic organizations (including development aid organizations) and industry. Regardless of whether a company is a pharmaceutical giant, a small start-up, a social enterprise, or one of the multitude of companies that fall somewhere in between these categories, industry requires a financial return, which in turn requires a sufficient market. Social enterprises, organizations that typically have dual missions of social and financial returns, may willingly accept lower financial returns as the trade-off for achieving the desired social returns, but financial stability and sustainability are still necessary in the long term. Impact investing [28], the practice of investing with a specific goal of social or environmental returns, has grown steadily as a fund-raising option for social enterprises, but even impact investors expect a financial return on their investments. Organizations such as the Global Impact Investing Network (GIIN) and Investors' Circle build out networks of impact investors, along with programs and platforms to bring social entrepreneurs and enterprises and impact investors together; however, even with these networks serving as catalysts and connectors, impact investors rarely select social enterprises that are pursuing big, expensive products with long development timelines and high failure rates. If you browse the portfolios funded by investors in these networks, you are likely to find medical devices, service delivery models, software solutions, and other companies in the medical and healthcare industries, but you are unlikely to find more than a handful of companies developing new drugs and vaccines.

So what happens in areas where the market is small or the buyers can't pay, as in the case of many infectious diseases that plague the developing world? Often this void is where governments with their development aid or research budgets and philanthropic organizations step in, focusing on funding projects and pipelines in areas where the market forces are not strong enough to drive private investment and industry attention. The major sources of funding for health in the development arena (including but not limited to funding for infectious diseases such as malaria, HIV, TB, etc.) include government foreign aid budgets; the World Bank and regional development banks; the Global Fund to Fight AIDS, Tuberculosis and Malaria; and the Bill & Melinda Gates Foundation. Much of this funding, however, is building infrastructure and healthcare and research capacity, supporting vaccination campaigns (e.g., GAVI, funded by many of the donors listed above, disburses over $1 billion/year for vaccine procurement and to implementing countries for the purpose of accelerating the introduction and uptake of new and underused vaccines [29]), and delivering already developed tools, interventions, and services (e.g., promoting Kangaroo Mother Care and exclusive breastfeeding), rather than supporting innovation in the discovery and development of new solutions to combat infectious diseases.

The development aid and philanthropic sectors are learning to take risks and fund innovative ideas. For instance, the Grand Challenges programs [30], a family

of programs fostering innovation to solve key global health and development problems, seek to engage the world's brightest minds to tackle the toughest problems in global health and development. Specifically, Grand Challenges Explorations (GCE), an initiative of the Bill & Melinda Gates Foundation, offers $100,000 awards to test out big ideas against specific and strategically focused challenges in global health and development. Another example is the Grand Challenges for Development program at USAID. The Saving Lives at Birth program offers "seed" grants of $250,000 to achieve proof of concept and reach prototype stage and "transition to scale" grants of up to $2.5 M to transition successful ideas to scale. After four rounds of funding innovation for mothers and newborns, for round five and going forward, a new level was added for "validation" funding, situated between the seed and transition to scale awards. The validation stage is intended to bridge the gap between achieving proof of concept and beginning the transition to scale. The addition of this stage indicates an understanding that, particularly in the global health arena, the gap between the initial innovation and impact at scale is difficult for innovators to bridge, in both the public and private sectors.

While these programs may be considered successful at the initial stage of sourcing great ideas, and targeting diseases that are not considered profitable in industry, none can yet point to a drug, vaccine, or diagnostic that received seed funding and has since completed development, manufacturing, and delivery to provide impact on the ground. In fact, the top award levels in these programs are barely sufficient to achieve preclinical proof of concept under standard development timelines and budgets. While many of these programs are too young to have delivered a truly new drug or vaccine based on standard development timelines, we should be starting to see a strong pipeline of ideas de-risked by this initial funding and ready for uptake by the pharma industry.

If development aid and philanthropy can provide useful sourcing mechanisms for innovation in drugs and vaccines for infectious diseases, collaboration and partnership with industry are likely the best path forward to turn these great ideas into impactful products.

How can we build structurally sound and sustainable bridges between grant-funded innovation and marketable end products?

3.2.1 Bridge Directly

Philanthropy and development aid can facilitate bilateral or multilateral partnerships and help create product development partners (PDPs) who have the expertise, capabilities, and capacity, both financial and human resources, to take ideas that have achieved proof of concept through development. For example, PATH Malaria Vaccine Initiative (MVI) is a global program that seeks to accelerate the development of malaria vaccines. MVI provides the technical expertise for malaria vaccines under development by overseeing clinical trials, establishing private sector partnerships for manufacturing, supporting regulatory capacity in malaria-endemic countries, and other activities to support the discovery and development

of malaria vaccines. The PDP structure can provide a smooth link between innovative, grant-funded, early-stage projects and the development capacity needed to bring these ideas to fruition and ultimately impact at scale.

3.2.2 Strengthen the Support

When direct bridging is not an option or when it's not yet available, grant-funding organizations should consider how to provide their innovators with the support they need to understand the innovation ecosystem, how to successfully develop a product and build market demand, and how to take a successful product from field prototype to regional and potentially global scale. For example, USAID's Center for Accelerating Innovation and Impact is developing tools such as Idea to Impact: A Guide to Introduction and Scale of Global Health Innovations [31], along with a practitioners' workbook and innovator toolkit to provide information and real examples of sustainable interventions. For a more hands-on approach, they offer venture development training and access to other innovators, mentors, funders, and development experts through the Development^{X}Change and other venture development training programs. While these programs are just examples and may not be right for every grant-funding organization or every innovator, we should better educate and prepare innovators early in their work to consider the issues of delivery, uptake, and scale. Neglecting these long-term concerns early in the development process can result in time and funds wasted on a project that was never deliverable or scalable. Particularly in the context of drugs and vaccines, early considerations should include what criteria the target product must meet to be deliverable in the developing world (e.g., oral versus IV dosing, single versus multiple doses, vaccination schedules that match current vaccine programs) to be able to achieve the scale and impact we seek.

4 Conclusion

Although the concepts of frugal engineering and frugal innovation have shown some promise in particular engineering and innovation contexts within the developing world, neither speaks directly to the challenges posed by pharmaceutical development for infectious diseases. While neither of these concepts in their current iterations has direct applicability to pharmaceutical development for infectious diseases, with some modifications, we have shown how frugal innovation could be restructured to address more of the roadblocks facing innovation in global health challenges. Specifically, greater attention to evaluating the impact of a particular solution and building better bridges between grant funding and sustainable business are critical requirements for frugal innovation in these contexts.

Along with the need to refocus on evaluating impact comes a call for each of us, whether we are funders, innovators, government representatives, or just global

citizens, to collect and share better data around the potential and realized impact of our solutions. Merely reporting how many people have purchased an item, for instance, is not sufficient to know if an impact has been made. We must instead follow the chain farther to know whether the new diagnostic tool led to better diagnosis and treatment, the new drug regimen was followed and completed, or the new vaccine was administered to children in remote villages who might be at risk. Likewise, we must better understand the underlying burden of the diseases we seek to diagnose, treat, or vaccinate against to calculate the potential impact in lives saved, DALYs, or any other metric.

Similarly, without building better bridges between philanthropy and industry, we risk funding exciting, innovative ideas that ultimately languish in the lab. To translate these new ideas into new diagnostics, drugs, and vaccines, we need the help of those who understand the development, manufacturing, and delivery challenges. Without these bridges, much of the incentive for industry to pick up de-risked innovations from grant-funded projects disappears, and we are back where we started with industry focusing on development of products for those who can pay. If well designed, these bridges could develop into true symbiotic relationships, where the pipeline of innovative ideas funded by philanthropic or government aid dollars could funnel into the development pipelines of industry to yield a functional product more quickly than if we begin from scratch each time.

If we follow the principles of frugal engineering and innovation, effectively source and fund innovation in infectious diseases through grant-making organizations, and then effectively transition successful interventions through product development, regulatory approval, manufacturing, delivery, and scale, we can reduce or eliminate the burden of infectious diseases on the world's poor.

Disclaimer This book chapter was prepared by Rebekah A. Neal in her personal capacity. The opinions expressed in this article are the author's own and do not reflect the views of the Bill & Melinda Gates Foundation.

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Concluding Remarks

Arvind K. Chavali and Ramesh Ramji

In the Preface, we began this book with a discussion on the importance of frugal innovations in addressing the health needs of communities in resource-poor settings. We were inspired by the invention of the "Foldscope" – an origami-based paper microscope – to learn about other frugal tools that are being developed for diagnostic purposes to fight infectious diseases. As an innovation that seeks to simplify an existing technology (a light microscope) at a cost that is greatly reduced, the Foldscope can be classified as a "lean tool or technique" based on a framework posited by Tran and Ravaud [1]. Many of the chapters in this book discussed various types of lean tools and techniques, especially in the area of microfluidics that satisfy ASSURED guidelines from the World Health Organization for point-of-care use. Beyond that, a few chapters also touched upon the development of diagnostics for detecting pathogens in food and environmental samples as well as the role that philanthropy plays in continuing to help fund frugal innovations.

This book only scratches the surface of frugal bioengineering innovations currently under research and development with new lean tools being continually published in the literature. Only very recently, we came across yet another lean tool named "Paperfuge," which is particularly relevant as a diagnostic in the context of infectious disease. The Paperfuge is an ultra-low-cost (~$0.20), lightweight (~2 g), and power-free centrifuge made from paper that was demonstrated to achieve speeds of 125,000 rpm (with theoretical limits of up to 1,000,000 rpm). Experimental validations of Paperfuge showed that the device is capable of isolating malaria parasites from blood samples spiked with 7.5% *Plasmodium falciparum* parasitemia in 15 min ([2]).

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Given the focus of this book on diagnostics/lean tools and techniques, we did not cover in depth the other aspects of Tran and Ravaud's frugal innovation framework, which neatly classifies all frugal innovations into four different categories. Besides lean tools and techniques, the other three categories include "opportunistic solutions," "contextualized adaptations," and "bottom-up innovations" (see the Preface for description and examples). Below, we wanted to describe a few examples of recent advances in these three remaining categories. Importantly, while some of the examples below include diagnostics, many are focused on prevention or treatment of infectious diseases.

1. Innovations that make use of a widely available technology such as mobile phones or the internet are termed as opportunistic solutions [1]. In terms of diagnostics, one important example is cell phone–based digital microscopy [3, 4]. In on-the-field trials, cell phone devices have been used to quantitatively identify samples containing *Schistosoma haematobium*, *Schistosoma mansoni*, *Loa loa*, and *P. falciparum* [3]. Other advances in this space that are more focused on prevention and treatment include the use of SMS-based product checks for the authenticity of medication (to fight against counterfeit drugs; Sproxil.com) as well as the use of SMS to improve adherence to antiretroviral treatments [5].
2. Solutions that use locally available materials but repurposed for a novel clinical use are termed as contextualized adaptations [1]. In the Preface, we described the Solarclave for sterilizing surgical equipment [6]. Another example in this space includes the use of an over-the-counter mentholated topical ointment (Vicks VapoRub) for the safe and cost-effective treatment of toenail onychomycosis [7]. In a pilot study, of the 18 patients that were treated with Vicks VapoRub for up to 48 weeks, 15 showed a positive treatment effect and 5 patients demonstrated mycological and clinical cure at 48 weeks [7].
3. Tools and techniques born out of necessity and adapted to local settings are termed as bottom-up innovations [1]. We previously described solar disinfection of water to reduce the occurrence of diarrhea ([8]; see the Preface). Another related advance is the use of sari cloth filtration of water to reduce the incidence of cholera, which was demonstrated in a field trial in Bangladesh [9].

   Additional examples in each of the four categories are also listed here: http://frugal-innovation-medicine.com

To reiterate an argument that we made in the Preface, a key challenge to keep in mind as tools and innovations are developed across the entire frugal engineering spectrum – from lean tools and techniques to bottom-up innovations – is to ensure that the frugal technology is (a) built for scarcity, (b) scalable, and (c) sustainable [10]. In other words, the technology must be able to function in a severely resource-limited environment, must address a need with proven efficacy, and must suit local user preferences to allow for long-term adoption. Therefore, to enable researchers at universities and institutions around the world to produce and properly scale frugal technologies that truly address the needs of the poor, Nilsson and colleagues at the University of California, Berkeley, argue for an interdisciplinary approach [11]. To this end, the authors put forth an idea for a new

field titled “Development Engineering” with the aim of combining engineering innovation with an understanding for institutional gaps, market failures, cultural constraints, and behavioral biases [11]. Nilsson et al. suggest that formalizing a field like Development Engineering, which builds on concepts from engineering, development economics, behavioral science, and sociology, can help in terms of better aligning academic incentives with practices intended to achieve real-world impact. A key ingredient to the success of Development Engineering and the generation of sustainable frugal technologies will be collaborations between universities, NGOs, governments, and the private sector [11].

Ultimately, we hope that we have provided readers with a useful and informative introduction to the many aspects of frugal science. The frugal innovations highlighted in this edited book are vital to pushing the boundaries of science and engineering by demonstrating what may be possible to achieve in terms of diagnostic capabilities with simplicity, ingenuity, and minimal use of widely available resources.

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