Chapter 12
Diagnostic Innovations in Developing Urban Settings

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Health in Developing Urban Settings

There is a significant body of literature highlighting the importance of point-of-care diagnostics in improving health in developing countries [1]. Access to effective healthcare options can be severely limited in the developing world by the (typically) large distance between villages and appropriately stocked clinics and poor accessibility to skilled clinicians and physicians. This problem is often exacerbated by inadequate access to clean water, sanitation services, and a reliable source of electricity. The development of diagnostic tests, designed specifically for use in low-resource settings, would thus meet a substantial need. Urban and rural settings in the developing world are often beset by a lack of resources. The unique characteristics and different diagnostic needs of each setting, however, must be considered. Much attention is given to novel diagnostics designed for primary care settings in rural areas where patients are geographically far from the formal healthcare system, while urban areas are largely ignored. To understand the importance—and future—of diagnostics in developing urban settings, it is necessary to first understand the distinct ecosystem present there.

While the world is becoming more urbanized, there are significant differences behind the reasons for urbanization and the effects of urbanization on a city's residents in developed- and developing countries. Developed country urban areas and developing country urban areas have very different standards of public health, environmental health, and city planning. Between developing urban areas, there is important variability. For example, while urbanization appears to correlate with economic opportunities in many Latin and South American countries, this trend does not hold in Africa, where urbanization is more often driven by high fertility rates and people

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fleeing rural poverty. More importantly, developing urban areas have not benefited from the health improvements afforded by better housing and sanitation—as has occurred in developed urban areas. Instead, developing urban areas contend with an increasing burden of noncommunicable diseases in addition to persistent infectious disease. According to a 2000 World Health Organization article, the environmental change precipitated by urbanization has created novel environments for the spread of pathogens not typically found in those areas, adding to the burden of existing infectious disease. Unregulated slums in large cities, in particular, are susceptible to these effects [2].

An additional problem in developing urban areas is the large disparity between high-income and low-income populations. Income disparity without an engaged middle class can undermine public health services, leaving low-income patients with few affordable health options. High-income communities are typically more engaged in government and public decision-making than those in low-income groups, and they also are able to insulate themselves from the dangerous risk factors common in cities (e.g., pollution, poor health services, poor nutritional content of food). There is thus little incentive to improve public health systems that would benefit all levels of society. Without a middle class that is politically engaged but unable to afford the insulating solutions of the wealthy class, the unengaged poor are left with suboptimal public health systems and the burden of paying for health services through the private sector [2]. Therefore, affordable healthcare options are just as important in developing urban areas, as patients rely more on private providers of health care. Likewise, assumptions that point-of-care diagnostics are unnecessary in urban areas (where the distance to healthcare facilities is not as great as in rural areas) do not hold, though for different reasons that are outlined below. Other characteristics of an ideal diagnostic for rural settings, while still favorable, might be deprioritized depending on how health care is provided. To identify which characteristics are important in urban areas, we must first look at the types of diseases needing to be diagnosed.

Disease Classification and Diagnostic Needs

Diseases can be classified as either *communicable* or *noncommunicable*. Communicable diseases are those caused by pathogenic microorganisms (i.e., viruses, bacteria, and protozoa) and can be transmitted between people or animals, whereas noncommunicable diseases are those that cannot be transmitted to others. Both types of diseases can present as an acute sickness or a chronic, persisting affliction. Communicable and noncommunicable diseases present the healthcare system with very different challenges in terms of diagnosis and treatment. Communicable diseases primarily require diagnostic tools to identify a causative agent in order to inform proper treatment, while noncommunicable diseases, especially those of a chronic nature, require diagnostic tools for ongoing patient monitoring as well as initial diagnosis. Though the diagnostic requirements for each disease type differ, management of each disease type in developing urban settings would greatly benefit from innovation.

Communicable Diseases

Communicable diseases can spread through a population by a number of different mechanisms that result in the transmission of pathogens to humans: (1) direct transmission between people via contact with contaminated bodily fluids (e.g., rhinovirus or *M. tuberculosis*); (2) indirect transmission through environmental contamination, often with an origin in the feces of humans or animals (e.g., Giardia parasites or *E. coli* bacteria); (3) by a vector through a relatively unaffected host organism (e.g., malaria parasites carried by mosquitoes); or (4) by infected animals (e.g., rabies virus). Zoonotic pathogens—those agents transmitted to humans from nonhuman origins—account for greater than 60 % of infectious diseases that affect humans [3, 4]. In the case of zoonotics, the value of diagnostic tests rises beyond monitoring populations of animals (e.g., livestock and companion animals) to protecting human health, food sources, and the livelihood of farmers.

The focus of a diagnostic for a communicable disease is on the identification of a causative agent to inform clinicians and guide the selection of a treatment. Once an appropriate treatment has been determined, the need for further diagnosis is minimal; notable exceptions include chronic communicable diseases (e.g., hepatitis C), which might require ongoing monitoring [5]. In rural settings, a rapid diagnosis is important, because patients must travel long distances to centralized health facilities, and patient follow-up is difficult [6]. If they do not receive a diagnosis with their initial visit, it is unlikely that they will receive their results. These limitations, especially physical distance, do not necessarily apply in developing urban settings, but the need for rapid identification of communicable disease is just as important. In one study, less than 10 % of the patients testing positive for syphilis using a non-rapid diagnostic in Nairobi received treatment [7]. Additionally, poor public health and high population densities mean that hospitals are typically overburdened, and the time to treatment and quality of care can suffer [8]. One way to alleviate some of this burden is to push screening for communicable diseases to primary care facilities or the home through the use of point-of-care diagnostics. This strategy would allow for efficient triage outside of the hospital, separating those who need hospital follow-up from those who can self-treat. This potential improvement in care provision is dependent on innovations to bring the high quality of testing found at centralized hospitals to point-of-care platforms available outside of the hospital.

Below, we describe the potential for low-cost diagnostic tests to improve health care in developing urban settings using two model communicable diseases: enteric disorders and HIV/AIDS.

Enteric Disorders

Diarrhea, caused by infections from enteric pathogens, is a leading cause of mortality and morbidity worldwide [9] and affects young children disproportionately [10, 11]. The reduced availability of clean water and unsanitary living conditions in many developing urban environments (e.g., slums) exacerbates the spread of enteric diseases. The physiological symptoms of enteric disorders are easy to diagnose

(e.g., abdominal pain and/or diarrhea [12]), but infection by a number of pathogens [13]—bacteria (e.g., enterotoxigenic *E. coli*), viruses (e.g., rotavirus), and protozoa (e.g., cryptosporidium)—are known to cause this very common condition. Proper treatment requires identifying the cause of the symptoms. Antibiotics, for example, will not cure viral diseases and, when administered prophylactically, can lead to drug resistance [14] or advantageous colonization by dangerous flora [15]. Therefore, it is important for clinicians to use diagnostic tests to identify the specific pathogen before selecting a method of treatment. Two common test methods include molecular diagnostics and standard microbial culture techniques. These methods, however, are difficult to implement in low-resource settings, because they are either too expensive (e.g., PCR amplification of a gene sequence) or require well-equipped, centralized laboratories with highly trained personnel (e.g., culture). In many cases, easy-to-use point-of-care tests to classify the pathogenic microorganisms would be sufficient to influence healthcare decisions and select a course of treatment.

HIV and AIDS

Infection with HIV ultimately leads to AIDS, an incurable disease. While AIDS is no longer considered to be acutely fatal, it remains a global epidemic and affects developing urban environments disproportionately. The causes for this disparity are related to living conditions in comparison to those in rural or established urban settings: inhabitants of developing urban centers become sexually active at a younger age, have more sexual partners, and are less likely to adopt preventative strategies [16]. AIDS can be managed with a carefully administered therapeutic program to slow the progression of the disease and treat opportunistic disorders. Over six million people in the developing world are treated with highly active antiretroviral therapy (HAART) [17], antiretroviral drugs that inhibit a number of the important pathways in the replicative life cycle of the HIV virus. The result of effective treatment is a decrease in the number of HIV particles in the blood and an increase in the number of CD4+ cells that can be found in the blood; diagnostic tests for these markers can be used as a prognosis of the efficacy of HAART (vide infra). Another use of diagnostics in the treatment of HIV is to monitor the side effects of HAART. Antiretroviral drugs may cause hepatotoxicity [18]; therefore, rapid point-of-care diagnostic assays for liver function would help healthcare workers determine the safety and efficacy of treatment regimens.

Noncommunicable Diseases

Noncommunicable diseases are the predominant cause of death worldwide. According to a 2012 WHO World Health Statistics report, mortality rates from noncommunicable diseases are rising and account for approximately 60 % of deaths per year in low- and middle-income countries [19]. Therefore, these diseases are a

major threat to global health and economic development. While noncommunicable chronic diseases, such as cardiovascular disease, cancer, and diabetes may have once been thought of as developed-world problems, the reality is that the developing world accounts for the majority of this burden. In a 2007 paper in *The Lancet*, Abegunde et al. state that in 2005 over 80 % of global deaths from chronic diseases occurred in low- and middle-income countries and that the overall burden of chronic diseases was quickly increasing, driven by rapid population growth in developing urban areas [20]. According to the same paper, the cost of doing nothing to address the issues of heart disease, stroke, and diabetes—in terms of reduced economic output—will be $84 billion over a 10-year period, with millions of people unnecessarily dying during that time period. Requiring patients to utilize healthcare facilities for ongoing management of their disease is inefficient and unsustainable.

Unlike communicable diseases, whose causative agents are identifiable, the causes of noncommunicable diseases are more difficult to diagnose. Sources of noncommunicable diseases may be environmental, genetic, or due to lifestyle choices. Diseases caused by environmental factors—or those that cannot be directly controlled by an individual—include acute toxicity from the ingestion of heavy metals (e.g., tainted water supply) and the effects of malnutrition. Genetic diseases include autoimmune disorders (e.g., lupus or celiac disease), hemophilia, and sickle cell anemia. Diseases that arise from the lifestyle choices of an individual include cirrhosis (liver damage linked to alcoholism) and emphysema (lung damage linked to smoking tobacco). While it may be instructive to classify the causes of noncommunicable diseases in this way, a great number of them are caused by a combination of factors. Examples of such noncommunicable diseases include diabetes mellitus type 2 (lifestyle choices and genetics) [21], allergies (environmental and genetics) [22], and cancer (environmental, lifestyle, genetics) [23].

According to a 2005 analysis of the disability-adjusted life years (DALY) burden—a measure of the costs of mortality and morbidity—in developing countries, the most burdensome noncommunicable diseases are: cardiovascular diseases, diabetes, cancer, and chronic respiratory diseases [24]. Notably, an important factor in the large DALY burden for each of these diseases is their chronic nature, requiring ongoing monitoring and care. A prime example of this is diabetes, where a single test might identify a patient as diabetic, but one or more blood glucose tests per day are needed to maintain an individualized plan to manage a patient's health. The need for continual monitoring in parallel with an extended period of treatment creates a vastly different model of healthcare provision than that encountered during the management of communicable diseases. If patient monitoring is limited to health facilities, the patient burden on the health facility will continue to increase, overwhelming the facility and reducing quality of care for all patients. Despite the physical proximity to healthcare facilities in developing urban areas, at-home diagnosis and monitoring solutions must become an integral part of the healthcare system.

For certain noncommunicable diseases (e.g., cardiovascular disease, many cancers), initial diagnosis requires highly skilled personnel and centralized facilities with specialized instrumentation. Access to these resources is restricted in most developing urban areas, due to heavily constrained healthcare budgets. The emerging field of

biomarker discovery, however, is reforming this paradigm by enabling the development of rapid, point-of-care diagnostic tests for noncommunicable diseases. A prominent example is the quantitative measurement of cardiac troponins as a means to diagnose cardiac health [25]. Immunoassays for troponin have been developed successfully for a number of laboratory [26] and point-of-care platforms [27]. These point-of-care technologies, however, rely on portable electronic instruments and individual test cartridges. The high costs associated with these components have limited their penetration in developing world markets.

Diabetes

For many reasons, diabetes can be considered the archetypal noncommunicable disease. There are methods to diagnose, monitor, and manage diabetes, and diagnostic tests are available and in use globally. Before the first blood glucose reader was introduced in 1970, monitoring of glucose levels by patients was limited to urine dipsticks. This was suboptimal, as urinalysis could only detect blood glucose levels elevated above the renal threshold and with an inherent delay in the results. Despite these limitations in at-home monitoring, it was not until the mid-1970s that at-home use of blood glucose readers was considered, and even then, there were significant concerns as to their accuracy, ease of use, and affordability [28]. The concerns have been addressed through technological advances, and at-home blood glucose monitoring is now an integral part of managing diabetes in developed countries. Similar diagnostic strategies exist in the developing world, but significant challenges still remain due to the economic strain of frequent testing and treatment. The costs associated with diabetes—due to the aggregate prices of consultation, syringes, insulin, and testing—can be massive in comparison to an individual's yearly income. For example, the cost of treating diabetes can account for up to 75 % of yearly wages for those living in developing urban settings [29]. Further complications arise for individuals with tuberculosis infections, as there is a strong link between diabetes and an increase in the risk of developing active and difficult-to-treat tuberculosis [30]. Proper diagnostic tests for each disease would generate valuable, actionable information toward providing individualized health care, and affordable at-home monitoring solutions would improve service provision at overburdened hospitals.

Cancer

One of the most difficult diseases to diagnose in a point-of-care or low-resource environment is cancer. Environmental (e.g., melanoma caused by sunlight), lifestyle (e.g., lung carcinoma caused by smoking), and genetic factors (e.g., lymphomas) can cause cancer, which is a leading cause of death worldwide [31]. Tumors commonly are located within the body, and, as a result, cancers are diagnosed using sophisticated imaging technology—X-ray, computed tomography (CT), and positron emission tomography (PET) are three such techniques—that is not

typically available in the developing world. A combination of tumor biopsy and microscopy is a more accessible approach but requires significant expertise and infrastructure to employ. The majority of cancer cases in the developing world are detected at an advanced and untreatable stage—due to inadequate screening and early diagnosis services [32]. Biomarkers may be useful diagnostically for some cancers, such as prostate cancer (prostate-specific antigen) and ovarian cancer (CA-125). While the accuracy and specificity for these biomarkers are still in question [33, 34], a bladder cancer biomarker (NMP-22) [35] has been validated clinically and is used in FDA-approved point-of-care diagnostic assays (NMP22 BladderChek, Alere). It is apparent that true point-of-care diagnostic tests—using a combination of new methods to obtain biospecimens, biomarker discovery and validation, and technology development—would find immediate use in diagnosing and combating cancer.

Solutions to Better Diagnosis in Developing Urban Areas

There are several possible ways to improve the health of patients suffering from chronic or noncommunicable diseases in developing urban areas. For example, improving the existing healthcare system, reducing income disparity or improving the voice of low-income urban dwellers, and reducing environmental and behavioral risk factors, could improve patient health [2, 36]. Another method to improve patient care would be to empower patients and improve treatment efficiency through at-home diagnosis and monitoring tools. As the incidence of noncommunicable diseases continues to rise in developing urban areas, the development of novel, affordable, at-home diagnostics will become immensely important to prevent overburdening of hospitals and the associated impacts on patient care.

Characteristics of an Ideal Test

The World Health Organization Sexually Transmitted Diseases Diagnostics Initiative (WHO/SDI) developed the ASSURED criteria to determine if diagnostics are suitable for deployment in limited-resource settings [37]. ASSURED stands for Affordable, Sensitive (i.e., low probability of false negatives), Specific (i.e., low probability of false positives), User-friendly (i.e., easy to use, requiring minimal training), Rapid and Robust (i.e., short time to a result, stable and reliable at ambient conditions), Equipment-free, and Deliverable to end users. These settings, however, place more demanding design challenges on diagnostics that are not as critical in developed urban areas with reliable sources of power, refrigeration, supply chains, and trained personnel. Although these criteria were created to evaluate diagnostics for resource-limited rural settings, many of these criteria are also relevant for diagnostics designed for use in developing urban areas.

The *affordability* of a diagnostic is particularly important to consider when designing Point of Care (POC) tests for developing urban areas. As stated in the introduction, while urban areas have relatively more wealth than rural areas, there is a great disparity in wealth that causes urban dwellers to rely more on private providers of health care, which means affordability is still important. Ideally, the final cost of the diagnostic should be pennies and include the cost of materials, manufacturing, packaging, shipment, and storage. Manufacturing tests in a developing urban area for local distribution and use reduces a number of these costs and has the added benefit of bolstering the local economy. The cost of materials can be minimized by using relatively inexpensive components, such as plastics and paper-based materials.

Clinical *sensitivity and specificity* are two important performance characteristics of diagnostic devices. These characteristics are evaluated by comparing the results of the test to the results of a reference standard test (i.e., a "gold standard") for the same panel of samples. The clinical sensitivity is defined as the probability that a sample shown to be positive by the gold standard test will also test positive using the test under evaluation. The clinical specificity is defined as the probability that a sample shown to be negative by the gold standard test will also test negative with the test under evaluation. Designing POC diagnostics with both high sensitivity and specificity is desirable. Diagnostic tests should also provide reproducible results. The reproducibility or precision of a test is often reported as the coefficient of variation (CV), which is the standard deviation of a measurement normalized by the mean. This value is typically reported as a percentage; an ideal diagnostic has a %CV of zero. Although this goal cannot be achieved realistically, diagnostic manufacturers strive to minimize this value and generally achieve CVs less than 10 %. For example, Roche's handheld Accutrend® Plus meter measures blood glucose and cholesterol levels with a %CV of ~3 % [38], and Abbott's i-STAT measures cardiac troponin I in blood with a %CV of ~8 % [39].

User-friendliness is another characteristic that is important, regardless of the setting, and is especially critical for tests designed for home use by patients to reduce the burden on hospitals. A user should be able to administer a test successfully with little to no technical training. Ideally, the test should require as few steps as possible, little to no sample preparation, and be minimally-invasive and self-contained. The results should be available within minutes of applying the sample, and the readout should be clear and have a low likelihood of misinterpretation. A rapid time to result enables users to take immediate action to manage a chronic condition or illness or prevent spread of infectious diseases. Diabetics self-monitoring blood glucose is a classic example where rapid time to result is important for the effective management of a disease.

An ideal POC diagnostic should also be *robust* and dependable. Diagnostics often contain biological reagents that can lose activity when exposed to harsh environmental conditions. In developed urban areas, tests are transported and stored in controlled environments (i.e., the cold chain) that prolongs a product's lifetime. In developing urban areas, however, reliable refrigeration and controlled environments may be lacking (e.g., brownouts), and tests may be exposed to harsh conditions

ranging from freezing to tropical temperatures that degrade device performance. Therefore, reagent stability is an important consideration when designing tests for developing urban areas. One method of improving the activity of reagents is to dry and store them on fibrous membranes with additional stabilizers, such as proteins and sugars.

POC diagnostics for use in limited-resource settings should require *no external equipment* and little to no power to operate. These characteristics are less important in developing urban areas where adequate infrastructure exists and there is often access to relatively consistent power. However, requiring equipment often increases cost significantly, which could limit availability of the diagnostic or restrict it to well-funded hospitals instead of primary care or at-home testing. One of the aims of innovation in diagnostics for developing urban areas is to push diagnosis and monitoring into primary care and at-home testing in order to reduce burden on hospitals. Therefore, the cost of required external equipment can be very important, even in urban areas.

Ultimately, a diagnostic must be adopted by its target population in order to generate a benefit. Barriers to adoption can include an unintuitive user interface, operational complexity, or something as fundamental as failing to avoid cultural taboos. An example of a product failing after market introduction due to this simple, yet vital, consideration is the mosquito bed net [40]. In this case, users in certain sub-Saharan regions of Africa were expected to sleep under white bed nets, a color culturally associated with those who had recently died. In this case, simply changing the color of the nets addressed this taboo. By involving potential end users through user-centered design, diagnostic developers can minimize the risk that their product will not be used for cosmetic or other nontechnical reasons.

This section has shown that while an *ideal* test follows the ASSURED guidelines, not every characteristic must be met in order for a diagnostic device to be useful in developing urban areas. In addition to considering these criteria, innovative groups must also work closely with end users to identify the most important characteristics of a test and ensure that the user interface is intuitive and that the test is culturally acceptable and perceived as useful. If these additional criteria are not considered, a diagnostic test may not achieve widespread adoption.

Technologies

As previously discussed, there exists a real need for innovative diagnostic technologies that will allow for diagnosis and monitoring to be moved from overburdened hospitals to primary care facilities and at-home testing. Because of the unique challenges of providing health care in developing urban areas, innovation at the technology level is critically needed. The sections below describe POC tests that are commercially available or in development at the time of publication of this chapter. The advantages and disadvantages of each test or diagnostic platform will be discussed in context of its utility in developing urban areas.

Commercial POC Tests

Several technologies already on the market could have a positive impact in developing urban areas if their current limitations are addressed.

Electrochemical Readers

Glucometers

Untreated diabetes and its associated complications are burdens on budgets, families, and healthcare systems worldwide. Although diabetes mellitus type 2 is often considered a disease of the developed world, it is a growing global problem [41]. The burden of diabetes in developing urban areas can be reduced through the use of glucometers. The glucometer is a POC diagnostic device that enables diabetic patients to regularly monitor and control blood glucose levels—through insulin therapy and/or diet—to prevent acute and late-stage complications of the disease. It is one of the most ubiquitous and commercially successful POC tests on the market in developed countries.

Early POC blood glucose test technologies were developed in the mid-1960s by the Ames Division of Miles Laboratories (now part of Bayer) [42]. Its test strips, marketed as Dextrostix®, contained dried enzymes and chromagens that produced a visible, colored product in the presence of glucose. The user applied a drop of blood to the reagent-impregnated zone of the test strip, waited 1 minute, rinsed the blood from the strip, and matched the color of the test zone to a calibrated, color-coded read guide. Two examples of tests on the market that use visual color matching to estimate blood glucose levels include the BETACHEK® Visual by National Diagnostic Products and the Chemcard™ Glucose Test by Chematics, Inc.

These tests are affordable, use robust reagents, do not require external equipment or power, are portable, and provide rapid test results (~3 minutes). The tests are convenient to use in any location, deliverable to end users, and reported to be accurate and reproducible. According to company product literature, an independent study showed that the BETACHEK® has a high level of accuracy compared to a reference method ($R^2 = 0.977$) and a low percent coefficient of variation (<5 %). Although these tests have many advantages, they require multiple user steps to obtain results, and the visual readout by color matching is subject to user interpretation.

Most glucometers on the market today consist of battery-operated, hand-held electronic readers and single-use, disposable test strips. To measure blood glucose levels, the user inserts a test strip into the meter and applies a finger-stick drop of blood to the end or side of the strip. The digital display provides visual numeric readout of blood glucose levels. These glucometers are cost-effective, accurate, and easy to use. User interaction is minimized, and blood glucose measurements are obtained visually or audibly within seconds.

Glucometers use electrochemical measurements to determine blood glucose levels, and the electrodes and reagents necessary for these measurements are printed directly on a disposable test strip. A finger-stick drop of blood is delivered to the reagents and electrodes on the strip via passive wicking through a microfluidic capillary. Today's meters require less than 1 μL of blood and provide results in 5 seconds [43]. The prevalence of diabetes and the need for frequent blood glucose measurements create a high demand for glucose test strips. The strips are produced at a scale of approximately 10^{10} test strips per year, with single manufacturing lines producing roughly 10^6 test strips per hour [44]. These large manufacturing scales are achieved using well-established, high-precision printing and lamination technologies that drive the manufacturing cost down to pennies per test strip.

Digital glucometers have several advantages for disease management in developing urban areas, because they satisfy most of the ASSURED criteria. Although the electronic readers require batteries, they generally have low power consumption and long battery lifetimes. The main disadvantage of this technology is the pain associated with finger sticks to obtain blood samples for analysis.

Given the frequency of testing, maximizing user comfort has been (and will continue to be) a key focus in the development of glucometers. Requirements of blood sample volumes, and thus lancing depths, have been reduced to minimize the discomfort associated with finger sticks. Many glucometers function with blood drawn from alternative sites, such as the arm or thigh [45]. Another approach to reducing the discomfort associated with lancing devices and blood sampling is developing minimally invasive or noninvasive measurement techniques, such as sampling interstitial fluids or transcutaneous spectroscopy [43]. These devices have the potential to make continuous blood glucose measurements and enable closed-loop insulin dosing. Scientists are also developing ways to reappropriate electronic glucometers to measure analytes other than glucose in whole blood, such as cholesterol and lactate [46]. This approach involves making test strips with appropriate chemistries for a specific metabolite and determining the correlation between glucometer response and metabolite levels using standards.

Cholesterol/Triglycerides

Cardiovascular disease is emerging as a social and economic burden on the people and healthcare systems in developing countries [47]. Devices similar to glucometers are available for measuring triglyceride and cholesterol levels in blood, which are important markers for cardiovascular disease risk assessment. Two commercially available systems include Roche's Accutrend Plus and Polymer Technology Systems, Inc.'s CardioChek Plus. Both devices consist of handheld electronic meters and separate, disposable test strips that can measure a number of different analytes. The test strips contain enzymes and reactive dyes that produce changes in the reflectance of the test strip in the presence of analyte. The meters convert reflectance to a clinically relevant value, and the user reads the test result from the digital display. Both the CardioChek Plus and Accutrend Plus are FDA-approved and

CLIA-waived. These devices have similar advantages and disadvantages as glucometers; however, cholesterol and triglyceride levels do not need to be measured as frequently as blood glucose levels.

Lateral Flow

The lateral flow or immunochromatographic strip test is a well-established diagnostic platform developed to enable low-cost, rapid POC testing. The tests are designed for single use and enable qualitative or semiquantitative visual detection of a variety of analytes including proteins, small molecules, antibodies, nucleic acids, and viruses [48]. One of the earliest and quintessential lateral flow tests is the home pregnancy test. Most lateral flow tests do not require the addition of external reagents, making them straightforward to perform and user-friendly. The user simply adds sample, waits several minutes, and looks for the appearance of colored lines (i.e., sample and control lines) in the test window. Figure 12.1 shows a schematic of a traditional lateral flow test.

The main benefits of lateral flow tests are their simplicity of use and of readout. The user is typically required just to apply the sample (and perhaps a chase fluid), and the natural wicking potential of the material begins the test. Specifically, a liquid sample is applied to the sample pad, and the fluid wicks into the device by capillary action. The sample picks up a visual labeling agent or detection conjugate (e.g., antibody-gold nanoparticle or antibody-latex particle conjugates) stored in dry form in the conjugate pad. In a sandwich assay, binding between the conjugate and target analyte in the sample occurs as the fluid moves through the membrane. As the sample wicks along the membrane, the analyte-conjugate complexes are bound by antibodies or other capture agents immobilized at the test line on the membrane. At sufficient densities, the captured conjugates form a visible colored line on the membrane that is read by eye. Gold nanoparticle conjugates appear pink or red, and polystyrene or latex particle conjugates are typically dyed red or blue. The absorbent pad wicks excess sample and conjugate from the membrane. Lateral flow tests include a control line that should also be visible after running a sample. A lack of color at the control line indicates an invalid test. The user needs only to determine whether there is color present or not in the test area, meaning that even untrained users can perform these tests with relatively high accuracy.

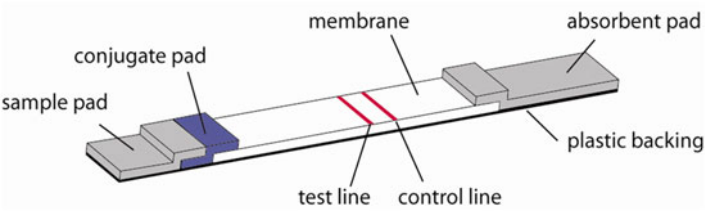

Fig. 12.1 Schematic illustration of the components of a lateral flow test

Lateral flow tests meet many of the ASSURED criteria, making them well suited for POC testing in developing urban areas. The tests are affordable (they are composed of relatively inexpensive plastic materials and porous membranes), and they are easily manufactured at high volumes and low cost using established printing, cutting, and lamination technologies. In many cases, the most expensive components of lateral flow tests are the biomolecular reagents; however, this cost can be minimized through high-volume production. Since the tests use capillary action to drive fluid flow and results are visually read, no expensive external equipment or power is needed. This makes lateral flow tests compact, portable, deliverable to end users, and easy to operate. The tests are also generally robust, can be shipped without refrigeration, and be stored for more than a year when appropriately sealed in pouches with desiccant [49]. A variety of biological samples are compatible with lateral flow tests including blood, plasma, urine, stool, and saliva. Samples with higher viscosity, such as stool or saliva, however, must be either mixed with a diluent or buffer before testing or chased with additional liquid to help it travel along the membrane.

Although lateral flow platforms have many attractive features, these diagnostic tools have several notable constraints. For example, detecting multiple analytes (i.e., multiplexing) on one test strip can be challenging, because different reagent sets can potentially interfere with one another, and the optimal test conditions (e.g., pH, blocking agents, and surfactants) for one reagent set may not be suitable for a different reagent set. It is also challenging to test limited sample volumes on lateral flow tests without the addition of a diluent or buffer. Lateral flow tests also have limited quantitative analysis capabilities.

Traditional colloidal gold lateral flow tests are typically limited to low nanomolar visual detection limits [50]. Greater analytical sensitivity (100–1,000-fold more) can be achieved using external readers, alternative conjugate labels (e.g., fluorophores), or additional signal enhancement techniques such as silver amplification of gold nanoparticle conjugates [6, 47]. These approaches, however, can increase assay cost and/or complexity.

In addition to pregnancy, other examples of commercially available lateral flow tests include assays for infectious diseases (e.g., influenza, HIV, dengue fever, hepatitis, gonorrhea, syphilis, chlamydia, and malaria), cardiac markers, and illicit drugs.

Emerging Technologies

Although glucometers and lateral flow tests are powerful diagnostic platforms, there is a need for more advanced POC devices that can perform complex, multistep laboratory-based analyses (e.g., immunoassays, cell counts). Ideally these systems should be portable, highly automated, and require little to no user interaction to obtain results. Devices that perform several laboratory procedures (e.g., sequential reagent addition, mixing, washing, and detection) are often referred to as “lab-on-a-chip” or “micro-total analysis systems.” Microfabrication and microfluidics have emerged as promising technologies to achieve this goal. The sections below describe

lab-on-a-chip technologies developed or in development that have potential for improving health in developing urban areas. The interested reader can find a comprehensive survey of companies using microfluidic and lab-on-a-chip technologies in a recent review article by Chin and colleagues [51].

Microfluidic Technologies

Microfluidic-based diagnostics are lab-on-a-chip devices that test for single or multiple analytes in less than a milliliter of sample. This is typically achieved by creating tiny (<1-mm-wide) channels in glass or plastic and using specially designed analyzers to move the sample and other fluids in a predefined manner to perform analyses. The origin of microfluidic device development dates back to the 1970s; however, it was not until the 1980s that academic interest in microfluidics began to intensify [52]. The main benefits of microfluidic technologies are their ability to manipulate small volumes of fluid, perform several analyses from one sample (i.e., multiplexing), perform complex operations (e.g., mixing and splitting), and achieve a higher assay sensitivity than lateral flow devices. Unfortunately, this typically comes at increased cost; most microfluidic diagnostics require an analyzer and fine-tolerance manufacturing. This section will outline some of the plastic- or glass-based microfluidic technologies currently in development that could have a large positive impact in developing urban areas.

Small Molecule Analyzers

Abbott's i-STAT blood analyzer is an example of a successful, commercially available POC diagnostic that uses active microfluidic technology. The i-STAT is used in over 1,800 hospitals worldwide and gives clinicians access to laboratory-quality data within minutes at the POC, which allows them to make critical treatment decisions in real time. The system consists of a handheld, battery-operated analyzer and single-use, disposable test cartridges to perform several different clinical chemistry tests and/or immunoassays. The cartridges have microfluidic channels for sample handling and microfabricated thin-film electrodes for making electrochemical measurements. Each cartridge contains stored calibrant and is designed to perform one or more (i.e., a panel) quantitative diagnostic tests on <100 μL of blood, including measurements of blood gases, electrolytes, lactate, glucose, creatinine, hemoglobin, hematocrit, coagulation parameters, and cardiac markers. The i-STAT has several features that make it easy to use and effective in emergency and critical care applications: no sample pretreatment steps, automated calibration, bar code scanning for automatic loading of user or patient information, a docking unit for downloading test results for electronic record keeping, and wireless transmission and sharing of test results. This device meets many of the ASSURED criteria; however, the high price of the analyzer limits the settings where it would be economical. Reduction in overall cost and cartridges designed specifically for the needs of primary care

facilities in developing urban areas would make this technology much more useful by allowing for the transfer of care from hospitals to primary care facilities. In its current form, however, this powerful technology is not a practical solution for at-home testing or for use in many urban and rural areas of the developing world (due to its cost).

Quantitative, Rapid Immunoassays

Immunoassays are a category of tests that detect the presence or amount of an analyte through the use of immune system antibodies selected to bind to the target analyte. Several technologies on the market are based on immunoassay principles. For instance, the previously mentioned lateral flow tests use immunoassay detection. Immunoassays can be performed in formats that are qualitative (e.g., lateral flow tests) or quantitative (e.g., enzyme-linked immunosorbent assays, ELISAs). ELISAs can be performed using a variety of biological samples and are often highly sensitive, but they are not well-suited for POC testing applications in developing urban areas. ELISAs require costly reagents, well-equipped labs, and trained personnel, and it can take hours or longer to obtain results. Claros Diagnostics recognized the limitations of traditional immunoassays for developing world applications and was founded in 2004 to create a low-cost, easy-to-use POC diagnostic platform that can perform complex, multistep immunoassays in minutes with high sensitivity and specificity. The platform consists of a photometric analyzer and inexpensive disposable plastic assay cassettes that contain networks of microfluidic channels and reagents to perform quantitative immunoassays. The analyzer uses pneumatic actuation to combine sample (whole blood or urine) and reagents in a controlled manner to perform multistep immunoassays with silver enhancement chemistries to achieve high analytical sensitivities. The analyzer comes in both a benchtop and battery-operated handheld unit.

In 2010, Claros obtained CE marking for a prostate cancer immunoassay test and is currently pursuing FDA approval with CLIA waiver for the US market. The platform has also been used to successfully diagnose HIV and syphilis in field trials in sub-Saharan Africa [53]. Although the technology was initially designed to offer a relatively low-cost method of acquiring lab-quality immunoassay test results at the POC in developing countries, the technology has also proven attractive for use in developed countries. Claros was acquired by Opko Health in 2011 and will expand its testing capabilities to include panels for infectious diseases, cardiology, neurodegenerative diseases, companion diagnostics, and women's health. The Claros platform strives to provide improved sensitivity and quantitative results when compared to lateral flow but with increased cost. Similar to the i-STAT, the higher cost and need to purchase an analyzer means that this technology will have limited utility in an at-home testing setting. Instead, with the development of test cartridges specifically aimed toward the needs of developing countries and depending on final cost, it has the potential to positively impact health care delivery at primary healthcare facilities in urban (and rural) settings.

Cell Analyzers

In addition to small molecule and immunoassay detection, microfluidic devices are being developed to perform cell-type analyses. One company developing POC diagnostics for cell-type analysis using a microfluidic platform is Daktari Diagnostics, Inc. Daktari is developing a robust, portable CD4+ cell-counting system (Daktari CD4) that allows physicians in remote settings to monitor the immune status of patients. Measuring CD4+ cell count is particularly important for monitoring the progression of HIV and AIDS and the effects of therapeutic treatments (e.g., highly active antiretroviral therapy). Flow cytometry is traditionally used to measure CD4+ cell counts in centralized laboratories. However, this testing is limited to only the best supported urban hospitals, because flow cytometers are expensive to purchase and maintain and require highly skilled personnel to prepare and test samples. A CD4+ cell diagnostic suitable for primary healthcare facilities would enable monitoring of HIV+ patients to be moved away from overburdened hospitals. This would result in more efficient screening for those patients in need of further care and streamline care provided at the hospitals.

The Daktari CD4 consists of a portable analyzer and disposable plastic microfluidic cartridges with on-board reagents stored in blister packs. The system uses a combination of microfluidic cell chromatography, cell lysis, and impedance spectroscopy to provide CD4+ cell counts from a finger-stick drop of blood. Sample preparation and reagent delivery are automated, thereby minimizing the need for user interaction. In comparison to flow cytometry, the Daktari CD4 system is more suitable for use in developing urban areas—it is more user-friendly, less expensive, and provides faster results. The Daktari CD4 is in development and undergoing performance evaluations.

An alternative approach to rapid CD4+ cell counting is being developed by Zyomyx. The Zyomyx platform uses separation by sedimentation and cell stacking in a disposable high-precision capillary to estimate cell counts. A measurement is performed by mixing a finger-stick drop of blood with antibody-particle conjugates (1-μm particles) and injecting the mixture into a closed-end glass capillary filled with a high-density medium. Results are interpreted visually within tens of minutes. This system has several advantages over competing technologies, including low cost, rapid time to results, no need for external equipment or power and it is simple to use. Given these advantages, this system has the potential to greatly improve the treatment and health of patients in developing urban areas.

Paper Microfluidic Devices

Within the last 5 years, paper-based microfluidics have emerged to provide an attractive platform for the development of easy-to-use, low-cost, portable, and disposable diagnostics for POC testing in resource-limited settings. Paper microfluidic devices were originally developed by Andres Martinez and Professor George Whitesides at Harvard University to address several limitations of traditional glass- or

plastic-based microfluidics. Paper-based microfluidics leverage the natural wicking properties of paper to drive fluid flow. Wicking eliminates the need for power or external pumps to actuate fluids and run samples, which reduces the cost of the tests and makes them compact, portable, easy to operate, and useful for at-home monitoring. Paper is also readily available, inexpensive, and relatively easy to manufacture and process. Despite all of these benefits, one significant limitation of paper is that a sample wicking through the paper will travel radially unless somehow constrained. This makes it difficult to control fluid flow and therefore perform anything other than simple assays, such as those performed on unidirectional lateral flow test strips. Martinez and Whitesides were able to overcome this limitation by patterning hydrophobic barriers in paper to direct fluid flow along desired paths [54].

Several methods to pattern paper have been developed in recent years. The original method described by Martinez et al. involved UV curing of a photosensitive polymer, SU8 [54]. Methods using other photosensitive polymers were subsequently developed by Whitesides [55]. Fenton et al. [56] and Fu et al. [57] describe cutting methods as a means of patterning paper (in these cases the edges of the substrate act as barriers). Li et al. [58] describe a method of patterning hydrophilic channels in a hydrophobic paper using plasma treatment. A very simple method called wax printing, described by Lu et al., uses a commercially-available printer and an oven to create hydrophobic barriers [59]. A version of this process developed by Carrilho et al. [60] and Diagnostics For All is described in more detail below.

The wax printing method of patterning paper leverages existing commercial printers with solid-ink technology, such as the Xerox ColorQube. This method of fabrication is scalable and can provide the volumes of devices needed to reach global markets. Figure 12.2 shows the process for making wax-patterned paper-based microfluidics using a solid-ink printer.

Devices are designed on a computer using standard graphics software, and the hydrophobic wax walls of the microfluidic channels are printed onto the surface of a sheet of paper. The printed paper is then heated to above the melting point of the wax so that it permeates through the thickness of the paper to create hydrophobic barriers. Channels patterned in this way wick microliter volumes of fluids by capillary action and distribute the fluids into test zones where independent assays take place, similar to the SU-8 patterned device shown in Fig. 12.3. Many different types of paper or porous materials can be patterned using this technique—including materials composed of cellulose, nitrocellulose, and nylon—such that the properties of the membrane can be selected for specific applications (e.g., filtering, wicking fluids, and storing reagents) and sample types (e.g., urine, feces, blood, or saliva).

More complex microfluidic networks are made by stacking layers of patterned paper and affixing them to one another with layers of patterned adhesive (Fig. 12.4) [61]. These multilayer microfluidic devices enable control of wicking fluids in three dimensions and provide access to a variety of fluidic operations, including splitting, incubation, and mixing. Figure 12.4 shows an example of a multilayer device with four sample inlets that divide each sample into an array of 16 individual detection zones on the bottom face of the device (256 detection zones total). The length of each fluidic channel connecting the sample inlet and the detection zones is equal

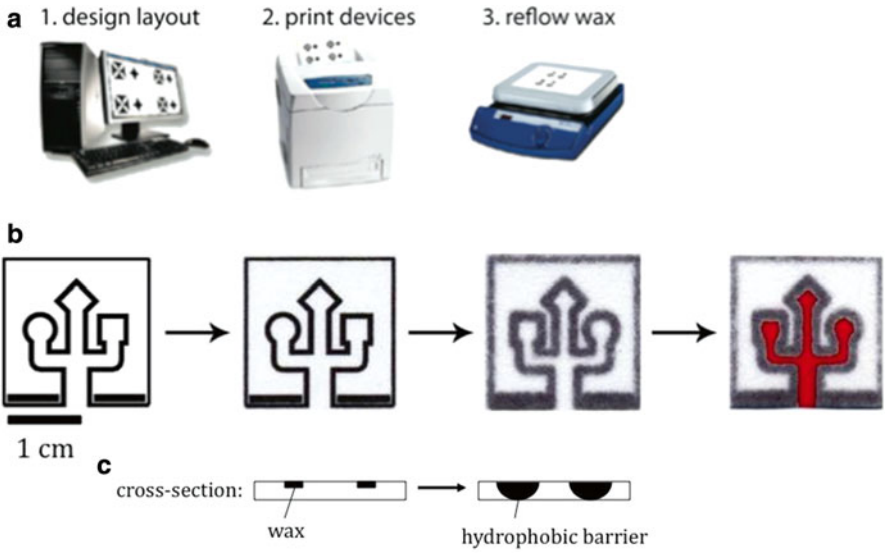

Fig. 12.2 Patterning hydrophobic barriers using wax printing. (**a**) Devices are prepared using inexpensive equipment: device layouts are designed using computer software (*1*), a commercially available printer prints solid wax ink onto paper (*2*), and a hot plate or other heat source reflows the wax to create hydrophobic channels throughout the thickness of the paper (*3*). (**b**) Demonstration of a three-channel device advanced from concept to working prototype. (**c**) Cross-sectional schematic illustration of a device before and after heating to reflow printed wax. Adapted with permission from Carrilho et al. [60]. Copyright 2009 American Chemical Society

Fig. 12.3 Single-layer patterned paper diagnostic test for glucose (*left*) and protein (*right*) in urine. The *arrow* indicates the direction of sample flow when dipped in urine. Reprinted with permission from Martinez et al. [54]. Copyright 2007 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

and ensures equal distribution of sample into each of the detection zones. The parallel nature of the fluidic conduits in this platform makes it particularly well-suited for multiplexed assays. Each detection zone, for example, could contain unique, independently optimized sets of reagents to perform a number of different assays on a single input specimen. Further complexity has been integrated into the paper

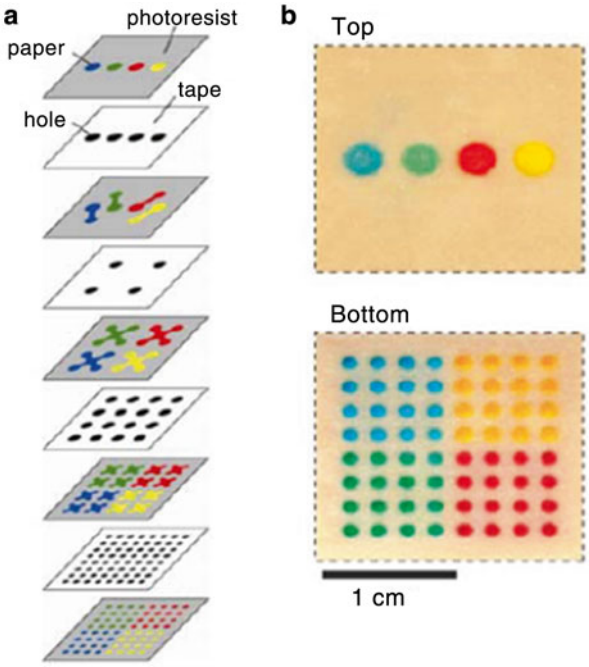

Fig. 12.4 Three-dimensional multilayer patterned-paper microfluidic device that splits single input samples into 16 equal volume detection zones on the bottom of the device. This design enables multiplexed detection of multiple analytes in parallel. Reproduced with permission from Martinez, A.W. et al. *Proc. Natl. Acad. Sci.* 105, 19606 (2008). Copyright 2008 National Academy of Sciences, USA

platform with the development of timing gates [62], valves [55, 61], and batteries [63]. Quantitative results can also be obtained by imaging test results using a scanner, digital camera, or mobile camera phone and analyzing the results with image analysis software. The latter method allows for integration with the powerful and promising field of telemedicine [64].

In addition to the colorimetric readouts demonstrated above, much work has been done proving the compatibility of paper microfluidics with electrochemical analyses. By screen printing conductive inks, such as silver/silver chloride or carbon black, electrodes can be patterned on the paper microfluidic devices [65]. These electrodes can be used for a variety of purposes, including electrochemical analysis of glucose [66], analysis of other analytes (e.g., cholesterol) using modified commercial glucometers [46], or to provide heat to specific areas of the paper microfluidic device [65].

Paper-based microfluidics is a highly-flexible diagnostic platform that is compatible with a number of different assay formats and readouts, and it is now moving from academia toward the market. Diagnostics For All (DFA), a nonprofit based in Cambridge, Massachusetts, is building off of the developments of the Whitesides Group at Harvard University; DFA aims to develop specific diagnostic tools for developing countries [67]. Traditional clinical chemistry assays and immunoassays have been successfully demonstrated on the patterned paper platform using electrochemical and colorimetric readouts. DFA's lead rapid diagnostic test is a colorimetric clinical chemistry assay for measuring liver transaminase levels from a finger-stick drop of whole blood [68, 69]. This low-cost, disposable test is targeted to help detect potentially fatal medication-induced liver damage in patients taking

antiretroviral medications (e.g., HIV patients) in resource-limited settings. Semiquantitative readout is achieved by visually comparing test results to a color-coded read guide that provides clinically actionable information at the point of care. In addition to the liver function test, DFA is developing several other clinical chemistry tests as well as several immunoassay tests for human health and agriculture and livestock applications.

Paper microfluidic devices are attractive, because they offer the capabilities of glass- and plastic-based microfluidics to perform complex assays but rely on the wicking properties of paper; therefore, they do not require external equipment to operate them. Their inherent low cost means that if they can demonstrate the requisite sensitivity and specificity, they have the potential to be of great utility in decentralized testing situations, such as primary healthcare facilities and at-home testing. Furthermore, because the technology is relatively new, there is still a great deal of innovation happening. This means that the capabilities of paper microfluidic devices are constantly expanding in many different ways, including providing quantitative results, performing assays not yet capable in simple rapid formats, and improving sensitivity through amplification steps. Their current characteristics, inherent low cost, and constant evolution suggest great potential for paper microfluidic devices designed to address healthcare problems in developing urban settings.

The Potential for Innovative Technologies

Developing urban areas face a sometimes daunting combination of disease burden and financial limitations on care. When viewed from a traditional, centralized healthcare delivery model, the potential for high-quality care of all residents can seem bleak. However, the same constraints making centralized healthcare models infeasible push for new models of healthcare delivery and drive the development of new, innovative technologies. As presented in this chapter, new microfluidic technologies that address the constraints of developing urban areas are in development and have the potential to greatly improve patient care and hospital efficiency. Furthermore, because several of these technologies are being developed specifically for developing countries—with involvement from potential end users—adoption could be higher than technologies developed for centralized healthcare models. In this respect, developing urban areas act as incubators for new technological innovation that could eventually move to developed countries if patient demand for at-home testing rises.

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