

Point-of-Care Diagnostics for Global Health

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Annu. Rev. Biomed. Eng. 2008. 10:107–44

First published online as a Review in Advance on March 20, 2008

The *Annual Review of Biomedical Engineering* is online at bioeng.annualreviews.org

This article's doi:
10.1146/annurev.bioeng.10.061807.160524

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1523-9829/08/0815-0107\$20.00

Key Words

low-resource settings, POC, infectious disease, lab-on-a-card

Abstract

Biomedical engineers have traditionally developed technologies in response to the needs of the developed world's medical community. As a result, the diagnostic systems on which they have worked have met the requirements of well-funded laboratories in highly regulated and quality-assessed environments. However, such approaches do not address the needs of the majority of the world's people afflicted with infectious diseases, who have, at best, access to poorly resourced health care facilities with almost no supporting clinical laboratory infrastructure. A major challenge for the biomedical engineering community is to develop diagnostic tests to meet the needs of these people, the majority of whom are in the developing world. We here review the context in which the diagnostics must operate, some of the appropriate diagnostic technologies already in distribution, and some emerging technologies that promise to address this challenge. However, there is much room for innovation, adaptation, and cost reduction before these technologies can impact health care in the developing world.

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1. INTRODUCTION: DIAGNOSTICS AND GLOBAL HEALTH

Diagnostics play several critical roles in health care in the developed world, such as providing appropriate and timely care to patients, ensuring safe blood banking, and providing crucial surveillance data for both emergency public health interventions and long-term public health strategies. The role of diagnostics is just as critical in the developing world and low-resource settings in the developed world, but in such settings the potential utility for point-of-care (POC) diagnostics is probably even greater. Although this has been recognized for quite some time (1), diagnostic technology development has not received the same financial support and dedicated resources as drug discovery and vaccine development have from either the public or the private sector.

POC: point-of-care

Several recent factors have led to increased support and attention from the donor/public sector and the private sector for the use of diagnostic technologies, including the rise of antibiotic resistance, the cost of effective drugs, the increased threat of an accelerated epidemic-to-pandemic transition of a communicable disease owing to globalization, and the HIV pandemic.

Another factor has been the rapidity of scientific and technological advances, such as those in genome sequencing and high-throughput antigen screening, coupled to proteomics and transcriptome analysis. These advances have accelerated the unraveling of disease pathogenesis at a molecular level and have identified pathogen and disease biomarkers. The emerging fields of microfluidics and nanotechnology promise exciting and integrated solutions to sample processing, assay performance, and analyte detection. These lab-on-a-chip or lab-on-a-card solutions have been recognized as an opportunity to bring accurate and sensitive diagnostic tests to the POC (2–5), in high-income countries and in low-resource settings, as well as in the developing world. Consequently, there has been an explosion in the past 5 years in publications about early stage technologies that attempt to overcome the hurdles and barriers of introducing diagnostics into the developing world. However, these obstacles are complex and must be identified early in the product-development process for these new technologies to impact the care patients receive, particularly in low-resource settings.

In this review we discuss factors that must be considered when developing diagnostic tools for low-resource settings, emphasizing emerging technologies that address at least some of these factors. Because of the overwhelming importance of infectious disease in the developing world (and all low-resource settings), we focus on the development of appropriate infectious-disease diagnostic technologies. However, many arguments discussed here are applicable to diagnostic tests for noncommunicable diseases such as cancer. Political, social, and health care infrastructures vary extensively between countries, and these factors are critical to the introduction of effective health care (6–9). However, as these broad factors have been recently reviewed in this journal (9), we concentrate on diagnostics, the related technical needs and technical barriers, and the ways in which biomedical engineering can address these concerns.

2. DIAGNOSTIC CAPABILITIES IN THE DEVELOPED WORLD TODAY

The criteria for commercially viable products in the developed-world market today are clinical usefulness (i.e., is the test necessary to inform patient care?) and cost (as determined by reimbursement rates). Although these same parameters are critical for global health, the technical approaches to address them are generally not directly transferable to low-resource settings. For example, a high-throughput, automated robotic processing instrument is generally not affordable or feasible in low-resource settings that lack the necessary laboratory infrastructure. However, certain technical methodologies optimized for high-resource settings could enable low-resource testing.

2.1. Location-Specific Diagnostic Capabilities

Patient-diagnosis requirements vary depending on the location where the test is performed, and in high-income settings, this has resulted in location-specific technology to match the required result turnaround time, specimen handling, and test complexity.

2.1.1. The modern hospital: centralized laboratory. The gold standard for the detection of the majority of infectious agents remains culture isolation of the bacterial or viral pathogen. This primarily results from the fact that the isolation of bacteria is necessary to perform drug-susceptibility

PCR: polymerase chain reaction

testing, and current molecular tests are not adequately multiplexed in a highly sensitive platform integrated with sample preparation. Therefore, culture remains necessary for comprehensive microbe identification, epidemiology, drug-resistance testing, patient triage, and nosocomial monitoring. This requires significant knowledge and training to correctly perform the appropriate testing algorithms and interpret results. Recently, automated systems have been developed that perform rapid (8 to 14 h) identification and antimicrobial susceptibility testing on a manually prepared inoculum (10, 11). Software-based identification systems are utilized to automate the identification (genus and species) of an organism. The adaptation of these systems to lower-throughput, lower-cost instruments could be useful in low-resource settings because this would lower personnel training requirements.

2.1.2. Reference laboratory. Clinical specimens are referred for reference laboratory testing when the methodology is complex, the immediate turnaround time is not critical, the specimen is stable during transport, and specific pathogens are detected or monitored. Reference laboratory testing enables specialized testing at a lower cost through batch processing using high-throughput robotic automation and large instrument assay step integration. This is particularly true of molecular diagnostic testing such as for HIV viral load monitoring or chlamydia nucleic acid-based detection.

For over 15 years, nucleic acid–amplification-based methods have been touted as the diagnostic approach of the future (12–15). Advantages include the use of sequence complementarity to enable improved specificity, target gene amplification to provide theoretical single copy detection, and much more rapid turnaround times than culture. There are a number of strategies to detect and amplify nucleic acids, including isothermal enzymatic schemes (16) and the polymerase chain reaction (PCR) (17). Despite the clear advantages of PCR, it took 10 years from its invention in 1983 until the first U.S. Food and Drug Administration (FDA)-approved PCR-based diagnostic test was introduced. This resulted, in part, from the practical issues of reducing the assay protocol complexity through instrumented automation, improving the method's interface with sensitive fluorescence-based detection, using improved thermostable enzymes for high-fidelity amplification, recognizing and incorporating controls for reaction inhibition, and controlling the cross-contamination that was found to be a significant risk of false positive results. Even today there are still only approximately a dozen FDA-approved PCR diagnostic tests. The issues that have slowed market penetration include higher cost relative to more traditional culture or immunoassays and the lack of integration of specimen nucleic acid extraction.

These same issues have even greater impact on the introduction of PCR for low-resource settings testing. Ironically, another problem for the global use of PCR stems from the high specificity of primer hybridization. Sequence variation due to mutation or genetic variability observed for various isolates can result in false negatives owing to the loss of primer recognition. This necessitates assay redundancy or multiplexing to guarantee the detection of all isolates. Reference laboratory PCR-based testing has only recently been refined to combine automated nucleic acid extraction with high-throughput robotic amplification for HIV viral load testing (18). Although run-to-run variability associated with manual sample processing is reduced, the platform uses very large and expensive instrumentation; reagent and consumable costs are from 12 to 14 dollars per specimen, and there is still approximately 1 to 2 h of hands-on technician time and 6 to 8 h for the total turnaround time. PCR technology will need to be adapted for low-cost, near-patient testing for use in low-resource settings.

2.1.3. The physician's office laboratory. Diagnostic methods utilized in physician's offices in high-income countries are most analogous to near-patient methods in low-resource settings. In

Table 1 FDA definition of a simple test^a

FDA definition of a simple test
Fully automated instrument or unitized, self-contained test
Uses direct unprocessed specimens/capillary blood (fingerstick), nasal swabs, or urine
Needs only basic, non-technique-dependent specimen manipulation, including any for decontamination
Needs only basic, non-technique-dependent reagent manipulation, such as “mix reagent A and reagent B”
Needs no operator intervention during the analysis steps
Needs no technical or specialized training
Needs no electronic or mechanical maintenance
Produces results that require no operator calibration, interpretation, or calculations
Produces results that are clear to read, such as positive or negative, a direct readout of numerical values, the clear presence or absence of a line, or obvious color gradations
Has test performance comparable to a traceable reference method, as demonstrated by studies in which intended operators perform the test
Contains a quick reference instruction sheet written at the educational level of the user

^aSimple as defined in Recommendations for Clinical Laboratory Improvement Amendments of 1988 (CLIA): CLIA Waiver Applications. <http://www.fda.gov/cdrh/oivd/guidance/1171.pdf>.

both settings, the dominant format currently in use is lateral-flow immunochromatographic immunoassay strip tests, despite the poor sensitivity levels (approximately 70%) observed for this technology. In higher-income settings, these tests are used because of the need for rapid turnaround times of 15 to 20 min. Also, because of the quality-control requirements of moderate to high complexity testing, the physician’s office laboratory generally only performs tests that are waived from the Recommendations for Clinical Laboratory Improvement Amendments of 1988. The FDA defines these waived methods as those that are “simple” and that have an “insignificant risk of an erroneous result.” Therefore, the FDA definitions of a simple test as given in the **Table 1** provide an excellent goal for the ideal near-patient diagnostic.

2.1.4. Emergency medical care (first responders). Similar to the physician’s office laboratory, first responders must use simple, rapid testing to rule out exposure to specific biothreat agents released into air, water, or onto surfaces (19). Lateral-flow, enzyme immunoassay (EIA) and PCR platforms have been developed for field testing. Their performance was recently verified for spiked water based on an evaluation of technology performance under specific, predetermined criteria and the appropriate quality-assurance procedures by the Environmental Protection Agency’s Advanced Monitoring Systems Center, one of six verification centers under the Environmental Technology Verification Program (20). In general the results were not sensitive enough to detect lethal dose levels in large volumes of water, especially for the immunoassay methods. Although the PCR platforms are ruggedized and adapted for field operation, they were inadequate in their ability to concentrate and purify nucleic acid at low concentration in large volumes of water. Improvements are mandated; progress in this area will no doubt have direct relevance for adaptation to diagnostics in low-resource settings.

2.1.5. The home. Home screening is a potential, but controversial, method to empower individuals to anonymously utilize screening for infections such as HIV or sexually transmitted diseases (21, 22). Effective control of the prevalence of infectious diseases requires better methods to identify infected individuals so they can be treated and avoid infecting others. Educated

EIA: enzyme immunoassay

ARI: acute respiratory infection

WHO: World Health Organization

STI: sexually transmitted infection

consumers in developed countries are increasingly demanding more diagnostic testing for personalized, evidence-based care. Proactive consumer-driven wellness monitoring is supported by leading medical societies and the American Medical Association because personal health behaviors are the primary determinant of disease, disability, and death (23) and are the primary drivers of health care costs (24). The diagnostic platforms necessary for home care should also enable better approaches to low-resource-setting tests.

2.1.6. The modern military. The U.S. government has made a large investment in new technologies aimed at insuring homeland security through the development of monitoring platforms that enable multiplexed detection of all possible biothreat agents. In addition, there are some close overlaps between the medical needs of the poorest developing nations and those of the military forces of developed nations. The military forces of these developed nations have, as one of their primary missions, extended operations in developing nations. Military forces have always had to keep their (combat and noncombat) personnel healthy and ready for extended operation in the developing world. Military and global health workers have a similar interest in highly multiplexed simultaneous detection of bacteria, RNA and DNA viruses, and toxins (25). The development of tools for medicine continues within the U.S. military at such organizations as the Telemedicine and Advanced Technology Research Center (<http://www.tatrc.org/>), which focuses on new technologies for medicine, and the Walter Reed Army Institute of Research, which has, for a century, had a major focus on tropical infectious diseases. For example, the Walter Reed Army Institute of Research recently announced the development and FDA approval of a new rapid malarial diagnostic test (26).

3. CLINICAL NEEDS AND USER REQUIREMENTS FOR POINT-OF-CARE TESTING IN LOW-RESOURCE SETTINGS

3.1. Disease Burden and Diagnostic Needs in Global Health

The burden of disease is commonly measured in terms of the disability-adjusted life year, a unit that accounts for the years of life lost due both to mortality and to disability as a consequence of the incidence of disease (27). Ischemic heart disease and cerebrovascular disease are the major disease burdens in high-income countries, whereas infectious disease is the major cause of mortality and morbidity in the developing world (28), accounting for more than half of all infant deaths there (29). Moreover, over 95% of deaths due to major infectious diseases [acute respiratory infections (ARIs), malaria, HIV, and tuberculosis (TB)] occur in developing countries, with by far the largest burden on Africa. Improved and appropriate diagnostics could lead to a large reduction of disability-adjusted life years resulting from the major causes of disease in low-resource settings (30–32).

In the absence of diagnostic tests, disease in low-resource settings is often treated based on clinical symptoms and local prevalence of disease. When the clinical symptoms are fairly characteristic of a disease, or the disease is fairly prevalent in the region, syndromic management of a disease can be cost-effective to a health system and has been recommended by the World Health Organization (WHO) for certain symptoms/diseases, such as malaria, sexually transmitted infections (STIs), and common childhood diseases. Syndromic management of pediatric ARIs as recommended by WHO was shown to significantly reduce the disease burden (33). Whereas this approach captures most patients requiring treatment, it also unnecessarily treats patients who do not require treatment. Equally important, this latter group of patients is not being treated for their disease. Syndromic management of disease may also accelerate drug resistance.

RD_T: rapid diagnostic test

Below we discuss current infectious-disease-related clinical needs for which there is an urgent necessity for new POC diagnostics. We also address some of the clinical and contextual challenges a new device would face in order to successfully address these clinical needs. A projection of global mortality and burden of diseases suggests that conditions common to higher-income countries such as heart disease, cerebrovascular disease, diabetes, and chronic obstructive pulmonary disease will become health priorities in lower-income countries by 2030 (34).

3.1.1. Malaria, tuberculosis, and HIV. HIV, TB, and malaria are the causes of major infectious-disease burdens that disproportionately afflict the developing world. Each disease presents unique and major challenges to the diagnostic development community because of its unique biology, as well as historical context.

3.1.1.1. Malaria. Approximately 300 to 500 million cases of malaria infection occur per year, resulting in approximately 2 million deaths per year, mostly in sub-Saharan Africa, of which approximately 1.2 million are infants under the age of five. Four species of the protozoon *Plasmodium* cause malaria in humans: *Plasmodium falciparum*, *P. vivax*, *P. malariae*, and *P. ovale*. Of these, *P. falciparum* causes the highest mortality and morbidity. Overdiagnosis and overtreatment for malaria in malaria-endemic regions come at the expense of the detection and treatment of bacteremia in febrile patients. This leads to high mortality rates in patients mistreated for malaria (35, 36).

In sub-Saharan Africa where there are limited laboratory resources, the majority of malaria cases are diagnosed based on clinical symptoms using algorithms such as the WHO Integrated Management of Childhood Illnesses. This approach of presumptive treatment can reach a sensitivity of 88% at a specificity of 66% (37). When chloroquine, a cheap and safe antimalarial drug, was still effective, this level of specificity and the resulting overtreatment were acceptable. However, chloroquine resistance has rendered it ineffective almost worldwide. The current effective artemisinin-combination-therapy drugs are much more expensive. Diagnostic tests can play a crucial role in minimizing unnecessary drug expenditure and in extending the useful therapeutic lifetime of current antimalarial drugs.

There are several approaches to diagnose a malaria infection (**Table 2**), and each method has its own benefits and limitations (38). Microscopy and lateral-flow rapid diagnostic tests (RD_Ts) are currently the only tests generally available in low-resource settings. Since the first microscopic observation of malarial parasites in human blood by Laveran in the 1880s, light microscopy has been the gold standard diagnostic test for malaria. When performed accurately (with a clean microscope, good quality slides, and good quality staining and by appropriately trained staff), light microscopy remains the cheapest quantitative, as well as the most specific, test for a current

Table 2 Malaria test performances in the public health service

Diagnostic test	Hospital results		% patients with negative test results, for which an antimalarial was prescribed	Reference(s)
	Sensitivity	Specificity		
Microscopy	50	96	48	41, 224
	71.3	92.8	51	
RD _T	95.4	95.9	54	41
Presumptive treatment	65–100	20–80	NA	37, 225, 226

For the microscopy and lateral-flow rapid diagnostic test (RD_T) data, a research microscope slide was prospectively collected and used as a gold standard against the reported test result by hospital staff.

infection of live malarial parasites. Unfortunately, limited health care infrastructure results in extremely poor performance of microscopy as a diagnostic tool for malaria (Table 2).

RDTs can provide accurate POC malaria testing, and with the higher artemisinin-combination-therapy costs, they have now become cost-effective (39, 40). However, this cost-effectiveness is dependent on health care providers responding to the RDT result. A recent study comparing malaria treatment practices in response to RDT results versus microscopy results showed that 51% of patients with negative malaria test results as diagnosed by microscopy were treated with antimalarials and 54% of patients with negative malaria test results as diagnosed by RDTs were treated with antimalarials (41). Thus the diagnostic test had no impact on health care providers' treatment behavior and the patient care. Several factors may contribute to this poor impact of the test on patient treatment, including a historical mistrust of test results and consequently a behavioral tradition of presumptive treatment, reinforced by presumptive treatment guidelines for malaria management, as well as the unavailability of optional treatments for patients presenting with fever (42).

3.1.1.2. Tuberculosis. There are approximately 2 billion people currently infected with TB, with approximately 8.8 million people developing TB and 1.6 million people dying of TB each year (43, 44). In 2006, the majority of new cases were in Southeast Asia, but the highest incidence rates and mortalities were in sub-Saharan Africa (43). TB is a major cause of mortality in HIV-infected people, contributing to the high burden of TB in sub-Saharan Africa where HIV is endemic. Additionally multidrug resistance is a cause for the rising incidence of TB in regions such as Eastern Europe. Although TB is generally thought of as a slowly evolving chronic illness, multidrug-resistant TB and newer, even more drug-resistant strains of TB (the so-called extensively drug-resistant strains for which there are no antibiotic treatments) can result in death within days in immunosuppressed individuals such as those with AIDS (45).

Sputum smear microscopy remains the main method for TB diagnosis, particularly in low-resources settings. It also remains a key component of the WHO strategy to control TB: directly observed treatment short-course strategy (46). There is also a recognition for the need to comprehensively strengthen laboratory facilities (47). Although specific, microscopy lacks the desired sensitivity, especially in HIV-compromised patients. Other major challenges in sputum smear microscopy are specimen collection and processing, both of which largely impact the sensitivity (48). These factors (combined with the limitations of performing microscopy in low-resource settings with poor quality reagents, unkept microscopes, and often a poor level of staff training) lead to an overall poor performance of this diagnostic approach.

The complexity of TB transmission and pathogenesis, with the confounding effect of HIV coinfection, calls for the use of several diagnostic technologies to address TB management: (a) an alternative or improvement to sputum smear microscopy for the detection of active TB; (b) an alternative or accelerated approach to current culture approaches, allowing wider use with faster return of results to patient; (c) a highly sensitive screening test, such as the tuberculin skin test, that is not compromised by HIV status for symptomatic patients to relieve clinic burden; (d) a cost-effective drug-resistance test; and (e) a screening tool for latent infection in asymptomatic patients. Detailed reviews of TB diagnostics needs and emerging solutions have recently been published (49).

3.1.1.3. HIV. An estimated 39.5 million people were living with HIV in 2006, 24.7 million of them in sub-Saharan Africa. An estimated 2.9 million deaths occurred because of AIDS in 2006, 2.1 million occurring in sub-Saharan Africa (50). WHO, together with the global health

Table 3 Summary of HIV tests and biomarkers currently used for HIV screening and management

Diagnostic tests	Biomarker	Clinical applications	Available platforms	Specimen requirements	Cost per test (USD)
Rapid HIV tests	IgG	Adult screening, antenatal screening as part of PMCT	RDT, agglutination	Whole blood, sera, or plasma	0.60–4.00
HIV EIA	IgG	High-throughput screening	EIA plates	Whole blood, sera, or plasma	0.27–3.00
HIV EIA	IgG/p24 combination	High-throughput screening	EIA plates	Whole blood, sera, or plasma	0.35–1.50
Confirmatory tests	IgG	Screening confirmation	IFA, LIA, Western blots	Whole blood, sera, or plasma	10.97–15.50
Molecular NAAT HIV test	DNA	Infant diagnosis, early acute phase HIV diagnosis	Isothermal amplification, PCR	Whole blood	10.00–30.00
Antigen detection	p24	Infant diagnosis, early acute phase HIV diagnosis	EIA	Plasma, dry plasma spots	10.00
Viral load	RNA	Infant diagnosis, early acute phase HIV diagnosis, HIV therapy monitoring	Isothermal amplification, PCR, branched DNA	Plasma (requires cold chain preservation), dry plasma spots (with higher limits of detection)	17.00–87.00
Viral load	Reverse-transcriptase activity	Infant diagnosis, early acute phase HIV diagnosis, HIV therapy monitoring	Colorimetric assay	Plasma	13.00–15.00
CD4 counts	CD4 cells	CD4 levels used to stage patients for ART therapy and monitor response to therapy; for children CD4 is required	(a) Flow cytometry, (b) Dedicated cytometry, (c) Manual affinity enrichment of CD4 with light microscopy for CD4 count, EIA	Whole blood and requires cold chain preservation if test cannot be performed immediately	(a) 1.00–25.00 (b) 2.26–20.00 (c) 3.00–8.00

The prices indicated in U.S. dollars are per test and were obtained from the WHO. ART, antiretroviral therapy; EIA, enzyme immunoassay; IFA, immunofluorescent antibody test; IgG, human immunoglobulin G; LIA, line immunoassay; NAAT, nucleic acid–amplification test; p24, HIV protein antigen; PMCT, prevention of mother-to-child transmission; RDT, lateral-flow rapid diagnostic test.

community, has made definite efforts to extend HIV treatment to low-resource settings. The challenge is to make treatment and required testing available where they are needed. New solutions are desperately required to achieve this.

A panel of tests targeting a range of biomarkers exists to diagnose and manage HIV (**Table 3**) (51–54). These include the detection of HIV-specific immunoglobulin G for the screening of adults, detection of the HIV antigen p24, and detection of HIV DNA and RNA for early HIV diagnosis and infant HIV diagnosis where anti-HIV immunoglobulin G antibodies cannot be used as biomarkers as these may originate from the mother. The onset of AIDS in HIV-positive patients is managed through antiretroviral therapy. At a first instance, CD4 cell count or percentage is used to dictate drug administration. However, HIV viral load should also be used where feasible (55, 56). With the exception of anti-HIV antibody detection and some manual CD4 tests that have

NAAT: nucleic acid–
amplification test

been developed, all current HIV-related tests require sophisticated laboratory infrastructures. Successful antiretroviral therapy rollout to low-resource settings requires the implementation of cheaper and easier-to-use POC CD4 and HIV viral load tests and POC specimen handling, storage, and transportation systems.

HIV also impacts the etiology of prevalent diseases in immunocompromised HIV patients. Coinfection of several diseases such as TB with HIV leads to higher levels of susceptibility, morbidity, and mortality. South Africa has had to modify the WHO guidelines for infant ARI management to account for the impact of HIV on ARI etiology (57). The possibility of drug–drug interactions and drug toxicity is a major challenge of HIV management in low-resource settings where diseases have traditionally been diagnosed and treated syndromically through vertical programs such as malaria-control programs (58). In regions with high HIV prevalence, prompt and correct treatment of patients is only possible with additional diagnostic tools for opportunistic diseases. This will minimize the incidence of drug-related toxicity and, equally importantly, restrain drug resistance in these diseases.

3.1.2. Sexually transmitted infections. Gonorrhea, syphilis, and chlamydia together account for over 500,000 new infections every day. Syphilis (caused by *Treponema pallidum*) at pregnancy is a major cause of stillbirths and neonatal mortality in developing countries, accounting for the deaths of 1 million babies worldwide each year (59, 60). Gonorrhea (caused by *Neisseria gonorrhea*) and genital chlamydia (caused by *Chlamydia trachomatis*) infections lead to pelvic inflammatory disease, infertility, and ectopic pregnancy. Additionally, bacterial STIs increase the transmission of HIV through increased viral shedding in genital secretions as well as susceptibility to HIV infection (61).

A major challenge in STI diagnosis is that disease can be mostly asymptomatic. The early detection of asymptomatic STI is essential to avoid morbidity and transmission with sexual partners and congenital transmission, but it requires widespread screening. In symptomatic cases of STIs, syndromic management is a cost-effective intervention, except in cases in which drug resistance is arising, such as with *N. gonorrhea*, which requires POC diagnostic tools with enhanced specificity to syndromic management. In the case of syphilis, POC tests that can distinguish active from past infections are needed. This may require the detection of more than one biomarker. In the case of chlamydia and gonorrhea, currently only nucleic acid–amplification test (NAAT)-based assays appear to attain the required sensitivity levels (62), so again the identification of higher-copy-number pathogen biomarkers for improved EIA-based RDTs or the development of affordable POC NAATs is required. The WHO Tropical Disease Research Programme (TDR)-based Sexually Transmitted Diseases Diagnostics Initiative has elaborated extensive guidelines for STI POC diagnostic test development and evaluation (62, 63). These efforts are also informing POC test development and evaluation for low-resource settings in general.

3.1.3. Neglected diseases. Neglected diseases, as opposed to the big three global diseases (HIV, TB, and malaria), are diseases that are regionally endemic and affect the poorest of the poor (64, 65). At a local level, they place a major disease burden on the population and are poverty-causing diseases. On a global level, the top thirteen neglected diseases together account for over half a million deaths annually and have a disease burden of 56.6 million disability-adjusted life years, higher than malaria and TB (65). Unfortunately there is significant overlap in endemnicity for these neglected diseases and the big three global diseases, leading to high levels of coinfection and increased susceptibilities for either disease or exacerbating mistreatment of disease in these populations. Diagnostics tests are required to identify and treat patients afflicted by the so-called neglected diseases.

For neglected diseases, the private sector has no market incentive to develop drugs, let alone diagnostics (66). In addition to the technical challenges for developing appropriate low-cost diagnostic tools for these diseases, there is the challenge of developing a sustainable environment for the manufacture and deployment of new diagnostics. These developments may have to come exclusively from the public sector or through creative public-private partnerships. In the case of visceral leishmaniasis, the availability of new drugs, the price reduction of current drugs, and a commitment from the governments of India, Nepal, and Bangladesh to eliminate the disease as a public-health burden by 2015 have resulted in renewed interest in visceral leishmaniasis diagnostic development (67, 68).

3.1.4. Blood transfusions. Between 8 and 16 million hepatitis B virus infections, 2.3 to 4.7 million hepatitis C virus infections, and 80,000 to 160,000 HIV infections a year result from unsafe blood-transfusion policies (69). Up to 150,000 pregnancy-related deaths could be prevented each year through access to safe blood. WHO recommends testing blood for HIV, hepatitis B virus, and hepatitis C virus as a minimum level of safety. Of the 148 countries recently surveyed by WHO, 41 countries do not test for one or more of these viral infections, accounting for over 40% of donated blood worldwide.

In developed countries, blood safety is ensured through postdonation centralized laboratory testing. This requires a robust infrastructure in terms of communication, specimen handling and transport, and laboratory facilities. Additionally, in countries where there is a low prevalence of bloodborne diseases, the wastage of blood, blood collection, and blood-storage materials does not have a significant impact on the overall system costs to ensure blood safety. This system, however, is neither logistically nor economically feasible in many low-resource settings, nor does it meet the clinical needs for many of these settings, where, owing to blood-preservation limitations, fresh blood is often required at rural hospitals. In such settings (e.g., rural district hospitals) where throughputs of blood donations are often less than 10,000 and even 1000 a year, high-throughput EIAs or NAATs are not cost-effective, and often not technically realistic (70). Owing to the high prevalence of hepatitis B virus, hepatitis C virus, and HIV in many developing countries, postcollection screening could lead to up to 25% wastage of blood units, resulting in large resource and blood material costs. For these reasons, predonation screening with POC tests may be more cost-efficient and appropriate for the clinical needs of low-resource settings (70–72).

Several RDTs have become available for the three major viral infections of concern in blood safety; however, NAATs can further shorten the infectious-agent exposure window in blood units. An additional benefit of NAATs is that they can respond faster to emerging diseases or regional antigenicity differences than EIA-based tests (73). A platform that could cheaply test for the essential three diseases at the POC and flexibly add regionally important tests would be beneficial in addressing safety issues in blood transfusions in low-resource settings.

3.1.5. Drug resistance. Treating patients for infectious diseases based on clinical symptoms and regional disease prevalence in low-resource settings makes economic sense when the treatment is cheap and there is little chance of drug resistance developing. The management of malaria and community-acquired pneumonia is an example of the implementation of highly sensitive but unspecific guidelines to treat or refer patients in low-resource settings. Unfortunately, drug resistance threatens to undermine these cost-effective approaches for all the major diseases in the developing, as well as in the developed, world. Diagnostic tests become a cost-effective health intervention when cheap drugs are no longer effective and efficacious drugs are expensive, by limiting the use of drugs only to those patients requiring them. The judicious use of drugs may extend the efficacy lifetime of these drugs. It is important to consider POC diagnostic testing as

a preventative health intervention with respect to drug resistance rather than just a cost-effective intervention after the fact.

Drug resistance has become a major concern in the management of malaria, TB, HIV, and bacterial infections (74–78) and already places a significant health and cost burden on developing countries (76, 79). Developing a coordinated effort to monitor drug resistance from local and regional to global levels has become a priority in efforts to maximize the use of effective drugs and minimize the use of no-longer-effective drugs. This will require integrated approaches such as the World Antimalarial Resistance Network (80). Faster, cheaper, and easier-to-use pathogen culture tools and molecular diagnostic tests can play critical roles in these efforts.

3.2. Laboratory Resources, Medical Staffing, and Laboratory Staffing Limitations

The WHO Sexually Transmitted Diseases Diagnostics Initiative developed a set of generic guidelines for the development of diagnostic tests appropriate for the developing world summarized under the acronym ASSURED (63):

- Affordable by those at risk of infection
- Sensitive (few false positives)
- Specific (few false negatives)
- User-friendly (simple to perform and requiring minimal training)
- Rapid (to enable treatment at first visit) and robust
- Equipment-free
- Delivered to those who need it

It is crucial for a diagnostic test developer to realize that these are just guidelines and that there are extensive, detailed, and particular requirements and specifications for each clinical application that must be identified and considered at the onset of a product development program. There is an increasing recognition that the limitations in laboratory capacity in low-resource settings go beyond the physical space that is a laboratory (47, 51, 81, 82). In this section, we discuss these limitations and their impact on appropriate product specification design.

3.2.1. Staffing constraints. In the developing world, a major challenge to strengthening the health care system is training and retaining qualified health care providers (**Table 4**). In some settings, entire rural district hospitals may be run by assistant medical officers (with 3 years medical training). Similarly, there is little financial incentive for a highly qualified medical laboratory technologist to work in rural district facilities, so these may be staffed by personnel with 2 to 3 years diploma training. Although this level of staff is amply qualified for handling easy-to-use POC tests, there is still a need for highly trained laboratory managers who can establish quality-assurance systems for the testing.

3.2.2. Laboratory infrastructure and resources. Typically funding for laboratory capacity building has been limited and not integral. The limitations of the laboratory capacity in low-resource settings go beyond physical constraints such as clean water, electricity, and refrigeration (**Table 5**) (47, 82). Understanding these constraints is important in developing appropriate POC diagnostics for low-resource settings because they should inform the product attributes.

Typically the procurement of supplies is unreliable and unstructured. Government-purchased supplies are often poor quality. There is often no external quality assessment (EQA) or laboratory staff to implement any form of quality control. Diagnostic tests consistently underperform in low-resource settings in the absence of EQA (47). The outcome of this underperformance is poor

Table 4 WHO figures for national physician and nurse density per 1000 population

Country	Physician density (per 1000 population)	Nurse density (per 1000 population)	Year
Malawi	0.02	0.59	2004
Mozambique	0.03	0.21	2004
Uganda	0.08	0.61	2004
Kenya	0.14	1.14	2004
India	0.60	0.80	2005/2004
South Africa	0.77	4.08	2004
Brazil	1.15	3.84	2000
Bolivia	1.22	3.19	2001
United States	2.56	9.37	2000
Denmark	2.93	10.36	2002
Spain	3.30	7.68	2003
Cuba	5.91	7.44	2002

Table 5 Laboratory structure constraints in low-resource settings informing product attributes

Laboratory infrastructure constraints in low-resource settings	Implications on point-of-care diagnostic product attributes
A wide disparity of laboratory facilities and capacities within a country and among countries	Careful consideration for the final user of the test is required.
Poor or nonexistent external quality control and laboratory accreditation systems	The test should be reproducible and provide clear and easy to interpret internal and process controls.
Unreliable procurement system leading to stock outs of key laboratory supplies	The test should require as few external reagents and supplies as possible.
Unreliable quality of reagents and supplies procured through national channels	The test should require as few external reagents and supplies as possible.
Lack of basic essential equipment	The test should require as little instrumentation as possible or provide its own instrumentation.
Lack of laboratory consumables	No assumptions should be made regarding supplies for specimen collection, storage, and handling.
Unreliable water supply and quality	This is extremely variable in different regions and seasons, and a device should not require external water if high quality is needed.
Unreliable power supply and quality	This is often tied to water supply. Devices requiring external power should account for long periods of time without network electricity supply and high variability as well as frequency of surges from the network electricity supply.
Inconsistent refrigeration capacity	This is associated with unreliable power supply. A test should be able to withstand large fluctuations in temperatures (from 40°C to 10°C) during transportation as well as sustained storage at 30°C.
Insufficiently skilled staff	The test should be easy to use and interpret.
Limited training opportunities	Any training requirements should be given special consideration for the introduction strategy.
Limited access to distributors' service maintenance staff	Any device should be robust with over 1 year half-life.
Poor waste-management facilities	The environmental impact of disposable, chemical reagents, and biohazardous materials should be considered.

accuracy of the diagnostic test, compounded by mistrust by the health care provider of the test results. The combined result of these two factors is poor clinical sensitivity and specificity of the diagnostic test, rendering it a cost to the health care system rather than a savings.

Physical constraints such as limited water, unreliable power sources, and vast temperature variations have large impacts on test performance. The simple removal of the requirement of sheath fluid in a flow cytometer, such as the Guava EasyCD4 system instrument, immensely impacts the accessibility of this technology to peripheral laboratories. Reagent stability to large temperature fluctuations both during transportation and during storage at the health care facility is paramount to successful uptake of a diagnostic test.

3.3. User Requirements

It is essential to understand the user requirements for a diagnostic product at the onset of a new technology development program. Doing so in low-resource settings presents unique challenges. These settings are extremely diverse within countries, let alone within regions or globally, and identifying the correct stakeholders is a challenge. The test users in low-resource settings can have varied levels of training because the scarcity of human resources often leads to the available health care providers taking on extraordinary capacities. For example, in more remote settings, community health care workers are empowered to collect simple clinical specimens and perform basic diagnostic tests that may not be allowed in more urban areas that require patients to use designated specimen-collection laboratories. The diversity of users and respective training levels requires the development of multiple research tools. Additionally, language can be an enormous confounder in interpreting data (83). There are often local and unique expressions to describe particular disease conditions. Patient behavior, such as preferred access to alternative medicine for certain symptoms, can locally impact the timeliness of patient presentation to a health care facility (84).

Researchers and product developers should carefully evaluate the location in the chain of health care facilities for a diagnostic test's introduction, given the extreme infrastructure constraints and staffing limitations in remote settings. Understanding the clinical interventions available, the users, the health care providers, and their respective levels of training at the different health care access points should inform the diagnostic test specifications and its target setting even within low-resource settings (**Table 6**). For example, a quantitative malaria test may be of little value to a community health worker, but may be more informative to a doctor higher up in the health care referral chain. Similarly, although throughput may not be so much of a requirement in a rural clinic, it becomes a much more attractive feature, along with the possibility of batch testing, in a diagnostic test at a district hospital.

Disease pathogenesis indicates how crucial a POC test is. In the case of suspected malaria (particularly in infants), a 24-h delay in tests results could cause a preventable fatality, so a POC test is required. For HIV diagnosis (with the exception of HIV testing of pregnant women), a prompt test result is less urgent, so central facility testing is a clinically feasible option, and the costs and benefits for this option need to be evaluated against POC testing.

For some diseases, the simplest, yet accurate, available test still requires significant staff training and EQA, in which case it may be easier to train a limited group of highly skilled staff at a central facility to provide accurate high-throughput testing such as PCR than to train a larger group of laboratory staff to consistently perform the simpler tests at more peripheral centers. Implementing a reliable centralized laboratory testing infrastructure can be a challenge. In Malawi, one study, evaluating a strategy to ensure the delivery of suspected TB specimens to a central reference laboratory, found that only 40% of the specimens arrived at the central facility (82, 85).

Table 6 Diagnostic practices in primary, secondary, and central health care facilities

	Primary	Secondary	Central
Setting	Community/rural clinic, dispensaries, pharmacies	District hospital	Urban hospital
Clinical staff	Community health care worker, midwife	Nurse, medical officer, doctor (limited)	Nurse, medical officer, doctor, specialist
Possible interventions	Referral of patients, delivery, first line of drugs (limited)	Referral of patients, delivery, authority to prescribe a broader drug range, basic surgery, basic diagnostics	Full delivery and treatment options including surgery
Diagnostic test users	Community health care worker, midwife	Nurse, microscopist, laboratory technologist (limited)	Microscopist, laboratory technologist
Specimens collected	Finger prick for slide smear, or dried blood spot	Finger prick	Finger prick
	Stool	Venous draw	Venous draw
	Urine	Stool	Stool
	Vaginal swab (if legally authorized to do so)	Urine	Urine
		Vaginal swab	Vaginal swab
		Nasal swab	Nasal swab
		Sputum	Sputum
		Urethral swab, cerebrospinal fluid (limited)	Urethral swab
			Cerebrospinal fluid
			Pleural fluid?
			Endocervical fluid?
Current diagnostic testing	Clinical symptom	Clinical symptom	Clinical symptom
	Lateral flow	Microscopy	Microscopy
		Lateral flow, agglutination, manual hematology, manual blood chemistry	Lateral flow, agglutination
		Bacterial culture (limited)	Enzyme immunoassay
		Enzyme immunoassay (limited)	Hematology
			Blood chemistry
			Bacterial culture
			TB culture
			Viral culture (limited)
			Nucleic acid–amplification test (limited)
Throughput per day	<10	<50	>25
Biocontainment	None	None or basic	Autoclave available

3.4. Cost Analysis and Cost-Effectiveness

Health care facilities have extremely low budgets in low-resource settings, particularly in remote settings where POC tests are most needed. In the absence of external funding sources, tests for most diseases must be extremely low cost to be affordable independent of the cost-effectiveness. Performing a cost analysis in low-resource settings and demonstrating cost-effectiveness present unique challenges. A POC diagnostic test for a particular disease is often a disruptive technology

simply because there previously was no diagnostic test at all. Additionally, funding sources, buyers, and resource costs are convoluted by a maze of purchasers and stakeholders composed of ministries of health, vertical national programs, nonprofit organizations, donations from funding organizations, and the informal private sector, all of which often work in the same country independently.

It is important to first ascertain if case detection as an intervention is cost-effective for a specific disease in a specific region (86). Researchers have performed several cost analyses for the use of diagnostic tests in low-resource settings, including cost-effectiveness evaluations (87–89). Factors that may influence the cost-effectiveness of a new diagnostic tool over current practices are the cost of the test, disease prevalence, the cost of treatment, the cost of mistreatment, and throughput. In the case of malaria, the incremental cost of artemisinin-based drugs has increased the cost benefits for implementing malaria RDTs in many settings, but this can be rapidly offset by the health care providers' behavior if they are ignoring the test results (41, 89). The cost savings in averting the emergence of drug resistance are much more pronounced in the cases of TB and HIV, in which the second line of drugs or case management is several orders of magnitude greater in cost than the current first line of drugs or case management.

Although cost-effectiveness studies often show that more complex assays (even microscopy over RDTs) can be more cost-effective when higher throughput is required, it is not clear if the true costs of sustaining a reliable and robust laboratory system with effective training and EQA in place are taken into account. Moreover, rollout of easy POC tests such as the RDTs also requires significant training for both the users and the health care providers responding to the RDT results (90).

4. TECHNICAL CHALLENGES IN DEVELOPING DIAGNOSTIC TESTS FOR LOW-RESOURCE SETTINGS

Global health diagnostic tests must have low complexity without sacrificing diagnostic accuracy in a format that is practical for low-resource POC settings. The complexity of a test includes the need for user interpretation, the level of training necessary, the number of manual manipulations, the number of user intervention steps required, and the instrumentation requirement. Result accuracy is measured by the limit of detection, clinical sensitivity, and clinical specificity. For a test to be practical for low-resource settings, it must be capable of POC testing, have rapid turnaround time, and be low cost. Many currently utilized tests could benefit from either alternative or improved technology through bioengineered solutions. Testing that is simple and low cost, such as lateral-flow RDTs, is often not quantitative and often either not sensitive or specific, or both. As complexity increases, so do cost and turnaround time, as shown by Lee & Allain's (70) blood-screening study (Table 7).

Table 7 Detection of hepatitis B virus (HBV) infectious blood units by different screening assays

Test	Limit of detection	Estimated % of HBV infectious blood units detected	Time to result	Price per test (USD)	Instrumentation
Agglutination	30–50 ng ml ⁻¹	54	<10 min	0.65–1.30	No instrument
RDT	5–20 ng ml ⁻¹	77	<30 min	0.75–2.50	No instrument
EIA	0.5–1 ng ml ⁻¹	97	~2.5 h	1.50–3.00	Instrument required
NAAT	20 IU ml ⁻¹ DNA	100	6–23 h	6.00–20.00	Instrument required

EIA, enzyme immunoassay; RDT, rapid diagnostic test; NAAT, nucleic acid–amplification test (70).

The challenge for bioengineering is to provide low-cost yet simple methods without sacrificing test accuracy. A prominent development trend in recent years has been to miniaturize and integrate existing diagnostics into a lab-on-a-chip format. This strategy potentially solves many issues by lowering test complexity in a platform that would be practical at the POC. Cost is also reduced by using lower reagent volumes that would be housed and stored within the chip. Although instrumentation may be required, it should be designed to be low maintenance, battery operated, and low cost.

4.1. Diagnostic Testing and Physical Constraints in Low-Resource Settings

The majority of testing for infectious disease currently found in low-resource settings consists of the direct detection of the infectious agent by microscopy and the detection of the pathogen-specific antigen or antibodies by RDTs or agglutination tests (**Figure 1**). Under ideal conditions, all these tests can perform extremely well, benefiting both the patient and the health care system. Unfortunately, all these platforms can also perform poorly in low-resource settings for the reasons

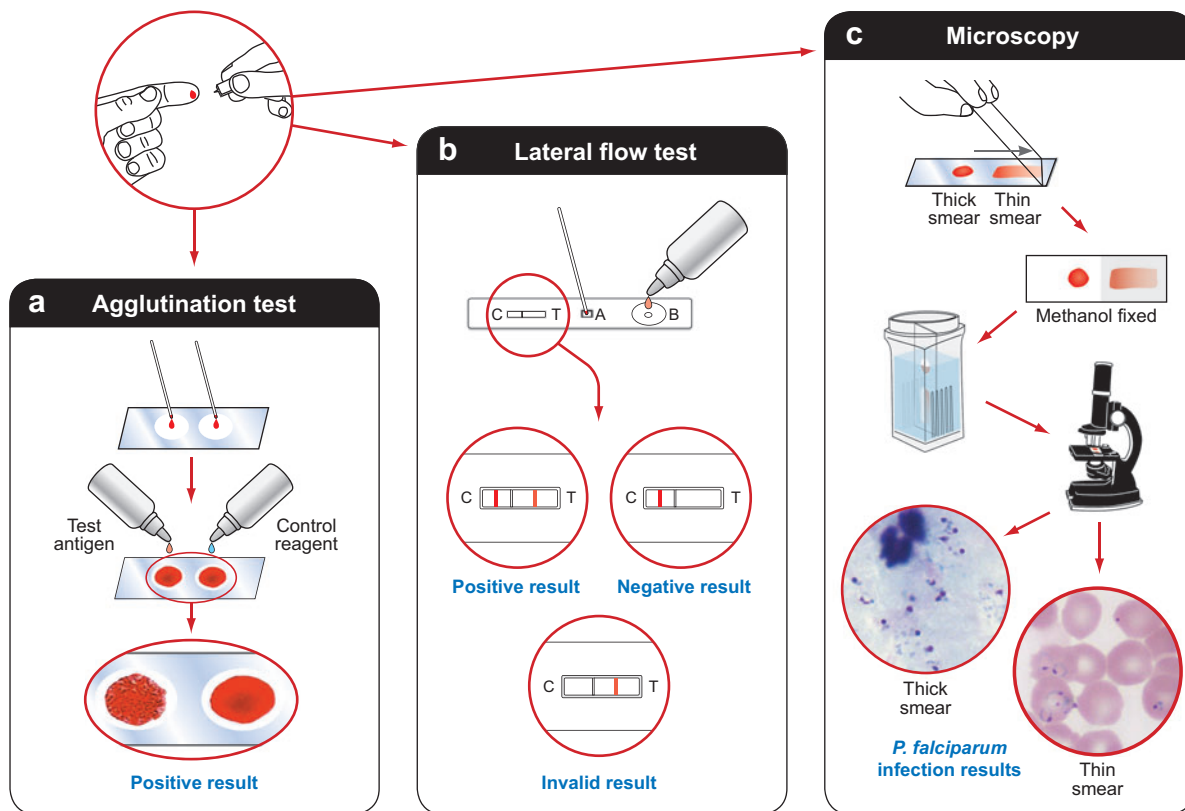


Figure 1

(a) Typical steps involved in an agglutination diagnostic test. In this figure, agglutination of red blood cells is observed similar to the results expected in an HIV-positive result from an HIV serology test. (b) Typical steps in a lateral-flow test (rapid diagnostic test) such as for malaria antigen detection. A is the specimen inlet, B the buffer port, C the control window, and T the test result window. A test result is only valid if a line is observed in the C window. (c) Typical steps for malaria light microscopy using Giemsa stain. Results for *Plasmodium falciparum* infection are shown in both the thick smear and the thin smear. The thin smear is methanol fixed such that the red blood cells remain integral.

discussed above, as well as for disease-specific reasons. For certain diseases, a range of tests is needed, providing options to meet the unique but diverse circumstances encountered within low-resource settings.

As mentioned above, microscopy remains the most economical, quantitative, and specific diagnostic platform for detecting live infections. Antigen detection tests or nucleic acid tests cannot distinguish live infections from recent infections. However, the importance of good quality slides, microscopes, and reagents (as well as regular training and evaluation of microscopists) is chronically undervalued. Over a century of poor microscopy performance has contributed to the culture of mistrust and undervalue of diagnostic test results by health care providers in low-resource settings.

Several tests utilize the visual observation of agglutination of either latex beads or red blood cells to detect either antigen- or pathogen-specific antibody (91–93). Agglutination tests have the benefit of being semiquantitative when tested on serial dilutions of specimen. These tests are cheap to manufacture, but, once reconstituted, the reagents have limited shelf life in nonrefrigerated conditions.

Lateral-flow tests or RDT technology remains the most successful new technology to impact POC testing in low-resource settings, and the uptake of RDTs is expected to continue rising for diseases such as malaria (94). In many cases (e.g., malaria and *C. trachomatis*), the sensitivity and specificity of RDTs may not be optimal, but they still are an improvement over current practices in the field. There is currently significant effort to develop quantitative lateral-flow tests as well as to enhance signal amplification, as reviewed by Chan et al. (95). These tests have not reached POC testing in low-resource settings. Reagent stability under the harsh conditions found in low-resource settings remains a major cause for test failure there (96, 97), as well as the manufacture and commercialization of poor quality tests.

An approach to introducing new technologies with minimal user-interface disruption is to combine the new technologies with agglutination, RDTs, or simple colorimetric outputs that are already in use in low-resource settings (98, 99). Significant effort has already gone into developing lateral-flow tests to allow visualization of nucleic acid–amplification signals. In the case of the NAAT, loop-mediated isothermal amplification, the output is turbidity, which is similar to agglutination and requires no additional instrumentation for signal visualization (100).

4.2. Specimen Collection

The impact of diagnostic test performance begins at specimen collection. Collecting the wrong specimen type can reduce the performance of a diagnostic test (101). For lower respiratory tract infections, the collection of sputum specimens remains a major challenge, especially in pediatric and HIV-positive populations (102, 103). In the case of sexually transmitted–disease screening, it has been important to evaluate the acceptability of different specimen-collection methods, as well as their impact on test performance (104).

Specimen-collection technologies assumed to be the norm in high-resource settings are most likely not available in low-resource settings. Ideally a test would be packaged with the optimal swab or specimen-collection device to ensure performance. The additional cost and possible regulatory-process ramifications are real deterrents for this. Certainly, the technologies should be evaluated with specimens collected under conditions encountered in low-resource settings.

4.3. Specimen Processing

In comparison to analyte detection, relatively little attention has been dedicated to developing approaches to process specimens that are appropriate for use in low-resource settings. Where this

has happened, it has been in integrated platforms such as lab-on-a-card. There is an enormous technology gap for developing low-cost, noninstrumented sample-processing technologies that can feed into a variety of downstream analyte-detection technologies. This approach would ensure multiple uses of the technology in a diversity of tests. Needs in sample-processing devices include rapid cell isolation from clinical specimens, rapid plasma separation from whole blood, white blood cell isolation from whole blood, and nucleic acid extraction from clinical specimens. Some exciting approaches for cell separation using physical differences, such as size shape and deformability in cell type, are emerging but have not resulted in commercial products (105, 106). An elegant approach employed for CD4 counting uses the relative shear-stress susceptibilities for binding CD4 T cells and monocytes to CD4 antibodies to specifically isolate immobilized CD4 T cells (107, 108). Similarly, although several interesting nucleic acid-extraction chemistries and technologies exist, there is not a single product that enables noninstrumented extraction of RNA or DNA.

In many cases in which the POC diagnostic test does not exist, simple technologies that stabilize the target analyte for shipment of the specimen to a central testing facility without requiring cold chain infrastructure can enable testing in low-resource settings that would otherwise not have been possible. Applications of such technologies include the use of dry blood filter spots to stabilize DNA for infant HIV diagnosis (109), RNA for HIV viral load testing and measles surveillance (110, 111), and antigen p24 for HIV diagnosis (112).

4.4. Instrumentation

Instruments in low-resource settings, above all, need to be robust to require low external maintenance. Instrument calibration and quality-control protocols and reagents should be simple to implement, and the reagents should be stable for long periods of time at high ambient temperatures. The most attractive instruments for uptake in low-resource settings are those that have multiple diagnostic applications and require minimal exclusive vendor source reagents (9), such as the light microscope. Instruments that require an exclusive reagent source are only likely to be taken up through a reagent rental model or under external funding opportunities. One advantage of the reagent rental-model approach is that the quality of the test reagents is controlled by a sole manufacturer. A major consideration, however, is that the sustainability, and practicality, of exclusive paired instrument-reagent systems presupposes the establishment of a reliable distributor and supply infrastructure in the low-resource setting.

A key accelerating force for dispersing high-end instrumentation into low-resource settings and adapting this instrumentation to low-resource settings has been the response to the HIV pandemic and initiatives, such as the U.S. President's Emergency Plan for AIDS Relief, to make antiretroviral treatment available in sub-Saharan Africa. To address the urgent need to perform accurate CD4 testing for antiretroviral treatment of HIV-positive patients, facilities have adopted flow cytometers at the district level in some cases across sub-Saharan Africa, and several manufacturers have developed simpler, more robust, test-specific flow cytometers. An additional long-term result of this initiative is the establishment of distributors and supply chains by several flow-cytometry manufacturers.

4.5. Use of Multiple Disease Markers for Clinical Disease Diagnosis

The molecular pathogenesis of disease is unique to each infectious agent. The onset, progression, and severity of clinical disease may be associated with different biomarkers over time. These may be both host and pathogen biomarkers. The time frame for the detection of any single biomarker associated with the disease may be limited. Target disease markers for a diagnostic test should be

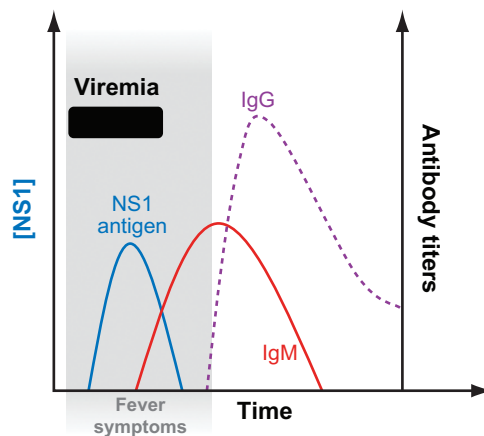


Figure 2

Typical biomarker profile for a primary dengue infection, in which viremia (viral load) and antigen levels are detected early during symptom presentation, followed by increasing immunoglobulin M (IgM) and immunoglobulin G (IgG) titers.

selected to diagnose a patient in the time frame he or she is most likely to present at a clinic and when intervention is most likely to have the best treatment outcome.

In the case of dengue infection, viremia and antigenemia are good early-fever-onset markers for disease diagnosis, but after days 4 and 5 of fever presentation, dengue-specific immunoglobulin M (IgM) detection may be a more sensitive test (**Figure 2**). IgM persistence beyond the presentation of fever symptoms has implications on the specificity of the marker for diagnosis of acute febrile patients. Multiplex diagnostic platforms that can detect multiple disease markers may increase the sensitivity of a test, but potentially at the cost of specificity. In the case of HIV screening tests, combining p24 antigen detection with anti-HIV antibody detection has created a highly sensitive test suitable for blood-safety testing (113).

For diseases with complex etiologies such as neonatal sepsis, the careful selection of disease markers must be tailored to the point of intervention targeted by the diagnostic tool. In identifying potential septicemia early in infection (when intervention has the most impact on the newborn's outcome), the infant's risk factors, clinical symptoms, and inflammation biomarkers are most relevant, and pathogen markers only become more relevant during later progression of disease, when hopefully the infant has been referred to a higher-level health care facility (114, 115).

4.6. Biosafety and Environmental Impact

Waste disposal in many developing-country clinical facilities is extremely rudimentary—often as basic as open outdoor incinerators for hospitals. Many supplies such as syringes are recycled either back into the clinic or even into the market as toys for children. Diagnostic tests appropriate for these settings must take into account their environmental impact.

The concept of biosafety is not widespread, and resources to ensure safe containment of biohazardous materials are often nonexistent. This is a particularly important consideration in settings that have high prevalence of infectious disease, particularly TB and HIV. One major problem is needle handling and disposal in developing-world clinics, which is a major challenge to resolve (**Figure 3**). Diagnostic tests should avoid contributing to these biohazard risks, particularly tests that do not explicitly inactivate pathogens as part of their procedure. A particular



Figure 3

Representative photographs to illustrate the importance of biosafety and environmental impact in medical product design for low-resource settings. Photos *a–c* were taken at health care facilities. (*a*) An open burning pit for medical waste in Senegal (PATH). (*b*) An incinerator overflowing with medical waste in Tanzania (PATH). (*c*) An open medical waste burner in Nigeria (PATH). (*d*) Needles and syringes in a public waste dump in India (Mark Koska).

concern is in the field of TB diagnosis, which requires handling and processing potentially contagious sputum specimens, as well as mycobacterial cultures. Either a safe means to package biohazardous components of a diagnostic test for shipment to a central facility with safe disposal resources needs to be implemented, or sustainable solutions to sterilize the biohazardous components need to be found. One group has demonstrated the effective use of a US\$50 solar cooker to disinfect 24-well TB culture plates (116) on the low-tech microscopic observation direct susceptibility assay developed to detect *Mycobacterium tuberculosis* from sputum samples and perform rapid, drug-susceptibility testing through microscopic observation of liquid TB cultures (117).

4.7. Regional and Population Variations on Diagnostic Test Performance

Many factors influence the performance of diagnostic tests, including the prevalence and incidence of disease in a population; the age of the patient; the acquisition of partial immunity; and coinfection with other diseases, especially HIV. In the case of TB, coinfection with HIV significantly drops the sensitivity of the sputum smear test.

In the case of malaria, the range of population susceptibilities to parasitemia complicates diagnosis. These are partly genetic but also result from partial immunity, acquired by frequent

exposure to parasite infection, and (in the case of pregnant women) additional susceptibility to placental malaria infection. Whereas NAATs and lateral-flow tests detecting parasite DNA and antigen markers, respectively, are highly specific tests for clinical malaria in susceptible populations (newborns, pregnant women, travelers, and in low endemic regions), they are less specific in high malaria-endemic regions and partially immunized populations (118, 119). A human host biomarker that, in conjunction with parasitemia, can confirm clinical malaria and not just latent infection would be extremely useful, but has not been identified.

In the case of serological assays and in the absence of specific antigens (such as for typhoid diagnosis and the most commonly used Widal test), regional background antibody titers must be determined to tune the cut-off titer for optimal sensitivity and specificity performance of the test in a specific population (120, 121). The complexities of disease pathogenesis and population variations require rigorous evaluation of a diagnostic test in the populations for which it is intended.

4.8. Standards for Evaluation

In a recent survey performed by WHO TDR, only 45% of the 85 countries that responded reported that they regulated in vitro diagnostics for infectious disease, not including tests used for blood banking, and of these, only 68% required clinical trial data (122). Of those that do not regulate diagnostic tests, most are developing countries. Many countries refer to the standards set by the U.S. FDA and the European Union to evaluate diagnostic tests. However, these often do not address the infectious diseases prevalent in many low-resource settings and are not appropriate for the clinical needs of these settings. As a result, there is often no robust set of criteria with which to select a diagnostic test for purchase and know its performance in the setting and the population on which it will be used. The WHO TDR and collaborating stakeholders are establishing a range of guidelines, both generic and disease specific, for evaluating infectious-disease diagnostics (122–126). These are, and will be, an invaluable resource for diagnostic test users in assessing available tests, but these guidelines will be equally important for test developers to consider early on in product development when establishing product specifications.

5. PLATFORMS FOR THE DETECTION OF MULTIPLE PATHOGENS: MULTIPLEX VERSUS SINGLEPLEX

5.1. Benefits and Risks of Multiplex Diagnostic Platforms

The term multiplex can be confusing as it has different meanings to an engineer, a molecular biologist, and a clinician. Generally, it refers to the simultaneous detection of more than one pathogen from a single specimen. From the clinical perspective, multiplexing is defined as a panel that provides for differential diagnosis because the same clinical symptoms generally occur due to infection from many etiological agents. One approach to multiplex reactions is to engineer the diagnostic device so that the specimen is split or aliquoted into separate reaction compartments (multiplexed as defined by an engineer). However, in many circumstances, the quantity of the targeted nucleic acid is limited so that dividing the specimen and using separate repeat analyses are often not possible. Molecular assay multiplexing refers to simultaneous detection by incorporating multiple PCR primers for nucleic acid amplification, or antibodies or antigens for immunoassays and detecting results within the same reaction. Although there may be good clinical rationale for a diagnostic panel, the engineering or assay strategies for multiplexing have technical risks that

Table 8 Advantages and disadvantages of a multiplex point-of-care diagnostic platform

Multiplexed assay format	
Advantages	Disadvantages
Panel of pathogens simultaneously detected	Panel varies by geographic location
Multiple pathogen detection at reduced cost per pathogen	Increase in per-assay cost
Reagent mixture reduces cost	Reduced assay sensitivity and specificity
Single specimen mixture detection	Lower copy target may not be detected
Single assay versus multiple	Increased assay complexity/method must detect RNA, DNA, and protein (antibody, antigen) from bacteria, viruses, parasites
Multiplex can incorporate redundancy, controls, subtyping, and drug resistance	Increased cost and assay complexity

can impact the cost, sensitivity, and specificity of the diagnostic test. **Table 8** summarizes the advantages and disadvantages of using a multiplexed assay format.

A significant challenge to the development of panels is that the relevant pathogen combinations will vary depending on the geographical disease-prevalence patterns. This will not only vary from country to country, but may also shift from year to year. Therefore, either totally comprehensive identification or an extremely flexible platform that can be readily changed is necessary. The validation of multiplexed platforms specific to geographic location and following changes to the panel is extremely problematic.

Multiplexed assay approaches that split the initial specimen do not decrease the per-analyte cost (i.e., a panel that detects six pathogens from one specimen fluidically split into six PCR assays will have a reagent cost equivalent to six individual assays). To save on reagent cost, investigators have attempted the combined detection of multiple pathogens within one reaction. For example, for PCR, the strategy for these multiplex reactions is the careful selection and optimization of specific primers that can function when combined in a single reaction (127, 128). A number of specific problems have been identified that limit PCR multiplexed primer reactions (129–131). Incorporating primer sets for more than one target requires careful matching of the reaction efficiencies. If one primer amplifies its target with even slightly better efficiency, amplification becomes biased toward the more efficiently amplified target, resulting in inefficient amplification of other target genes in the multiplex reaction. This is called preferential amplification and results in variable sensitivity and possible total failure of one or more of the targets in the multiplex reaction. Preferential amplification can sometimes be corrected by carefully matching all primer sequences to similar lengths and GC content and optimizing the primer concentrations, for example, by increasing the primer concentration of the less efficient targets. For the multiplexed combination of immunoassay antigen, antibodies, or multiple nucleic acid primer sets, a frequent artifact results from cross-reactivity or nonspecific binding when detection reagents are combined. This results in false positive or background signals that must be taken into account when adjusting the assay specificity. For PCR reactions, the reaction kinetics and efficiency are altered when more than one reaction occurs simultaneously. Each multiplexed reaction for each different specimen type must be optimized for the $MgCl_2$ concentration and ratio to the deoxynucleotide concentration, KCl concentration, Taq polymerase concentration, thermal cycling extension, and annealing times and temperatures. There is competition for the reagents in multiplex reactions, so all the reactions plateau earlier. As a consequence, multiplexed reactions in general are less sensitive and less specific than the corresponding simplex reactions.

5.2. New Technologies Permitting Multiplex Diagnostics

Recent significant technical improvements for both proteomic and nucleic acid methodology will better enable multiplexing or reactions. Chimeric primer designs have been shown to correct preferential amplification. Each primer contains a 3' region complementary to sequence-specific target recognition and a 5' region comprising a universal sequence. Using the universal-sequence primer permits the amplification efficiencies of the different targets to be normalized (132, 133). A number of detection approaches enable the simultaneous detection of multiple products within one reaction to discriminate and detect each target. For PCR reactions, the PCR products can be labeled so as to be detectable by spectroscopic, photochemical, biochemical, immunochemical, or chemical means. For example, amplification can be monitored in real time using multiple fluorescent dyes incorporated with a self-quenching probe design (133, 134). Here, the number of multiplexed targets is limited by the number of dye or other label moieties distinguishable within the reaction. As the number of different fluorescent moieties to be detected increases, so does the complexity of the optical system and data analysis programs necessary for result interpretation. For PCR reaction products, in addition to dye color, melting temperature has been used as an additional dimension of multiplexing (135).

To expand upon the multiplexing capability, amplified products can be hybridized to a solid phase followed by fluorescence detection. For example, a consensus PCR assay followed by a reverse-line blot hybridization assay has been shown effective for detecting and genotyping human papilloma virus (136). The capture and detection of up to several hundred pathogens can be accomplished on either a planar platform or microarray (137) or coupled to fluorescence-labeled polystyrene beads, as in the Luminex suspension array technology (138). These proteomic or DNA highly multiplexed platforms offer the potential for the simultaneous detection of many pathogens. However, their current cost will need to be substantially reduced for their practical use, especially in low-resource settings.

The clinical progression of most infections (e.g., measles and dengue) results in viremia early on, followed by fever, during which virus particles or nucleic acid is best detected by PCR. However, within a few days post-fever onset, the immune response clears circulating virus as the IgM immune response occurs. At this time, PCR can be negative while IgM is detected. Therefore, a strategy that combines both PCR and IgM detection should increase diagnostic sensitivity for patients at various times after the onset of fever. Lindegren et al. (139) demonstrated this to be the case for dengue. Therefore, combining molecular and immunoassay targets is another type of multiplexed approach. **Figure 4** illustrates one such platform under development for low-resource settings.

6. EMERGING TECHNOLOGIES APPROPRIATE FOR APPLICATION IN POINT-OF-CARE TESTING IN LOW-RESOURCE SETTINGS

6.1. Imaging and Image Analysis

Given that microscopy remains one of the most critical technologies for a wide range of diseases in the developing and developed world, it is inevitable that further developments in imaging and imaging analysis will continue to be applied to global health problems. Sample preparation for imaging is currently a roadblock requiring trained personnel, but inexpensive microfluidic systems could be designed to facilitate that aspect of imaging, allowing less-trained personnel to get reproducible results.



Figure 4

Solid model of the fever panel instrument (the DxBox) under development by a consortium (University of Washington, PATH, Nanogen, Micronics, Invetech) led by the authors with funding from the Bill & Melinda Gates Foundation. The DxBox combines both polymerase chain reaction and immunoassay-based detection for an orthogonal approach to near-patient clinical diagnosis of fever agents. It is battery operated, accepts a finger-stick blood specimen, and automates all steps within a microfluidic disposable. Although this is a conceptual model, it was designed with engineering input following initial biological reaction verification and sourcing of low-cost miniaturized instrument components for fluid movement, heating, and end-point optical detection. Similarly, the disposable is modeled for miniaturized fluidic circuitry and conversion from verified laminate prototype subcircuits to an injection-molded disposable that can be manufactured at low cost.

6.1.1. Imaging. Today, optical imaging by trained personnel is still the most trusted technique for definitive diagnosis of the two biggest killers in the developing world—malaria and TB. As mentioned above, high-magnification (100×) transmission light microscopy is still considered the definitive test for malaria. It is used to identify and count the number of malarial parasites in stained blood smears. Because this process is slow and relies on the training of the technician, it is well suited to some form of automation. For TB, the auramine stain method requires fluorescence microscopy. Although it is unlikely that any future conventional transmission or fluorescence microscopes will be better designed than those produced in the past 15 years, there have been great strides in reducing the cost of microscope construction based on the use of plastic parts. Decent optical microscopes coupled to charge-coupled-device cameras can now be purchased from multiple vendors for less than US\$500. One important driver for reducing the cost of cameras has been the incorporation of simple digital cameras into cellular phones. Such cameras, similar to decent webcams, can be purchased for less than US\$100 retail, so the potential for assembling complete camera-based imaging systems that interface with personal computers for a few hundred dollars is now within reach. The availability of bright blue light-emitting diodes and lasers offers the potential for making inexpensive complete imaging systems for fluorescence imaging as well.

6.1.2. Image analysis. Taking an image is only part of the problem. WHO, for example, suggests that at least 100 fields must be examined before making a definitive diagnosis of malaria (140). It would clearly be advantageous to have an automated, inexpensive, and robust system that could collect all the images and analyze them. There are many commercial image-processing packages that could be used to create algorithms capable of identifying stained pathogens in images and

counting them. Such image processing has been incorporated into automated histology instruments for the central laboratory for some time. Furthermore, the nearly ubiquitous data-capable cellular-phone networks in the developing world could allow such image analysis to occur either next to the microscope or remotely after sending the images to a site equipped either with a human or electronic-image analysis system. Such a development would make microscopic pathogen identification in remote sites much more practical than it is today.

6.2. Flow Cytometry

The ability to rapidly count cells on the basis of morphology or the presence of specific surface antigens has great value in many conditions, in particular in staging the progression of HIV infections into full-blown AIDS (141). The optimal tool for such type-specific cell counting has been flow cytometry, which has generally been financially out of reach for small laboratories in the developing world and has been out of the question for remote sites. For this reason, there are new low-cost and simplified conventional flow-cytometry instruments being introduced into clinical laboratories at this time, as recently reviewed by Baum et al. (142). In the past decade, there has been great interest in microfluidic approaches to flow cytometry (143–151). However, none of these microfluidics-based flow-cytometry instruments has made it to market. Flow cytometry may be superseded as the leading candidate for enumerating CD4⁺ lymphocytes by novel approaches from the Rodriguez group. This group's two approaches combine simple microfluidics with either fluorescence imaging (152) or conventional transmission light microscopy combined with particle counting (by eye, if need be) (107, 108). This latter approach, in particular, seems well suited to low-resource settings.

6.3. Immunoassays

Immunoassays have been used in one form or another for over 50 years. The basics of ELISA (enzyme-linked immunosorbent assay) have dominated the use of laboratory immunoassays since it was adopted over 20 years ago. Although ELISA is an excellent method in its usually 96-well plate format, it is not well suited to use outside the sophisticated climate-controlled laboratory with highly trained personnel. To bring immunoassays to global health, researchers have developed and deployed a few types of RDTs. Most are based on the lateral-flow immunoassay, or immunochromatographic test strip. These are most commonly seen in the developing world in the form of the home pregnancy test kit, which (despite the retail costs of the test) are extremely inexpensive to manufacture. The antibodies and other reagents store well on the nitrocellulose materials used in the flow strips, so room-temperature storage for a year is easily achieved. These are relatively fast (a few minutes) and require little sophistication on the part of the user—often running these assays is as simple as dipping the strip into a body fluid and waiting for the signal (and an internal positive control) to develop. A major failing of these tests is that they are rarely quantitative. Although this may be adequate for some diagnostic purposes, it is not adequate for all. It is possible that some lateral-flow tests, if read by a photometric reader, could be used at least semiquantitatively.

There is probably no assay type that has been subject to more implementation in novel formats than immunoassays. Almost every optical, electrical, and mechanical transducer imaginable has been coupled to immunoassays. Many of these will remain laboratory curiosities because their transducers are impractical. However, there are some that may ultimately prove useful. Some, such as the diffusion immunoassay for measuring the concentrations of small molecules, are inherently microfluidic but are extremely rapid (a few seconds), so they may justify the additional expense

of requiring sophisticated flow control (153–160). Others, such as the recently developed dry cantilever assay from the Manalis group (161), have exquisite sensitivity but no track record of holding up to complex samples.

6.4. Microfluidics

The field of microfluidics, or the manipulation of small volumes of fluids in microfabricated channels, and the related analytical field known as lab-on-a-chip (or micrototal analysis systems) are growing rapidly. Microfluidics has been reviewed recently (162–166), as has its rapidly increasing application to medical diagnostics (167, 168). It has been applied to almost every aspect of analysis of complex biological fluids. One of the most exciting uses for microfluidics in lab-on-a-chip application is in the automation of sample preparation. These sample-preparation tasks are often difficult to perform in laboratories in low-resource settings and are impossible outside of laboratories, so some of the greatest advances in moving diagnostics to the periphery of the health care system can be made in this way (3). Microfluidic systems have been used for continuously fractionating components of blood (105–108, 145, 169–172) and for nucleic acid extraction upstream of PCR (173), as well as for the purification of small molecules upstream of an immunoassay (3, 174). Some new techniques, such as pinched-flow fractionation, show particular promise in simply but effectively fractionating cells by size (175).

The use of microfluidics for blood analysis was recently reviewed in this journal (105), and there has also been a recent review of lab-on-a-chip devices for global health applications (2). Microfluidic devices have been designed for most common laboratory bioassays, including PCR (176, 177) and immunoassays (107, 178–196). In a few cases, microfabrication allows a technique such as PCR to be carried out efficiently (197).

Although these developments in microfluidics are good news, not all microfluidic devices lend themselves to the cost goals of the global health context. Depending on the final system's goals, the cost of some microfabricated devices may put them out of range for end users in the developing world. Relatively few microfluidic approaches have low cost as a central requirement of the technology (198). Using microfluidic devices as part of entire disposable systems, with or without a permanent instrument to drive the fluids and make quantitative readings, is probably the most practical direction for global health. This requires that the microfabricated devices ultimately be manufacturable using methods such as injection molding that allow production of large numbers at very low per-part cost.

6.5. Nanotechnologies

Nanotechnology has been a popular term to describe things with submicron dimensions. The nanotechnology that can be differentiated from more conventional chemistry and biochemistry that has been closest to being applied in diagnostics has generally involved nanoparticles for labeling, including novel versions of well-studied metal nanoparticles (199–203), simple or complex magnetic nanoparticles (e.g., for sample capture), quantum dots (bright fluorescent labels of protein size) (204–207), core-shell nanoparticles (metal-dielectric complex particles for high absorbance) (208), and the use of many of these in imaging. For nonimaging diagnostic applications, the most used have been quantum dots as replacements for fluorescent molecules for labeling secondary antibodies. However, because these do not lend themselves to the conventional ELISA format, they have been restricted to research applications. However, they may soon be found in use in lateral-flow tests. The Stayton group (209, 210) has been pushing the use of stimuli-sensitive nanoparticles in sample conditioning with applications in diagnostics for global health. A critical

SPR: surface plasmon resonance

issue in the applicability of these nanotechnologies to global health diagnostics will be whether the cost of these nanotechnologies can be kept low. Many of the uses in nanotechnologies for in vivo imaging will certainly prove valuable for medicine in the developed world, but the high cost of imagers themselves may well delay introduction into the developing world.

6.6. Surface Plasmon Resonance

There are several detection technologies that have been in the laboratory for some time that may make the transition to laboratory diagnostics in the next few years. Among these is surface plasmon resonance (SPR), which has been used in large laboratory instruments for more than a decade (211). SPR is a method of measuring the refractive index of thin film within a few hundred nanometers of a metal surface. If the metal surface is coated with a chemically selective coating, the binding of molecules to that surface can be detected without labels. Because it is not specific to a particular type of chemistry, SPR has been adopted for many binding assays, including nucleic acid detection (212, 213) and immunoassays. A variant of SPR that utilizes imaging of the required metal surface to allow multichannel detection also shows promise (214), and it too can be used for assays of different types (215)—it is a versatile platform of any binding assay. This will particularly be true if the imager itself can be made small and portable (216–223), and the imager can be easily and reversibly coupled to inexpensive disposables that perform all the fluidic functions and sample processing without the need for user activity.

6.7. Requirements and Challenges of New Technologies

The central challenges in adopting new technologies for use in diagnostics in global health are related to the location at which the technologies will be used and the users. These technologies must operate under conditions akin to a battlefield and by end users who are often poorly trained. To address the environment, the technology must be robust in all ways, and none of it (instrument or disposables) can require the sorts of storage conditions that we take for granted in laboratory medicine in the developed world. It must also require a minimum of training on the part of the end user and, ideally, be tolerant of failure on the part of the end user to use it properly. Issues such as making sure the test has internal controls that lock out a reading of the test if it has not been performed properly (or has failed because of long or extreme storage conditions) need to be considered. In other words, even if the test itself is simple, the test system may have to be complex to eliminate erroneous readings. All this must be accomplished at a minimum cost if the technology is to be of any use to the developing world. This is no small challenge.

7. SUMMARY

Current needs for accurate diagnostic methods for global health are critical. Existing methods are failing to meet the need, either because they are not accessible to health care workers in the developing world or because they are not affordable. Some new technologies, such as lateral-flow immunoassays and related RDTs, have recently made great progress in assisting beleaguered health care workers. However, additional test-system sophistication will certainly be needed to meet some of current and anticipated diagnostic needs. Of the new technologies, perhaps the most promising at the moment is microfluidics, as it has the potential to add capabilities for sample processing and complex fluidic handling while being compatible with inexpensive materials and fabrication methods. A major challenge for biomedical engineers in the next decades will be to translate recent research in these areas into affordable technologies available at the periphery of

the health care system. Developing a diagnostic test that can be adopted in low-resource settings and that has a cost-effective impact on health care requires that technology developers engage with the clinical need and the end users in low-resource settings at the early stages of product development.

DISCLOSURE STATEMENT

P.Y., G.J.D., and J.G. collaborate on the development of a microfluidic diagnostics platform with funding from the Bill & Melinda Gates Foundation. J.G. is the Chief Technology Officer of Micronics, Inc. He has also collaborated on the following grants: grant awarded to the University of Washington–led consortium for the development of a unique point-of-care diagnostic platform for use in the developing world, funded by the Bill & Melinda Gates Foundation; two NIAID-funded grants awarded to PATH for the development of near-patient point-of-care diagnostics for detection of enteric pathogens and STD diagnosis.

ACKNOWLEDGMENTS

We acknowledge support of many granting agencies [including the Bill & Melinda Gates Foundation, the NIH (NIDCR, NIBIB, NIAID, etc.), and USAID] and our respective colleagues and students at the University of Washington, Micronics, and PATH.

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