A young lady waits to be seen at a clinic in rural Liberia, West Africa.
Acknowledgments

The Diagnostics Innovation Map: Medical Diagnostics for the Unmet Needs of the Developing World

Copyright © 2010 BIO Ventures for Global Health

This report was written by Priya Mehta and David Cook, with the support of Health Advances, LLC.

Authors’ note:

We thank the Bill & Melinda Gates Foundation for its financial support. We are grateful to the team at Health Advances: Kristin Pothier, Sonia Gupta, Paula Ness Speers, Philina Lee, Kimberly Howland, Mark Speers, and Donna Hochberg for their hard work and significant contributions to this document. We thank Christopher D. Earl for his continued contributions and valuable feedback. We also thank our Scientific Advisory Board for their feedback and support: N. Leigh Anderson, Deborah Burgess, David Kelso, Alan Magill, Francis Moussey, Rosanna Peeling, Mark Perkins, Mark Reynolds, William Rodriguez, Samuel Sta, John J. Sminsky, Amy Wong, and Paul Yager. We thank all those interviewed during the course of this project for their time and effort to ensure the accuracy of the report.


Contents

Glossary of Diagnostic Terms .................................................. 4

Executive Summary .............................................................. 9

Chapter 1: Project Objectives and Methodology ......................... 12

Chapter 2: Context: The Need for Novel In Vitro Diagnostics
for Neglected Diseases ......................................................... 15

Chapter 3: Diagnostic Requirements in the Developing World ....... 23

Chapter 4: Innovative Diagnostic Technologies .......................... 31

Chapter 5: Incentives for Diagnostics Innovation
in the Developing World ...................................................... 54

Conclusion: A Call to Action .................................................... 59

Appendix I: Organizations Interviewed ................................. 62

Appendix II: Examples of Specific Diagnostic Unmet Needs
for Neglected Diseases ....................................................... 64

Appendix III: Researched Technology Highlights ..................... 74

Appendix IV: References ....................................................... 102
Glossary

Assay A term used to describe the procedure used for conducting a diagnostic test.

Analyte Entity or target that is being analyzed. Can be an ion, a protein, a cell, a molecule, etc.

Antibody Also known as immunoglobulins. Gamma globulin proteins that are found in blood or other bodily fluids of vertebrates, and are used by the immune system to identify and neutralize foreign objects, such as bacteria and viruses.

Antigen Any molecule that binds specifically to an antibody.

Biomarker A biological marker of disease.

CD4/CD8 Cell surface markers that are used in HIV viral load testing.

Central laboratory Hospital laboratory staffed by trained lab technicians performing high volume, moderately complex tests on highly automated instruments.

Deoxyribonucleic acid (DNA) A nucleic acid that contains the genetic instructions used in the development and functioning of all known living organisms and some viruses. DNA consists of two long polymers of simple units called nucleotides, with backbones made of sugars and phosphate groups joined by ester bonds. These two strands run in opposite directions to each other.

Endonucleases Enzymes that cleave the phosphodiester bond within a polynucleotide chain, in contrast to exonucleases, enzymes that work by cleaving nucleotides one at a time from the end of a polynucleotide chain.

Enzyme Usually a protein, that catalyzes (i.e., increase the rates of) chemical reactions.

Enzyme-linked immunosorbent assay (ELISA) A biochemical technique used mainly in immunology to detect the presence of an antibody or an antigen in a sample. In ELISA an unknown amount of antigen is affixed to a surface, and then a specific antibody is washed over the surface so that it can bind to the antigen. This antibody is linked to an enzyme, and in the final step a substance is added that the enzyme can convert to some detectable signal.

Immunoassay A biochemical test that measures the concentration of a substance in a biological liquid, typically serum or urine, using the reaction of an antibody or antibodies to its antigen.

In vitro diagnostics (IVD) Medical device products including instrument and reagents that utilize a variety of methods and formats to perform tests on human samples in order to assess disease risk, diagnose a condition, or monitor a patient’s health.

Isothermal reaction A chemical reaction going to completion at one temperature; not needing a change in temperature to continue reaction to completion.

Lateral flow test A simple device intended to detect the presence (or absence) of a target analyte in sample. Lateral flow tests are a form of immunoassay in which the test sample flows along a solid substrate via capillary action, and are often produced in a dipstick format.

Light-emitting diode (LED) A semiconductor light source.

Lysis The breaking down of a cell, often by viral, enzymic, or osmotic mechanisms that compromise its integrity. A fluid containing the contents of lysed cells is called a “lysate.”

Microfluidics Deals with the behavior, precise control, and manipulation of fluids that are geometrically constrained to a small, typically sub-millimeter, scale.
**Molecular diagnostics (MDx)**  A discipline of laboratory medicine involving the use of testing procedures to measure DNA and RNA. Molecular diagnostics utilize techniques of molecular biology to diagnose infectious, genetic, and toxicological diseases.

**Monoclonal antibodies (mAb or moAb)**  Monospecific antibodies that are the same because they are made by one type of immune cell which are all clones of a unique parent cell. Given almost any substance, it is possible to create monoclonal antibodies that specifically bind to that substance; they can then serve to detect or purify that substance.

**Multi-analyte test**  Type of multiplexing in which multiple analytes are measured and each analyte measured corresponds to one distinct diagnostic answer.

**Multiplexing**  Simultaneous measurement of multiple analytes in single reaction vessel.

**Nucleic acid**  Macromolecule composed of chains of monomeric nucleotides. These molecules carry genetic information or form structures within cells. The most common nucleic acids are deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Nucleic acids are universal in living things, as they are found in all cells and viruses.

**Nucleotide**  Molecule that, when joined together, makes up the structural units of RNA and DNA.

**Oligonucleotide**  A short nucleic acid polymer, typically with twenty or fewer bases.

**Photodetectors**  Sensors of light or other electromagnetic energy.

**Point of care (POC)**  Testing performed at the point of patient care. This includes physician office POC testing outside the central laboratory.

**Polymerase chain reaction (PCR)**  A technique to amplify a single or few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.

**Reference laboratory**  Commercial organizations offering laboratory services to physician offices, clinics, and hospitals.

**Ribonucleic acid (RNA)**  A nucleic acid that consists of a long chain of nucleotide units. Each nucleotide consists of a nitrogenous base, a ribose sugar, and a phosphate. RNA is very similar to DNA, but differs in a few important structural details: in the cell, RNA is usually single-stranded, while DNA is usually double-stranded; RNA nucleotides contain ribose while DNA contains deoxyribose (a type of ribose that lacks one oxygen atom); and RNA has the base uracil rather than thymine that is present in DNA.

**Sample preparation**  Processes by which a representative piece of material (sample) is extracted from a larger amount and readied for analysis.

**Sample to result automation/ processing**  A fully automated process which allows the operator to input a sample and walk away. The instrument returns a result with no further intervention.

**Thermal cycling**  A temperature modulation process developed to improve the performance, strength, and longevity of a variety of materials.

**Transcription-mediated amplification (TMA)**  An isothermal nucleic-acid-based method that can amplify RNA or DNA targets a billion-fold in less than one hour’s time.

**Turnaround time (TAT)**  The turnaround time of an assay within the context of an instrument or the laboratory procedure beginning with the sample being loaded onto the instrument and ending with the result being recorded on the instrument. This can also be called the time to result.
We need improved in vitro diagnostics for neglected diseases affecting the developing world: Diagnostics have the potential to save hundreds of thousands of lives each year by detecting disease in its early stages. Early diagnosis ensures that patients get the right treatment quickly and curbs the over-use of inappropriate medicines, critical in the fight against drug-resistant bugs. Unfortunately, health care providers in poor countries lack basic diagnostic tools that would be taken for granted in wealthy countries. Despite this, less than 5% of annual spending on research and development (R&D) for neglected diseases is allocated to diagnostics. This must change. Improved in vitro diagnostic products, coupled with access to therapies, can save hundreds of thousands of lives per year.

It is critical that diagnostics for the developing world be suitable for resource-poor settings: In the developing world, diagnostics are needed in a wide range of settings—from well-equipped hospitals to the most basic rural clinics. In this report, we focus on the diagnosis of infectious diseases through “in vitro diagnostics,” in which a biological or chemical analysis is performed on a fluid or tissue sample from a patient to identify the presence, absence or changed quantity of specific “analytes,” or molecular indicators of disease. Depending on the setting, useful in vitro diagnostic products will take several forms—from large instruments in urban laboratories to point-of-care (POC) tests that can be used in the most remote rural regions. In order to benefit the majority of developing world patients—those who live in resource-poor settings—diagnostic tests must meet certain requirements. Tests must be cheap, small, and robust, capable of operating without clean water or electricity and of withstanding heat, humidity, and transport, and simple enough to be used by minimally trained health care workers. Only then will the majority of individuals in the developing world have access to needed diagnostic tests.

New technologies have the potential to yield significant breakthroughs in our ability to diagnose neglected diseases in resource-poor settings: Technological innovations are emerging that could address many of the unmet diagnostic needs for neglected diseases. These innovations are developing in four categories, listed below in their likely chronological sequence. The categories are grouped according to current technological advances and a projected development timeline:

- **Simpler instruments based on the adaptation of existing detection platforms:** There is an opportunity to develop simpler and smaller instruments relying on existing detection technologies. This is largely an engineering challenge to simplify, automate, and miniaturize existing platforms. One of the economic drivers of this trend is the shortage of trained medical technicians, which creates an imperative to simplify instrumentation even in the developed world.

- **Improved sample preservation and management:** Despite the need for POC testing to improve health care delivery for rural populations, central lab testing will be the predominant mode in the near term. For this reason, sample preservation technologies will prove extremely valuable over the near- to medium-term. Samples will be stabilized for transport at ambient temperature from rural settings to urban settings.

- **Development of new detection technologies:** In the medium- to long-term, POC diagnostic platforms based on new detection technologies that sensitively measure diagnostic markers will be developed. These innovations hold the potential to dramatically change the diagnostic paradigms in the developing world. Some of these new diagnostics may be able to offer broad menus of tests in a variety of sample types that previously required multiple detection technologies. In addition, many of these detection technologies will simplify process steps like sample prepara-
tion by decreasing background signal arising from the biological sample. There will be a class of detection technologies that are broadly applicable to multiple analytes—including proteins, nucleic acids, whole bacteria, virus, and small molecules—and these have the potential to simplify the number of platforms required to address the needs of the developing world.

- **Discovery of novel biomarkers**: The discovery of new biomarkers—molecules that are indicative of disease—will have the least predictable timeframe. In the developing world, the need for new biomarkers falls into several classes: First are those diseases for which a relevant biomarker has not yet been identified; Second are the diseases for which known biomarkers require difficult-to-obtain samples; Third are the biomarkers that represent only a subset of infectious organisms responsible for disease; and finally, there are biomarkers that depend on a host response, which can be variable and therefore lack sensitivity. With adequate investment, new biomarkers can be discovered to support the development of assays that can be run on increasingly capable diagnostic platforms.

**Risk and cost-sharing collaborations between donors, industry, and the public sector will be fundamental to advancing diagnostic development**: Promoting technical advances and commercializing new diagnostics for the developing world will require risk- and cost-sharing collaborations between diagnostic companies and not-for-profit organizations. Donor investment will be needed to initiate, and in some cases to sustain, these efforts. New market-based incentives providing industry with the promise of attractive financial returns must be implemented. Equally, companies must consider the real potential of this market, due largely to the rapid expansion of private-pay health care markets in the emerging economies of India, China, Brazil, and South Africa. A POC platform that is robust, portable, and low cost will also have the attributes needed to enable new indications in developed world markets—serving, for example, as a basis for testing for cancer and swine flu in middle and high income countries. Addressing the broad range of unmet diagnostic needs of the developing world will require bold investment decisions and forward-thinking collaborations between for-profit and not-for-profit organizations, donors, and governments, in addition to technical and scientific breakthroughs.

**There is a need for additional analysis to inform investments in diagnostic development for neglected diseases**: Additional analysis must be done to identify financial models that will support industry investment in developing novel diagnostics for neglected diseases and to bring coherence to donor funding of these efforts. BIO Ventures for Global Health is committed to working with the range of players involved in the discovery, development, and commercialization of novel diagnostics—from for-profit companies to not-for-profit development organizations to donors—to catalyze investments and partnerships that will lead to better and faster diagnostic innovations.
At BIO Ventures for Global Health (BVGH), our goal is to help companies find ways to participate in global health. We believe it is essential to engage private-sector innovators—particularly entrepreneurial biotechnology companies with a track record of developing new products—in the effort to prevent, diagnose, and treat neglected diseases in the developing world.

BVGH undertook this report in order to better understand diagnostic requirements for resource-poor settings and to explore how recent technological advancements in the developed world might be used to improve patient care and save lives in the developing world. The report explores some of the most innovative current and emerging diagnostic technologies and provides insight into how these technologies might be applied to developing world settings.

The report aims to provide:

- An understanding of unmet diagnostic needs for neglected diseases and the performance requirements for diagnostics used in resource-poor settings

- An overview of the value that novel diagnostics can bring to health care in resource-poor settings

- Examples of innovative lab-based and point-of-care (POC) technologies and analysis of how these recent advances could address unmet diagnostic needs in the developing world

- A resource to aid in forging new partnerships to accelerate the development of diagnostics for neglected diseases

This report is informed by more than 60 interviews conducted with the executive teams of diagnostic companies and global health experts. The companies highlighted in the report are those that were determined to be the most innovative in their approach and those whose technologies have the greatest potential for application in the developing world. Companies were identified through informational interviews with scientific experts in the diagnostic field, including members of our Scientific Advisory Board.

Primary research was supported with reviews of relevant clinical, technical, and global health research literature as well as information provided on company Web sites. In conducting this research, we identified similar published work and tried to leverage, rather than duplicate, prior efforts. Our sources are referenced and annotated in the bibliography at the end of the document.

To ensure the integrity of our findings, the research was periodically reviewed and refined by our Scientific Advisory Board, comprised of thought leaders in diagnostics development and global health. We are grateful for the contributions of our advisory group:

- N. Leigh Anderson, PhD, Founder and CEO, Plasma Proteome Institute

- Deborah Burgess, Senior Program Medical Officer, Bill & Melinda Gates Foundation

- David Kelso, PhD, Professor of Biomedical Engineering, Northwestern University
The Scientific Advisory Board has endorsed the objectives of this report and provided critical feedback throughout the process. However, the content of the report—and any errors—are the responsibility of BIO Ventures for Global Health and not the Scientific Advisory Board.

This report is intended to build on the existing public knowledge of unmet diagnostic needs of the developing world (see Appendix II) and to identify examples of promising technologies that may be able to address those needs. As such, it is not intended to be an encyclopedic accounting of all diagnostics currently used in the developing world nor of all novel technologies in development that may have applicability to the needs of the developing world. Wherever possible, we have attempted to build on the previous work conducted by others in this field.

As a result of this report, we hope that diagnostic developers will have a better understanding of how their technologies might be able to apply to neglected diseases that affect patients in resource-poor settings. We also hope that public sector organizations looking to collaborate with the private sector in developing novel diagnostics will have the information necessary to form such partnerships and advance this cause.

The list of the organizational affiliations of the individuals interviewed for this project can be found in Appendix I. A list of the main secondary sources reviewed in the course of this research is compiled in Appendix IV.
Chapter 2
Context: The Need for Novel In Vitro Diagnostics for Neglected Diseases

The Value of Novel In Vitro Diagnostics for Neglected Diseases

Patients in developing countries suffer from a wide range of maladies, many of which are familiar to the developed world. But they also are afflicted by a range of infectious diseases such as malaria, tuberculosis (TB), and African sleeping sickness that are rare or nonexistent in the developed world. These infectious diseases kill over 10 million patients in poor countries every year, many of whom live in rural settings that lack basic economic and health care infrastructure. In this report, we focus on the diagnosis of these infectious diseases through “in vitro diagnostics,” in which a biological or chemical analysis is performed on a fluid or tissue sample from a patient to identify the presence, absence or changed quantity of specific “analytes,” or molecular indicators of disease.

Improved in vitro diagnostic products, coupled with access to therapies, can save hundreds of thousands of lives per year. For example, determining whether an infant is infected with HIV is currently impossible with available diagnostic technologies due to interference of anti-HIV antibodies transferred from the mother. A novel HIV diagnostic test that could detect infection in infants without this interference—with 90% sensitivity and 90% specificity—has the potential to save between 800,000 and 2.5 million lives per year if it is made widely available and coupled with use of antiretroviral (ARV) therapies. Similar impact projections have been calculated for a wide range of diseases afflicting the developing world (Figure 1).
### Figure 1: Examples of Potential Lives Saved with New or Improved Diagnostics

<table>
<thead>
<tr>
<th>Infectious Disease Area</th>
<th>Clinical Decision Point</th>
<th>Sensitivity/Specificity/ Turn-around Time (TAT)</th>
<th>Sample Type</th>
<th>Potential Impact</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute Lower Respiratory Infection (ALRI)</td>
<td>Identification of children aged &lt;5 years with bacterial ALRI</td>
<td>95% sensitivity 85% specificity &lt;1 hr TAT</td>
<td>Saliva Urine Dried blood spot</td>
<td>≥405K adjusted lives saved per year</td>
</tr>
<tr>
<td>HIV/AIDS</td>
<td>Identification of HIV in infants &lt;12 months</td>
<td>90% sensitivity 90% specificity &lt;1 hr TAT</td>
<td>Heel stick blood Dried blood spot Saliva</td>
<td>~180K DALYs per year if 5% of the population has access to ARVs ~2.5M DALYs per year if 100% of the population has access to ARVs</td>
</tr>
<tr>
<td>Diarrheal diseases</td>
<td>Detection of G. lamblia, C. parvum and entero-aggregative E. coli in children &lt;5</td>
<td>90% sensitivity 90% specificity &lt;1 hr TAT</td>
<td>Feces Vapors</td>
<td>Reduce prevalence of diarrheal-related stunting by 12.5% 2.8M DALYs saved per year if the cost of treatment is $6</td>
</tr>
<tr>
<td>Malaria</td>
<td>Diagnosis in febrile children aged &lt;5 in sub-Saharan Africa</td>
<td>95% sensitivity 95% specificity &lt;5 min TAT</td>
<td>Finger-prick blood Urine Saliva</td>
<td>~1.8M adjusted lives saved per year 396M unnecessary treatments averted per year</td>
</tr>
<tr>
<td>TB</td>
<td>Diagnosis of active infections, with or without concomitant HIV infection</td>
<td>85% sensitivity 97% specificity &lt;1 hr TAT</td>
<td>Sputum</td>
<td>400K lives saved per year</td>
</tr>
</tbody>
</table>

A detailed table identifying disease-specific unmet diagnostic needs is presented in **Appendix II**.

Yet despite these pressing unmet needs and estimates of lives that could be saved in developing countries with improved diagnostic tests, the investment in diagnostics designed for the developing world has been meager.

---

**Insufficient Investment**

Historically, most of the attention given to the diseases of the developing world has focused on the development of novel vaccines and therapeutics for malaria, TB, and HIV/AIDS. In 2008, $2.96 billion was spent globally on research and development (R&D) of new neglected disease products. National Institutes of Health (NIH) was the top funding body, contributing over $1 billion to research, and the Bill & Melinda Gates Foundation was the second largest funding source, investing over $600 million. With a collective investment of $365.3 million, the pharmaceutical industry was the third largest investor in R&D for neglected diseases. Of the total $2.96 billion R&D spend, however, only $119 million was allocated to diagnostic research for specific diseases and platform development—just over 4% of the total spending on novel products for neglected diseases. For two of the most prevalent diseases confronting developing countries—HIV/AIDS and malaria—less than 3% of total research spending was allocated to diagnostics. Of the more than $1 billion spent on HIV/AIDS R&D in 2008, only 2.2% was spent on research for novel diagnostics, across all assay types. Likewise, only 1.4% of the $542 million spent on malaria was allocated to diagnostics. The largest neglected disease R&D funder, the NIH, spent only ~1%, or $6.2 million, of its total neglected disease allocation on diagnostic research, specifically on Chagas disease, cholera, dengue, malaria, and TB. The total investment in TB diagnostics across all developers was slightly larger than average as a percent of total R&D spending, with 10% of the $446 million allocated to diagnostics. For all of the 36 neglected diseases assessed in the 2009 G-Finder report, “Neglected disease research & development: new times, new trends,” there is an enormous gap between the investment in therapeutics and vaccines compared to diagnostic tools.

---

*Ibid, 52.*


**Primary Uses of Diagnostics**

Diagnostic tools are essential for efficiently and cost-effectively diagnosing illness, selecting appropriate treatment, monitoring chronic diseases, and tracking the prevalence and impact of disease globally.

---

### Detection of Disease

Detection of disease enables treatment for those in need, resulting in lower morbidity and mortality rates. This can save lives and money by avoiding more costly care likely to be incurred as untreated patients become sicker, as well as avoiding productivity and income losses due to illness. For example, detection of TB in HIV patients is a particularly pressing need. The most commonly used method for TB detection, sputum microscopy, identifies fewer than 50% of TB cases in patients co-infected with HIV. TB is the main cause of suffering and death in people with HIV/AIDS, because TB progresses quickly in patients whose immune system has been destroyed by HIV/AIDS. Earlier and more accurate diagnosis of active TB is necessary to improve treatment outcomes for individual patients and reduce transmission. In addition, early diagnosis has been shown to have benefits in disease prevention as well as treatment.

---

Selection of the most appropriate treatment or the avoidance of inappropriate treatment: for the diagnosed disease, followed by monitoring of treatment efficacy, increases the chances of successful therapy. Selection of the appropriate treatment is important particularly for diseases where drug resistance is a significant problem, or where different strains of the infecting pathogen exist, warranting different treatment protocols. In many parts of sub-Saharan Africa, childhood fever is presumptively treated with anti-malarial drugs. This can promote drug resistant strains of malaria, threatening the long term viability of existing treatments. Another example is TB, where it is important to determine the drug susceptibility of the pathogen. Multi-drug resistant (MDR) TB is on the rise and creating major challenges for treatment efficacy. However, current tests can take weeks to define whether or not an infection is drug resistant, thus delaying the selection of appropriate treatment and furthering the spread of drug resistant infections. An MDR TB Surveillance report published by the WHO in 2008 estimates that the global proportion of resistance among all TB cases is 11% and climbing.

Monitoring treatment effectiveness for diseases requiring lengthy therapy is an important role for diagnostics even after a treatment regimen has been selected. Monitoring therapy effectiveness in diseases such as HIV/AIDS, where treatment is chronic, can help identify issues with treatment compliance or declining efficacy of the prescribed regimen due to the emergence of drug resistant viral strains. This allows caregivers to either re-educate the patient on how and why to comply with his or her treatment or to change treatment regimens for a better outcome.

Enabling surveillance programs that seek to identify and track the incidence and prevalence of diseases in various geographic areas is a vital public health role for diagnostics. Enterotoxigenic E. coli (ETEC) serves as a striking example of where improved surveillance is greatly needed. ETEC is the leading known cause of childhood diarrhea (estimated to be 14% of all childhood diarrhea) and is responsible for nearly 400,000 deaths per year—second only to rotavirus. Despite ETEC’s considerable disease burden, it has low recognition as an important cause of disease because of major gaps in epidemiological knowledge, particularly at the country level. These gaps create uncertainty around the severity of the problem and hamper disease awareness. Increasing recognition of ETEC as an important cause of disease will be critical for ensuring vaccine uptake, and will likely require detailed epidemiological data at a country level. Because the bacterium is difficult to culture, and since the symptoms of infection are similar to other types of diarrhea, a novel diagnostic test will greatly enhance the ability to collect the epidemiological data needed. A new field diagnostic that could be used to study incidence and correlate with clinical outcomes would enable donors to better deploy critical resources around an ETEC vaccine.

Determining which diseases are present in various geographies and whether they are increasing or decreasing in incidence helps guide the allocation of human, financial, and medical resources. For example, the WHO Global Influenza Surveillance Network enables the organization to recommend content of the influenza vaccine for the subsequent season by collecting patient samples and submitting viruses to collaborating centers for antigenic and genetic analyses. Updating this information is necessary to select an appropriate strain for the following year’s vaccine content and protect individuals against perpetually evolving viruses. This is a critical role for diagnostics to play.

---


Progress to Date

Though there is clearly a pressing need for additional investment in developing novel diagnostic tests for the developing world, significant progress has already been made. There are many organizations and individuals working to develop innovative new diagnostics suitable for use in the developing world. The United States’ NIH funds diagnostic test development through its Small Business Innovation Research (SBIR) grants. The Wellcome Trust supports diagnostic research and development, as does the United States’ Centers for Disease Control and Prevention (CDC) and the defense departments of several developed countries. Importantly, the Bill & Melinda Gates Foundation has focused its “Grand Challenges” grants that address inequities in global health on diagnostic development. There are also key global health organizations working to advance the development of diagnostics for neglected diseases. In particular, the UNICEF/United Nations Development Programme/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR) focuses on the development of new tools, including diagnostic tools, to control tropical diseases. TDR’s diagnostic-development efforts for TB resulted in the creation of the Foundation for Innovative New Diagnostics (FIND) in 2003. FIND is the only not-for-profit organization dedicated solely to the development of diagnostic tests for infectious diseases. PATH, an international global health nonprofit organization is also working on the development of point-of-care (POC) diagnostics, and has recently collaborated with the University of Washington to create the Center for Point-of-Care Diagnostics for Global Health (GHDx Center), which will identify and support innovative and promising diagnostic technologies that could have a significant impact on public health outcomes in the developing world.

These efforts have yielded significant technological progress as well. Resource-poor regions of the world have benefited greatly from immunochromatography (“dipstick”) technology, which has led to the development of lateral flow tests for malaria, HIV and syphilis. These tests are widely used because they are inexpensive, easy to transport, and provide a quick result. HIV tests are crucial to identifying individuals infected with the virus, a first step to broad use of anti-retroviral drugs. Indeed, use of anti-retroviral compounds
has increased by 10-fold between 2003 and 2008, and 4 million people in the developing world are now receiving this life-saving therapy (AIDS Epidemic Update, 2009, http://data.unaids.org/pub/Report/2009/JC1700_Epi_Update_2009.en.pdf). Remarkably, the number of diagnostic tests for HIV available in the developing world doubled from 2007 to 2008. The proliferation of tests for malaria has been even more remarkable: www.rapid-diagnostics.org lists 38 rapid diagnostic tests for malaria as of February 2008. However, immunochromatography technology has been widely copied and there are unfortunately few regulatory barriers to introducing substandard products into developing countries. The wide range of products means that there is a wide range of quality, and some products fail due to the fact that they are not designed to withstand the extremes of temperature and humidity in the developing world. So while there has been important progress, many challenges remain.

### Progress Has Already Been Made

Rapid diagnostic tests for HIV have made a major impact on diagnosis and treatment in the developing world since their introduction in the early 1990s. These immunochromatographic (or lateral-flow) assays detect antibodies to HIV-1 and HIV-2, and a more recent generation of tests also detects the p24 protein of the viral capsid. They provide qualitative information on a person’s HIV status with sensitivity comparable to laboratory-based ELISAs, typically in less than 20 minutes. The development and production of these tests was driven by market demand from HIV treatment and control programs and blood banks in the developing world.

The importance and potential impact of improved and novel diagnostics continues to increase as:

- The arsenal, availability, and cost of treatments for each disease expands
- The complexity of disease increases with drug-resistant strains and opportunistic infections in sick populations
- The developing world’s health care infrastructures improve with more capable caregivers
- Non-governmental and governmental agencies and donors recognize the power of diagnostics to contribute to improved health care in the developing world

Now, more than ever, is the time to focus on developing novel diagnostics products for the developing world.
Chapter 3
Diagnostic Requirements in the Developing World

Instruments that perform in vitro diagnosis vary in complexity and cost, from simple benchtop solid state “clinical chemistry” analyzers to multi-million dollar automated instruments that can run thousands of tests an hour in a reference laboratory. Despite this wide range of methodology, complexity, and automation, in vitro diagnostics being developed today almost all depend on the integration of multiple technologies—sample capture and preparation, analyte isolation and detection, signal generation and measurement, and data analysis. As a result, only rarely will a single technical breakthrough lead to a major advance in products available on the market. Instead, in vitro diagnostics evolve and improve over time as each of their component technologies is refined.

This technical challenge is compounded in the developing world. Unlike the developed world, where patients are evaluated primarily in a hospital or doctor’s office, diagnostic testing in the developing world is needed in a very wide range of settings—from a well-equipped hospital to the most basic rural clinic. The most resource-poor settings are likely to be challenging, if not hostile, to sensitive instruments and reagents. There, diagnostic tests must be simple, robust, and low-cost, to overcome many barriers to their implementation, such as the shortage of trained technicians and medical personnel, lack or reliable electricity and clean water, and extreme environmental conditions (i.e., high temperatures, dust, and humidity).

Depending on the setting, useful in vitro diagnostic products will likely take several forms—from large instruments in sophisticated laboratories in urban areas to point of care tests encompassing small benchtop instruments that can be used in decentralized health centers to rapid tests that do not require instrumentation and can be transported by care providers to the most remote rural regions. Developing suitable diagnostic tests for diseases of the developing world also requires a detailed understanding of the target product profile for a particular diagnostic, given the unique combination of market segment, test setting, test purpose and geography. These and other determinants of developing world diagnostic test design are outlined below in Figure 4.

Figure 4: Determinants of Diagnostic Test Design for the Developing World

<table>
<thead>
<tr>
<th>Clinical Knowledge</th>
<th>Technical Specifications</th>
<th>Available Infrastructure</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Disease and pathogen-specific information</td>
<td>• Sensitivity and specificity</td>
<td>• Electricity</td>
</tr>
<tr>
<td>• Biomarker availability by sample type (blood, serum, urine, sputum, saliva, stool, mucosal swab)</td>
<td>• Throughput</td>
<td>• Water</td>
</tr>
<tr>
<td>• Patient characteristics</td>
<td>• Shelf life</td>
<td>• IT and telecommunications</td>
</tr>
<tr>
<td>• Availability of specimen banks for test development and validation</td>
<td>• Cost</td>
<td>• Maintenance</td>
</tr>
<tr>
<td></td>
<td>• Portability</td>
<td>• Health care provider training</td>
</tr>
<tr>
<td></td>
<td>• Waste generation</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Geographic Factors</th>
<th>Regulatory Requirements</th>
<th>Other Considerations</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Prevalence and incidence</td>
<td>• Barriers to entry</td>
<td>• Financial Constraints</td>
</tr>
<tr>
<td>• Species and strain variability</td>
<td>• Exclusion of substandard competitors</td>
<td>• Government budgets</td>
</tr>
<tr>
<td>• Cultural factors that encourage or discourage testing</td>
<td>• Requirement for in-country studies</td>
<td>• Donor support</td>
</tr>
<tr>
<td>• Availability of therapeutics</td>
<td>• External agency evaluations</td>
<td>• Cost of therapy</td>
</tr>
</tbody>
</table>

| | | • Sustainability of business model for manufacturer, distributor, and health care provider |

Chapter 3
Diagnostic Requirements in the Developing World

Instruments that perform in vitro diagnosis vary in complexity and cost, from simple benchtop solid state “clinical chemistry” analyzers to multi-million dollar automated instruments that can run thousands of tests an hour in a reference laboratory. Despite this wide range of methodology, complexity, and automation, in vitro diagnostics being developed today almost all depend on the integration of multiple technologies—sample capture and preparation, analyte isolation and detection, signal generation and measurement, and data analysis. As a result, only rarely will a single technical breakthrough lead to a major advance in products available on the market. Instead, in vitro diagnostics evolve and improve over time as each of their component technologies is refined.

This technical challenge is compounded in the developing world. Unlike the developed world, where patients are evaluated primarily in a hospital or doctor’s office, diagnostic testing in the developing world is needed in a very wide range of settings—from a well-equipped hospital to the most basic rural clinic. The most resource-poor settings are likely to be challenging, if not hostile, to sensitive instruments and reagents. There, diagnostic tests must be simple, robust, and low-cost, to overcome many barriers to their implementation, such as the shortage of trained technicians and medical personnel, lack or reliable electricity and clean water, and extreme environmental conditions (i.e., high temperatures, dust, and humidity).

Depending on the setting, useful in vitro diagnostic products will likely take several forms—from large instruments in sophisticated laboratories in urban areas to point of care tests encompassing small benchtop instruments that can be used in decentralized health centers to rapid tests that do not require instrumentation and can be transported by care providers to the most remote rural regions. Developing suitable diagnostic tests for diseases of the developing world also requires a detailed understanding of the target product profile for a particular diagnostic, given the unique combination of market segment, test setting, test purpose and geography. These and other determinants of developing world diagnostic test design are outlined below in Figure 4.

Figure 4: Determinants of Diagnostic Test Design for the Developing World

<table>
<thead>
<tr>
<th>Clinical Knowledge</th>
<th>Technical Specifications</th>
<th>Available Infrastructure</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Disease and pathogen-specific information</td>
<td>• Sensitivity and specificity</td>
<td>• Electricity</td>
</tr>
<tr>
<td>• Biomarker availability by sample type (blood, serum, urine, sputum, saliva, stool, mucosal swab)</td>
<td>• Throughput</td>
<td>• Water</td>
</tr>
<tr>
<td>• Patient characteristics</td>
<td>• Shelf life</td>
<td>• IT and telecommunications</td>
</tr>
<tr>
<td>• Availability of specimen banks for test development and validation</td>
<td>• Cost</td>
<td>• Maintenance</td>
</tr>
<tr>
<td></td>
<td>• Portability</td>
<td>• Health care provider training</td>
</tr>
<tr>
<td></td>
<td>• Waste generation</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Geographic Factors</th>
<th>Regulatory Requirements</th>
<th>Other Considerations</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Prevalence and incidence</td>
<td>• Barriers to entry</td>
<td>• Financial Constraints</td>
</tr>
<tr>
<td>• Species and strain variability</td>
<td>• Exclusion of substandard competitors</td>
<td>• Government budgets</td>
</tr>
<tr>
<td>• Cultural factors that encourage or discourage testing</td>
<td>• Requirement for in-country studies</td>
<td>• Donor support</td>
</tr>
<tr>
<td>• Availability of therapeutics</td>
<td>• External agency evaluations</td>
<td>• Cost of therapy</td>
</tr>
</tbody>
</table>

| | | • Sustainability of business model for manufacturer, distributor, and health care provider |

Chapter 3
Diagnostic Requirements in the Developing World

Instruments that perform in vitro diagnosis vary in complexity and cost, from simple benchtop solid state “clinical chemistry” analyzers to multi-million dollar automated instruments that can run thousands of tests an hour in a reference laboratory. Despite this wide range of methodology, complexity, and automation, in vitro diagnostics being developed today almost all depend on the integration of multiple technologies—sample capture and preparation, analyte isolation and detection, signal generation and measurement, and data analysis. As a result, only rarely will a single technical breakthrough lead to a major advance in products available on the market. Instead, in vitro diagnostics evolve and improve over time as each of their component technologies is refined.

This technical challenge is compounded in the developing world. Unlike the developed world, where patients are evaluated primarily in a hospital or doctor’s office, diagnostic testing in the developing world is needed in a very wide range of settings—from a well-equipped hospital to the most basic rural clinic. The most resource-poor settings are likely to be challenging, if not hostile, to sensitive instruments and reagents. There, diagnostic tests must be simple, robust, and low-cost, to overcome many barriers to their implementation, such as the shortage of trained technicians and medical personnel, lack or reliable electricity and clean water, and extreme environmental conditions (i.e., high temperatures, dust, and humidity).

Depending on the setting, useful in vitro diagnostic products will likely take several forms—from large instruments in sophisticated laboratories in urban areas to point of care tests encompassing small benchtop instruments that can be used in decentralized health centers to rapid tests that do not require instrumentation and can be transported by care providers to the most remote rural regions. Developing suitable diagnostic tests for diseases of the developing world also requires a detailed understanding of the target product profile for a particular diagnostic, given the unique combination of market segment, test setting, test purpose and geography. These and other determinants of developing world diagnostic test design are outlined below in Figure 4.

Figure 4: Determinants of Diagnostic Test Design for the Developing World

<table>
<thead>
<tr>
<th>Clinical Knowledge</th>
<th>Technical Specifications</th>
<th>Available Infrastructure</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Disease and pathogen-specific information</td>
<td>• Sensitivity and specificity</td>
<td>• Electricity</td>
</tr>
<tr>
<td>• Biomarker availability by sample type (blood, serum, urine, sputum, saliva, stool, mucosal swab)</td>
<td>• Throughput</td>
<td>• Water</td>
</tr>
<tr>
<td>• Patient characteristics</td>
<td>• Shelf life</td>
<td>• IT and telecommunications</td>
</tr>
<tr>
<td>• Availability of specimen banks for test development and validation</td>
<td>• Cost</td>
<td>• Maintenance</td>
</tr>
<tr>
<td></td>
<td>• Portability</td>
<td>• Health care provider training</td>
</tr>
<tr>
<td></td>
<td>• Waste generation</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Geographic Factors</th>
<th>Regulatory Requirements</th>
<th>Other Considerations</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Prevalence and incidence</td>
<td>• Barriers to entry</td>
<td>• Financial Constraints</td>
</tr>
<tr>
<td>• Species and strain variability</td>
<td>• Exclusion of substandard competitors</td>
<td>• Government budgets</td>
</tr>
<tr>
<td>• Cultural factors that encourage or discourage testing</td>
<td>• Requirement for in-country studies</td>
<td>• Donor support</td>
</tr>
<tr>
<td>• Availability of therapeutics</td>
<td>• External agency evaluations</td>
<td>• Cost of therapy</td>
</tr>
</tbody>
</table>

| | | • Sustainability of business model for manufacturer, distributor, and health care provider |
Developing World Diagnostic Requirements by Level of Infrastructure:

To understand the unique requirements of diagnostic technologies for the developing world, it is helpful to first consider the range of settings in which tests are administered. We can broadly categorize test settings into three categories, differing by the level of infrastructure available: Moderate/Advanced, Minimal, or None. The chapter will close with a broad overview of requirements for diagnostics in the developing world. Diagnostic needs of specific neglected diseases in the developing world are summarized in Appendix II.

1. Moderate/Advanced Infrastructure:

Patients located in urban centers with moderate-to-advanced infrastructure often have access to hospital-based or centralized labs in urban health clinics that may be able to utilize a variety of high-throughput diagnostic instruments. In the developing world, moderate-to-advanced infrastructure environments are characterized by:

- Access to electricity and clean water
- A laboratory with minimal or good equipment, such as centrifuges, refrigerators, or microscopes.
- Robust IT systems
- Strong quality assurance programs
- The presence of trained technicians, physicians, and nurses, although training and expertise varies by site

2. Minimal Infrastructure:

Testing sites outside of urban hubs often occurs at health clinics with minimal infrastructure. In Africa, even urban settings may be under-resourced while in Latin America and Asia, it is generally rural health clinics that possess minimal infrastructure, characterized by:

- Unreliable availability of electricity and clean water
- No laboratory or minimal laboratory
- Few technicians or physicians, but access to health workers with minimal expertise

3. No Infrastructure:

In rural Africa and in parts of Latin America and Asia, patients may not have access to facilities with even minimal infrastructure. Most often, patients are treated via community or home health care services with no infrastructure, characterized by:

- No access to electricity or clean water
- No laboratory facilities available
- No trained health workers

Each of these settings impose distinct diagnostic requirements, and the relative importance of developing tests for each setting varies widely across geographies. The need for smaller, more resource-efficient diagnostics is greater in Africa—where more than 70% of the population is located in settings with minimal or no infrastructure—than in Latin America, where only 10% of the population resides in minimal infrastructure or no-infrastructure settings. Developing countries’ populations are becoming increasingly urbanized. The UN has projected that sub-Saharan Africa will increase from 38% urban in 2000 to 55% urban in 2030. Even so, addressing the need in resource-poor settings with minimal infrastructure will remain critical even beyond that date.

---

Nevertheless, the vast majority of patients in sub-Saharan Africa are living in minimal or no infrastructure settings. And in Asia, the 42% of the population living in no or minimal infrastructure settings corresponds to a staggering population upwards of a billion and a half individuals. There are immense challenges associated with delivering health care to patients living in areas with little or no access to laboratory equipment, trained health care workers, and unreliable electricity and clean water. To reach patients living in these settings, diagnostic tests must be inexpensive, easy to use and interpret, robust, and require few external resources.

Figure 5: Access of Patients to Care by Infrastructure Category

<table>
<thead>
<tr>
<th>Region</th>
<th>No Infrastructure</th>
<th>Minimal Infrastructure</th>
<th>Moderate/Advanced Infrastructure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Africa</td>
<td>25%</td>
<td>47%</td>
<td>28%</td>
</tr>
<tr>
<td>Asia</td>
<td>13%</td>
<td>29%</td>
<td>58%</td>
</tr>
<tr>
<td>Latin America</td>
<td>5%</td>
<td>5%</td>
<td>90%</td>
</tr>
</tbody>
</table>

Diagnostics made available in minimal infrastructure or no infrastructure settings would ideally meet the following parameters,\(^{18}\) recognizing that there are tradeoffs inherent in product development:

**Figure 6: Typical Requirements for Diagnostics in Minimal Infrastructure Settings**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Target</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cost of Goods Sold (COGS)</td>
<td>Will vary, but as low as $1-2 per test</td>
<td>COGS must be low enough to enable sustainable production at an affordable purchase price for developing world buyers. Acceptable price points will vary by indication, value to the health system, and cost of therapy.</td>
</tr>
<tr>
<td>Power</td>
<td>No electricity required</td>
<td>Disposable or rechargeable batteries (including solar-powered) if possible</td>
</tr>
<tr>
<td>Water</td>
<td>No running water required</td>
<td>Filtration of non-potable water may be feasible, but all devices to filter water must be included in kit</td>
</tr>
<tr>
<td>Refrigeration</td>
<td>None required</td>
<td>Reflects power and facility requirements</td>
</tr>
<tr>
<td>Stability</td>
<td>1-2 years at 40-45°C and ≥70% humidity</td>
<td>Distribution channels may be inefficient, leading to a need for long shelf-life in uncontrolled environments</td>
</tr>
<tr>
<td>Training</td>
<td>Minimal or none required</td>
<td>Desired: no training required; Acceptable: minimal training required</td>
</tr>
<tr>
<td>Interface</td>
<td>Non-language dependent</td>
<td>Intuitive interface that allows unsophisticated user to implement assay and understand diagnostics result</td>
</tr>
</tbody>
</table>

In the context of these constraints, it is easy to understand the potential value of point-of-care (POC) diagnostics. POC tests make diagnosis simple and fast by enabling accessible samples to be quickly analyzed outside a laboratory. In fact, in an ideal world, one could imagine that all tests might benefit from a POC format if they were able to achieve the same accuracy and low price as the best assays currently run on large, central lab-based platforms. Not surprisingly, however, there are typically trade-offs to make when moving from lab-based tests and equipment to POC tests.

Given the diversity of settings in the developing world—from densely populated cities to remote rural villages, there is no single answer to whether POC or lab-based tests are superior. In remote rural settings, POC diagnostics that can be readily administered by a minimally-trained healthcare worker, or even self-administered, are the required solution. This is because point of care theoretically affords the ultimate “test and treat” care model, reducing the likelihood of losing a patient to follow up. For clinical indications such as malaria or TB, where a delay between diagnosis and treatment can be life threatening, coupling of POC diagnostics with delivery of therapy is critical. Conversely, in situations where patient loss to follow-up is not a critical concern because the result is not immediately actionable, or in situations where the patient is located near the laboratory, then a central laboratory test may be preferable, given the advantages in specificity, sensitivity, and costs.
At times, lower performance of a POC test relative to a comparable lab-based diagnostic can be acceptable, given that the overall treatment rate is higher. For example, in a study by Gift et al., the lower sensitivity of a rapid test was found to be more than offset by a higher treatment rate of patients relative to a lab-based test, so that more diagnosed patients ultimately received treatment following the POC test. Based on the assumptions outlined in Figure 7, the POC antenatal syphilis test could prevent up to 417,000 additional cases each year.

**Figure 8: The Rapid Test "Paradox"**

A rapid test performed at the point of care, even if less sensitive than a lab-based molecular test, can result in accurate diagnosis and treatment of more patients overall.

---

**Nucleic Acid Tests: 2-3 Weeks, Test to Results**

- Sample
- Testing
- Shipment
- Notification
- Physician

\[
\text{Sensitivity (90\%) \times Return Rate (70\%) = 63\% Treated}
\]

**Rapid Tests: <40 min**

- Sample
- On-Site Testing
- Treatment

\[
\text{Sensitivity (70\%) \times Return Rate (100\%) = 70\% Treated}
\]


---


This understanding of different test settings and the tradeoff between POC and lab-based tests leads to a segmentation of the unmet diagnostic needs in the developing world, driven primarily by the varying levels of infrastructure available in different test settings:

- **Moderate/Advanced Infrastructure Settings:** Equipment used in labs with moderate to advanced infrastructure in the developing world needs to be cost-effective and relatively easy to use, as constraints on budgets and availability of trained technicians continue to confront the urban centers. Fortunately, companies in the developed world are pursuing simplified diagnostic equipment for lab-based settings in the United States to deal with constraints on the supply of trained lab technicians. However, these products remain unaffordable for most of the developing world. Improving the affordability of these instruments to enable their introduction into the developing world is critical to meeting the needs of the health care systems. Reducing the cost of service and maintenance and the costs of training will also be critical to enabling the overall affordability of introducing these diagnostic tests.

- **Minimal Infrastructure Settings:** In settings where there is only minimal infrastructure available—such as village or town health clinics—the imperative is for a small number of affordable benchtop instruments that are easy-to-use, robust, and require minimal water and energy. In these settings, limitations on the availability of trained technicians, resources and budgets require creative and efficient solutions to the diagnostic unmet needs.

- **No Infrastructure Settings:** Finally, in the most rural and remote settings, the need for cheap, rapid POC diagnostic tests that are easy to interpret and require no instrumentation is critical.

The understanding of the broad requirements for diagnostics in the developing world and how they vary by test setting leads to the conclusion that developing world markets have inherent limitations to the number of different diagnostic instruments that can be adopted. Scarce funding, the lack of trained technicians, and distribution challenges limit the number of diagnostic instruments that can be effectively introduced into these markets. To increase access to diagnostic tools and optimize health outcomes, industry and the global health community must work towards developing a small number of instruments based on highly flexible technology platforms that can address a variety of biomarkers. Further detail on this proposed approach is outlined in Chapter 5 of this report.
Chapter 4
Innovative Diagnostic Technologies

Several breakthrough technologies have transformed in vitro diagnostics in the past thirty-five years. First was the invention of monoclonal antibodies (MAb) by Köhler and Milstein in 1975, for which they won the Nobel Prize in 1984. Monoclonal antibodies, combined with major advances in fluorescent labeling and chemistry, made it possible to detect minute amounts of disease-related protein biomarkers in blood, cells, or tissue. Next was the invention of the polymerase chain reaction (PCR) in the mid-1980s by Kary Mullis (Nobel laureate in 1993), which allowed the “amplification” of a few molecules of DNA or RNA by many orders of magnitude into diagnostically measurable quantities. The success of the Human Genome Project and the subsequent determination of the complete genomes of many globally important infectious agents have provided a vast array of potential target sequences that could be used to measure the onset and progression of disease. Today, most in vitro diagnostics for identifying infectious diseases are based on either MAb detection or nucleic acid amplification.

Using these technologies, technological innovations are emerging that have the potential to address many of the unmet diagnostic needs for neglected diseases and that may yield diagnostics suitable for use in the developing world. The innovations will develop in four categories, listed roughly in their likely chronological sequence, based on the technological advances and their likely development timeline.

• **Simpler instruments based on the adaptation of existing detection platforms:** First, with the potential to be realized in the shortest timeframe, is the opportunity to develop simpler and smaller instruments relying on existing detection technologies. This is largely an engineering challenge to simplify, automate, and sometimes miniaturize existing platforms and should therefore generate useful instruments in the near-term. One of the economic drivers of this trend is the shortage of trained medical technicians, which creates an imperative to simplify instrumentation, even in the developed world.

• **Improved sample preservation and management:** Despite the need for point-of-care (POC) testing to enhance delivery of health care to rural populations, central lab testing will be predominant in the near term. In fact, several of the experts interviewed in the course of this study estimated that it is often less expensive to buy and equip transport vehicles than it is to develop a new POC test. For this reason, sample preservation technologies will prove extremely valuable over the near- to-medium term. Samples will be stabilized for transport at ambient temperature from rural settings to urban settings. Enhanced sample preservation will take advantage of the increasingly large installed base of centralized lab equipment, which supports testing for diseases such as tuberculosis (TB) and HIV therapy, and the purchase of which is supported by donors such as PEPFAR and the William J. Clinton Foundation Health Access Initiative (CHAI).

• **Development of new detection technologies:** In the medium- to-long term, diagnostic platforms based on new detection technologies that sensitively measure diagnostic markers will be developed. These innovations hold the potential to dramatically change the diagnostic paradigms in the developing world. Some of these new diagnostics may be able to offer broad menus of tests in a variety of sample types that previously required multiple detection technologies. In addition, many of these detection technologies will simplify process steps like sample preparation by decreasing background signal arising from the biological sample. There is also a class of detection technologies that are broadly applicable to multiple analytes—including proteins, nucleic acids, whole bacteria, virus, and small molecules—and these have the potential to simplify the number of platforms required to address the needs of the developing world.
• **Discovery of novel biomarkers**: The discovery of new biomarkers—molecules that are indicative of disease—will have the least predictable timeframe. In the developing world, the need for new biomarkers falls into several classes. First are those diseases for which a relevant biomarker has not yet been identified. For example, there is no definitive biomarker for demonstrating cure of Chagas disease after therapy. Second are the diseases for which known biomarkers require difficult-to-obtain samples. For instance, diagnosing stage II human African trypanosomiasis (HAT) requires spinal fluid. Third are the biomarkers that represent only a subset of infectious organisms responsible for disease. Finally, there are biomarkers that depend on a host response, which can be variable and therefore lack sensitivity. Certain TB diagnostics, for example, measure the human T cell response to the pathogen which can vary depending on the genetic make-up of the patient. Further hindering biomarker research is the lack of specimen banks that enable access to patient samples. With adequate investment to resolve these various issues, biomarker discoveries will support the development of assays that can be run on an increasingly capable armamentarium of instruments.

The remainder of this chapter highlights and characterizes a sampling of emerging diagnostic technologies. These technologies are grouped into the four categories described above – those that represent the adaptation of existing detection platforms, those that represent advances in sample preservation and management, those that rely on entirely new detection technologies, and those that will drive advances in biomarker research and discovery. These technologies are not intended to represent an exhaustive listing, but rather to highlight some promising solutions that can be harnessed to address the major needs in the developing world. The technologies described in this chapter were selected based on the guidance of our Scientific Advisory Board and are in no way endorsed by BIO Ventures for Global Health; there are additional technologies described in Appendix III.

Note: A summary of these technologies and a summary of how they rate on various key requirements of diagnostics for resource-poor settings can be found in Figure 20 at the end of this chapter.

### Adapting Existing Detection Platforms

#### Nucleic Acid Detection Technologies

The evolution of in vitro diagnostics based on amplification and detection of nucleic acids over the past two decades has changed the way many diseases, in particular infectious diseases, are diagnosed in the developed world. When a pathogen is present in a test sample, nucleic-acid based tests can offer superior sensitivity and specificity over immunological methods due to their ability to detect a few target molecules. As a result, one benefit of this type of test is the early detection of disease. Depending on the method used, nucleic acid amplification can also offer the advantage of quantitative information on the amount of the pathogen present. This is important, for instance, in monitoring patients with HIV during therapy. Despite these benefits, nucleic acid-based tests have traditionally been limited by long turnaround time, requirements for complex and expensive central lab equipment, multi-step sample preparation, costly reagents, and the need for a skilled operator. Several new nucleic acid detection platforms currently in development are automating sample preparation and amplification procedures and performing tests in a self-contained, single-use cartridge. By reducing the number of steps and the potential for contamination, POC nucleic acid-based tests are becoming plausible. Ideally
such platforms would decrease turnaround time, while preserving the sensitivity and range of applications offered by gene amplification. A final hurdle to meet developing world needs, which has not yet been solved, is to provide these tests in a cost-effective manner so that they can be made widely available.

The most advanced example of molecular testing that has been implemented outside of the central laboratory is the GeneXpert System offered by Cepheid. The system offers automated molecular testing from sample preparation through interpretation in one hour and can process difficult samples such as sputum and whole blood. Cepheid’s system is amenable for use in a regional laboratory of a developing country, given that the system does not require a water supply, and cartridges are stable for approximately one year at room temperature. While the system has been designed with reduced power consumption in mind, commercial systems today require 100-240 volt power.

The Cepheid GeneXpert System uses an instrument and assay cartridge that integrates nucleic acid extraction, amplification, and detection with reduced turnaround time compared to conventional central laboratory procedures. Instruments are designed to process one, four, or 16 samples in independently programmed procedures. Cartridges accommodate a range of sample volumes, enabling the concentration of purified nucleic acid to enhance detection sensitivity. The sample prep step utilizes an ultrasonic lysis procedure to facilitate processing of difficult samples. The system provides an option for nested polymerase chain reaction (PCR) analysis to enhance sensitivity and provide confirmatory analysis of target sequences. The system utilizes six optical channels so that up to six sequence targets, including controls, can be analyzed in a single assay.

Cepheid has also made strides in providing diagnostics specifically for the developing world. It established a non-profit arm of the company for distributing products to the developing world. It has a sputum-based TB test (GeneXpert MTB/Rif), developed in collaboration with the Foundation for Innovative New Diagnostics (FIND). GeneXpert MTB/Rif is a qualitative test designed for rapid detection of Mycobacterium tuberculosis (Mtb) and assessment of rifampicin resistance, a useful surrogate marker for multi-drug resistant strains of TB. Results are available in less than 2 hours using a cartridge that performs the analysis from sputum specimen to a definitive TB result. This device should be able to identify TB, and also the likelihood of drug resistance, which will help alleviate the increasing burden of drug-resistant TB strains. Cepheid has also developed commercial tests for MRSA/MSSA, Clostridium difficile, Group B Streptococcus, and enterovirus.

Despite this progress, however, the cost of the Cepheid technology remains high. The value of the progress Cepheid has made is to show the ability of such novel technologies to advance the detection of major developing-world pathogens, but the broad adoption of these tests will depend on breakthroughs in reducing cost.
Nucleic acid detection that depends on PCR has a requirement for cycling between high and low temperatures to alternately denature DNA and allow for primer annealing and DNA synthesis. Thermal cycling adds complexity to the instrument and increases assay time. An alternative is to use methods that work at a single temperature, so-called isothermal amplification. BioHelix’s IsoAmp® provides molecular detection in an isothermal (65°C) reaction by using an enzyme to denature the DNA product and enable subsequent rounds of synthesis. The approach—named isothermal helicase dependent amplification (HDA) technology—uses a helicase, an enzyme to unwind double-stranded DNA; exposure of the single-stranded target region allows primers to anneal. DNA polymerase extends the 3’ ends of each primer using dNTPs to produce copies, which independently enter the next cycle of HDA. An important innovation is the rapid detection of amplified products by vertical flow using a disposable BioHelix Express Strip (or BESt™) cassette. Technology improvements being evaluated include integrated sample prep and dry reagents to improve stability and shelf life. BioHelix offers rapid Staphylococcus and MRSA detection, for research only, and products are in development for C. difficile, HIV, Herpes simplex virus, Chlamydia trachomatis and Neisseria gonorrhoeae, and Factor V Leiden.

**Figure 9: BioHelix’s IsoAmp Technology**

Ionian Technologies has also developed a proprietary isothermal amplification technology, called NEAR, for rapid POC diagnostics. The reactions are very fast, typically being complete in five minutes under isothermal reaction conditions. This allows for small, inexpensive portable devices to be used as an instrument platform. Further, the assay is sensitive down to 10 copies or less of the target and is tolerant to a variety of sample matrices. Ionian has received funding from the Bill and Melinda Gates Foundation to develop assays for chlamydia, gonorrhea, and TB, and is also developing products for other IVD applications, food safety, and agriculture and biodefense applications.

Ionian’s amplification method is based on the extension of oligonucleotide primers at a temperature significantly above their melting point, or the temperature in which a DNA double helix forms. The double helix between primer and target is stabilized by binding and subsequent extension by a DNA polymerase in the presence of a sequence specific endonuclease. The endonuclease nicks the product strands to release a short oligonucleotide product of 21-28 nucleotides (nt). This oligo serves as a primer for subsequent synthesis and enables the polymerase to repetitively extend additional short, 21-28 nt products. Ionian has developed a lateral flow readout with visual results for certain applications.

Gen-Probe Inc. was the first company to commercialize nucleic acid-based tests and has been developing molecular diagnostic products for more than 26 years. Gen-Probe has developed a Closed Unit Dosing Assay (CUDA) system for the analysis of microbial contaminants in water samples. CUDA is a portable testing system that is designed to deliver assay results in approximately one hour. CUDA integrates sample preparation, isothermal amplification, and detection in a single-use cartridge with a portable instrument applicable to developing world settings.
Gen-Probe has an integrated portfolio of assay technologies that support its fully automated “sample to answer” instrument strategy, including transcription-mediated amplification (TMA), chemiluminescent and real-time fluorescent detection chemistries, and sample preparation methods utilizing hybrid capture for purifying target nucleic acids from complex biological samples such as blood and sputum. Recently, the CUDA system along with intellectual property and know-how was transferred to a privately funded company, Roka Biosciences. Gen-Probe retained rights to all clinical diagnostic applications of the CUDA system.

**Figure 10: CUDA Technology**

A variety of other isothermal amplification methods are being developed. The Foundation for Innovative New Diagnostics (FIND) is developing loop-mediated isothermal amplification (LAMP), targeting molecular diagnostics for malaria, TB, and human African trypanosomiasis (HAT). As these diverse methods mature and gain validation, we can anticipate new and cost-effective approaches to molecular diagnostics for the developing world.

**Antibody-Based Methods**

Immunoassays are diagnostic tests that use the specificity and binding affinity of antibodies to detect biomarkers. Immunoassays can detect antibodies made in response to an infection, or they can directly detect the product of a pathogen, such as a protein. Immunoassays use relatively well-understood technology with important applications to diseases of the developing world. The immune system responds to most infections by developing antibodies, and these antibodies are both abundant and readily detected using immunoassay methods. One limitation of antibody detection is that the presence of an antibody is not necessarily indicative of an active infection because antibodies can be detected in blood long after the infectious agent has been cleared by the immune system. An alternative is to use an immunoassay to directly detect an antigenic protein made by the microbe in question. However, direct antigen detection is often limited by the concentration of low-abundance antigens. Another challenge for immunoassays is that a common format in the developed world, called an enzyme-linked immunosorbent assay (ELISA) is not suitable for most developing world applications, due to the need for complex readers, expensive reagents, and well-trained technicians.

The “lateral flow immunoassay” format attempts to simplify complex ELISA methods. In the lateral flow assay, a specimen is applied to a membrane strip. Fluid from the sample — such as urine, saliva, or serum — flows across the strip, which contains a capture antibody specific to the biomarker. The antigen-biomarker conjugate is then captured by a secondary antibody immobilized on the membrane. When sufficient biomarker is bound, a line materializes on the membrane, indicating a positive test. A common example of the lateral flow test is the home pregnancy test sold as an over-the-counter diagnostic in the developed world. These simple and relatively robust devices have been adapted to several developing world indications, including malaria and HIV.

21 Further details on the lateral flow immunoassay format can be found at: http://www.rapid-diagnostics.org/tech-lateral.htm
Lateral flow tests are also being modified for use with simple detection instruments to minimize subjective interpretation of results, provide an electronic record of the test result, and produce quantitative readouts. One example is the Triage instrument developed and marketed by BioSite, now owned by Inverness Medical. Inverness also makes important lateral flow tests for malaria (BinaxNOW) and HIV (Determine HIV 1/2).

One emerging company focused on handheld readers for lateral flow tests is Alverix, Inc. Alverix’s business model is to develop inexpensive, hand-held electro-optical devices and to partner with companies who have expertise in developing and marketing lateral flow assays. Its hand-held instruments enable electronic connectivity even in remote locations and can detect multiple analytes on a single strip. Despite their simplicity, the Alverix devices utilize sophisticated image processing algorithms to provide quantitative results and eliminate guesswork from interpretation of lateral flow assays. Target markets include physician offices, retail clinics, and in-home diagnostics. An initial instrument is available with a price point of $100, and future designs could substantially lower cost and improved portability. Alverix hopes to deliver improved sensitivity and quantitative results, while removing user subjectivity from assay interpretation.

Claros Diagnostics, Inc. was founded with the goal of developing microfluidics-based low-cost, robust POC diagnostic platform suitable for use anywhere, including low-resource settings in the developing world. The company has already developed a handheld, battery-operated analyzer specifically for field use in low-resource settings in a collaboration led by Professor Samuel Sia of Columbia University. To do so, the company had to overcome two critical issues that have historically impeded the commercialization of microfluidics-based diagnostics in the developing world: cost and robustness. To address these issues, the Claros team has pioneered a number of innovations. Examples include:

- The use of injection molding to fabricate microfluidics components. Injection molding enables high volume production at very low cost, but historically has not been capable of delivering the tolerances required for a microfluidics application. Claros has developed a proprietary method of making the tooling capable of delivering the exacting specifications required for a microfluidic device.

- The use of an approach that uses air bubbles to enhance the function of the device. Typically, microfluidic assays can fail as a result of the introduction of air bubbles in the channels of a device; the Claros approach not only tolerates bubbles, but uses them to enhance the function of the device. For instance, bubbles are introduced during sample washing, and the resulting discrete fluid phases separated by air-water interfaces provide better washing than would a continuous fluid stream.

- The use of robust and inexpensive instrument components such as light-emitting diodes (LEDs) and photodetectors. Simple optics are utilized to monitor in-process steps and measure assay endpoints, thereby achieving real time quality control.

- The use of inorganic redox chemistry detection to deal with the high heat and humidity in developing world environments, thereby avoiding the problems of enzyme denaturation. Protein analytes are detected through silver reduction chemistry, much like silver staining is used in immunohistology.
Claros scientists have created an immunoassay “development kit” that potential collaborators could use to develop a test for a developing world indication that would be compatible with the Claros platform. Claros intends to promote their platform with multiple developers while retaining control of manufacturing know-how. This is expected to enable broad diffusion of their technology while securing the interests of their shareholders. The company is focusing on well-defined opportunities in patient care, tailoring the test menu and specifications to meet the specific requirements of the final users. As an initial product for the developed world, they will provide a multiplex test for use by urologists in the management of prostate cancer. A related approach in the developing world is to focus on a panel for maternal health, combining HIV, syphilis, and anemia testing in a single disposable. The market research to support this product was funded by a Bill & Melinda Gates Foundation grant to RTI International to assist Claros in understanding the product requirements for various developing world market needs.

**Chemistry-Based Methods**

Access to basic medical care, including standard clinical chemistry and hematology, as well as kidney and liver function tests via a fast, affordable, and portable diagnostic technology would fill an important void in developing world health care. Abaxis’s Piccolo Xpress analyzer helps fill this void by providing general chemistry analysis. The Piccolo Xpress delivers chemistry results from 100uL of whole blood, serum, or plasma in about 12 minutes with the precision and accuracy of larger laboratory analyzers. The Piccolo Xpress uses a broad menu of self-contained, single-use reagent panels — which includes comprehensive metabolic, lipids, liver, kidney, electrolyte, and glucose monitoring. The system is portable (11.2 pounds, the size of a shoe box) and can be operated from a 15 V, 5 Amp DC power source. Originally designed for NASA, it is used routinely by the military, veterinary offices, physician offices, and small hospitals and is suitable for developing world settings that don’t have access to a convenient central lab. The company is not currently focused on developing world indications per se, but is currently working on improvements to its reagent discs to avoid the need for refrigeration.

**Figure 11: Claros Diagnostics Technology**

Top Image: The Claros benchtop analyzer for use in physician offices.

Bottom Image: A handheld, battery-operated analyzer, developed specifically for field use in low-resource settings in a collaboration led by Professor Samuel Sia of Columbia University. Collaborators included Columbia University, Pratt School of Art & Design, Smart Design, and Claros.

**Figure 12: Abaxis’ Piccolo Xpress Technology**
Combining Immunoassay and Nucleic Acid-Based Methods

One important way that current technologies are being adapted for developing world settings is by combining multiple detection technologies into a single instrument. This is in response to the limitations on the number of different instruments that can be effectively mobilized in the field to perform needed tests in remote settings. Each additional instrument at a remote site adds to the burden of technician training, supply chain logistics, and repairs and maintenance. An ideal solution for the developing world might be to have a small number of platform instruments that have the ability to perform multiple tests based on a common technology. For instance, one platform for immunoassay detection and another for nucleic acid detection could address a large percentage of the diagnostic needs of the developing world if the platforms were sufficiently flexible to accept multiple assays using the same disposable cartridge design.

It may even be possible to go one step further and develop a single instrument that is capable of running both immunoassay and nucleic acid-based assay tests. The DxBox is a prime example of a system that aims to improve turnaround time for immunoassay and molecular testing in the same platform. DxBox is a lab-on-a-card approach currently being developed by a consortium led by the Yager Laboratory at University of Washington together with the Stayton Laboratory, PATH, Micronics, Inc., and Epoch Biosciences (formerly Nanogen, Inc.).

The initial product concept focuses on diagnosis of infectious agents that cause febrile illness. The presentation of fever can lead to many possible treatments depending on the infectious agent, but in many regions, children receive a presumptive clinical diagnosis based on whatever disease is most prevalent in their region. This can lead to inappropriate treatment for patients actually suffering from a different malady, and can enhance the development of resistance to antibiotics and antimalarials, creating public health risks for the community as a whole. Initial development of the DxBox has been funded through the Bill & Melinda Gates Foundation’s Grand Challenges Program in Global Health initiative. Micronics has commercial rights to the system, and the eventual instrument is designed to offer several advantages over existing technology including: minimal sample volume requirements (one to two drops of blood), automation of whole blood processing, and immunoassay and molecular testing with a single instrument. The immunoassays are expected to achieve ELISA-like sensitivity and specificity, and complete molecular assays including 40-cycle PCR reactions may be completed in as little as 30 minutes. Nucleic acid-based assays are expected to be capable of detecting pathogen RNA as well as DNA. The overall turnaround time is intended to be 30 minutes, which would be within the time constraints needed to test and treat within the same patient visit. Shelf life under tropical conditions will be maximized by storing reagents like buffer and ethanol in blister packs and packaging dried analyte-specific reagents on the card.

To perform assays on the DxBox, which will be a portable battery-powered reader and controller, finger-stick blood will be applied to a disposable card and pneumatic control by the controller will divert the sample for immunoassay and/or nucleic acid testing. Sample processing, including nucleic acid extraction, will be self-contained within the card. For the nucleic acid test, fast turnaround times will be enabled by shuttling small sample volumes between areas on the card held at different temperatures rather than heating and cooling in place.

A panel for febrile illnesses is in development and immunoassay reagents for detection of malaria, measles, dengue, typhoid, and Rickettsial infections are being validated with reference to commercially available kits. Given the ambitious nature of the DxBox project, we anticipate that cost will be a challenge for broad-based uptake.
Improving Sample Preservation and Management

While point-of-care testing will be attractive when it is available, a significant amount of current funding is being mobilized to enhance the capabilities of central labs with current technology, with the goal of providing testing services to outlying areas. In many cases, this strategy is adequate because test results are not required to make immediate clinical decisions for individual patients. Monitoring patient response to HIV therapy or determining whether a patient with active TB has a drug-resistant infection, for example, are situations where immediate results are not required. In the near-term, an interesting strategy is to send samples collected in the field for testing to a centralized laboratory. Under this model, the sample may not be tested for hours or even days while it is being transported. Sample preservation – typically requiring refrigeration – would be needed to ensure the integrity of the measured analytes, which would otherwise degrade over time. Eliminating the need for refrigeration would broaden access to diagnostics that currently require sample transportation to labs.

New chemistries have emerged that can enable samples to be preserved without refrigeration for several days and even withstand the high ambient temperatures common in the developing world. One of the major benefits of this approach is that it permits testing using laboratory instrumentation that is available today without requiring new product R&D.

- **Sierra Molecular Corporation** has developed the AssayAssure® family of sample stabilization chemistries for whole blood, genital tract swabs, and urine. The United States’ Centers for Disease Control & Prevention has used the AssayAssure® Molecular Swab Kit for epidemiology studies in both Asia and Africa, doing primary detection and taking viral load measurements from samples self-collected by female patients. For whole blood, the chemistries preserve lymphocyte cells, cell surface markers, intracellular proteins, and nucleic acids in a single tube for up to seven days without refrigeration or the need for fractioning. Because the chemistries stabilize cells and inhibit nucleic acid degradation in sample matrices rather than relying on lysis and precipitation, one blood sample can be used for multiple tests (e.g. molecular testing, flow cytometry, and even culture). The chemistries stabilize bacterial and viral targets, as well as intracellular RNA. In the HIV context, this means that a single blood sample can yield HIV primary diagnosis, CD4 counts, viral load measurement, and viral genotyping.

- **Qiagen, Life Technologies, and Thermo Fisher Scientific** also offer sample preservation chemistries typically geared towards developed world lab use, but which can be used in developing world settings as well. For example, Qiagen’s PAXgen offers RNA stability in blood samples at room temperature for several days and is commercially available today.

- Finally, a relatively simple approach to sample preservation has been to dry samples onto filter paper. This method has been employed throughout the developing world to increase patient access to HIV viral load testing. **Whatman** makes customized collection devices to facilitate sampling and allow customization, depending on the analyte to be detected from the sample.

Sample preservation may not be an option for all sample types or clinical settings, particularly when immediate feedback to patient or caregiver is required. Even for those indications where sample preservation is feasible, one stumbling block has been the sheer lack of method validation. Performing studies to validate sample preservation and expediting regulatory approvals for these technologies could significantly enhance patient access to diagnostic testing by making available central laboratory testing possible for patients in remote areas. For patients in areas with minimal or no infrastructure, the path to improve access to diagnostics in the near-term may be through improvements in sample preservation and transportation.
Developing New Detection Technologies

New detection technologies hold the potential to revolutionize the diagnostic paradigm in two ways: 1) Eliminating the need for process steps inherent in other detection technologies, and/or 2) Addressing broader menus of tests that have traditionally required more than one detection technology. There is admittedly a gray area between “exploiting existing detection technologies” and “creating new detection technologies” since some of the technologies listed in this section rely, for instance, on immunoassays. For the purposes of this report, if the new technology requires significant engineering breakthroughs, it has been categorized as a new detection technology.

A challenge for novel detection technologies is that commonly used specimens such as blood and sputum contain substances that interfere with many tests. This drives the need for extensive and costly purification methods. An alternative is to minimize purification by creating tests that can measure biomarkers directly in urine, saliva, and genital or nasal swabs. These samples are both easier to obtain and easier to purify than whole blood or sputum. The tradeoff is that these samples may contain lower concentrations of the desired biomarker and therefore more sensitive detection techniques are required.

Another advantage of higher sensitivity is that a small volume of sample can be tested. This is an important consideration in the developing world where phlebotomy is sometimes unavailable due to the lack of trained technicians and supplies. Moreover, obtaining large volumes of blood from infants and young children is almost never feasible. More sensitive detection methods may enable evaluation of blood obtained from finger or heel sticks, or it may enable the use of blood instead of sputum. Sputum is a difficult sample to obtain reliably, especially from children. A current challenge in TB biomarker discovery is to identify indicators of active infection in blood to obviate the requirement for sputum, but this may require ultra-sensitive detection methods.

One example of a technology that may enable the detection of low concentration analytes is the Single Molecule Array Technology (SiMoA™) in development by Quanterix, a venture-backed company based in Cambridge, MA. The SiMoA technology allows the behavior of thousands of individual molecules to be observed simultaneously, rather than an ensemble average of many molecules as with existing technologies. This capability permits the detection of analytes, including proteins, at levels far below the capabilities of traditional clinical immunoassay platforms. While the current instrument will require laboratory conditions and skilled operators, it has potential to evolve into a simpler device, with longer-term investments in technology development.
In the SiMoA assay, a dilute sample is applied to an array containing thousands of femtoliter-sized wells etched into an optical fiber bundle. Each well is isolated from neighboring wells and can be used to trap, at most, a single analyte molecule. Detection is performed by imaging the arrays using a proprietary instrument comprised of a light source, optics and digital camera. A simple software algorithm is used to count fluorescent wells containing trapped molecules to determine the analyte concentration according to Poisson statistics. Time to result is currently several hours. However, a consumable is currently in development that will enable a 30-60 minute turnaround time. The company has demonstrated that SiMoA is 1000-fold more sensitive than a traditional ELISA for the detection of several proteins. Quanterix is developing SiMoA technology for a variety of applications in the areas of oncology, inflammation, neurological, cardiovascular and infectious disease.

Another challenge for the developing world is that many testing modalities necessitate extensive manual sample preparation, often requiring trained technicians or ancillary equipment unavailable in resource-poor settings. For example, molecular tests require extraction and purification of nucleic acids prior to amplification. Whole blood for immunoassay must be fractionated to remove cells and hemoglobin that will interfere with detection of serum proteins. For a test to be adopted in the developing world, manual sample preparation must be greatly reduced or eliminated so that minimally trained health care workers can perform testing. Fortunately, the goal of reducing sample preparation is also a key objective for the developed world, both to reduce labor costs and to minimize the need for skilled technicians. Many companies are therefore aiming to create detection technologies that are insensitive to interference from the biological matrix and do not require sample preparation.

Traditionally, methods that depend on light are used to detect many endpoints in clinical diagnostics. Detection methods can include fluorescence, phosphorescence, chemiluminescence, or a color change due to a chemical or enzymatic reaction. Optical methods face particular challenges under the rigors of developing world conditions. Dyes and enzymes can degrade in the high temperature storage conditions of the developing world. This degradation can lead to higher assay background or lower sensitivity. Optical approaches may also be very sensitive to contaminants. For instance, most biological samples contain autofluorescent compounds, which drive the need for high sample purity when fluorescence is used – increasing the complexity of sample preparation on the front end.
Axela’s dotLab technology uses light, but instead of measuring emission or absorption of a sample, it relies on a physical property, diffraction, to allow quantitative measurements of an analyte. Diffraction does not depend on the bulk properties of the sample, and is thus much less sensitive to interference from the biological matrix. dotLab’s relative simplicity paired with high sensitivity make it a promising technology for future use in the developing world.

The technology functions based on disposable diffraction gratings containing regular arrays of immobilized antibodies or other ligands specific to the target(s) of interest. The test sample flows over the diffraction array and the analyte binds to the regular array causing a change in the diffraction of incident light. The geometry of the system is such that light impinges the bottom of the sample, and the refracted light is not absorbed by the bulk solution. Thus, an analyte does not need to be purified from whole blood prior to analysis. Internal controls allow a real-time assessment of diffraction due to the sample itself, enhancing the ability to distinguish signal from noise. Axela’s technology reaches a picomolar limit of detection while allowing for at least 8-plex multiplexing. The system employs a benchtop reader, so its use is currently restricted to a traditional lab setting. Turnaround time, from 10 minutes to 2 hours, depends on both the concentration of the analyte and the volume of the sample. This timing is compatible with same-visit results in most areas of the developing world. Applications in development currently include Influenza (rapid multiplex strain detection), Bovine Respiratory Disease (BRD) including PI3, BRSV, BVD, IBR, food borne zoonosis including campylobacter, escheria coli, salmonella, and shigella species, and strongylodiasis, leishmaniasis and cystercerosis. The open platform allows anyone to develop assays using this detection technology.
Other detection technologies do not depend on light at all. These approaches share the common theme of measuring physical changes in a sample. Measurements of mass, magnetic properties, diffraction or electrical potential may allow development of more robust systems, which could eventually serve as platform technologies upon which a broad range of assays are commercialized. While not all of the non-optical technologies are simpler than optical systems, one or more may be more amenable to meeting developing world market needs.

**Vivacta** is a venture capital financed company based in the United Kingdom. Its novel piezofilm detection system enables rapid, quantitative immunoassay measurements. The primary advantages include: the ability to run the assay using 30 μL of whole blood, compatible with a fingerstick, without any sample preparation. The limit of detection is comparable to laboratory-based instrumentation and turnaround time can be as short as 10 minutes. The current instrument is a portable, benchtop reader that could be adapted to run on battery power, does not require running water, and can function without ambient temperature control.

To perform the Vivacta assay, a drop of blood is applied to a disposable cartridge and inserted into the reader. The cartridge is fabricated from a PVDF polymer with a thin indium tin oxide surface layer, the piezofilm. The piezofilm is coated with covalently bound antibody specific to the analyte in question, much like the surface of an ELISA plate. An important feature of the cartridge is that piezoelectric materials can be induced by heating to exhibit a change in electrical potential. Within the cartridge, a pump mixes the blood and resuspends dried secondary antibody labeled by carbon, an inherently stable material. Analyte is bound to the capture antibody on the piezofilm and also to the secondary antibody, forming a sandwich. Unbound secondary antibody is distributed through the solution. An LED light source, whose wavelength has high absorption by the carbon label and minimal absorption by red blood cells, is flashed on the sample. The carbon label absorbs light and through internal conversion it generates heat. Label bound to the piezofilm via the sandwich of antibodies and analyte causes local heating of the piezofilm, resulting in a voltage change proportional to the amount of label bound. Unbound label merely gives up its energy to the surrounding blood, without an effect on the piezofilm. The instrument quantifies the voltage signal and calculates the analyte concentration. Internal positive, negative and calibration controls are included in separate wells in the disposable cartridge. Results are displayed on a touch-screen. The device relies on 25V of electrical power and the company believes it could also be run on rechargeable batteries.

The first application under development for the Vivacta technology is measurement of TSH for thyroid function, with plans to develop assays for use in acute myocardial infarction. The company has also considered infectious disease applications, for which piezofilm detection could offer the inherent advantages of sensitive, quantitative detection without a need for sample preparation.

**Figure 16: Vivacta Technology**
T2 Biosystem’s magnetic resonance technology can detect multiple analytes, and can analyze samples of different types, all in one platform. The technology is robust: reagents are dryable with predicted cartridge shelf life of one to two years without refrigeration. The reagents (composed paramagnetic iron particles) are inexpensive and T2’s goal is for disposable cartridge costs to be less than $1. A benchtop analyzer is in development now, and a POC instrument is anticipated.

The basic premise behind T2’s technology is its use of miniaturized magnetic resonance to enable high sensitivity, non-optical detection. In short, the sample, which can be unprocessed whole blood, urine, water, saliva, or nasal swabs in solution, is added to a cartridge and inserted into the bench-top instrument. The instrument comprises a low-field, low-cost permanent magnet and a radio frequency coil for detecting the T2 spin signal from the water in the sample. In the cartridge, the sample mixes with paramagnetic iron nanoparticles coated with covalently bound, analyte-specific ligands, such as an antibody or a DNA oligomer. Binding of the analyte (which can be proteins, viruses, bacterial cells, DNA or a small molecules) causes the iron particles to aggregate. This clustering of paramagnetic iron particles creates local changes in the applied magnetic field. These local irregularities affect the nuclear spin properties of diffusing water molecules, resulting in a characteristic change in their T2 spin relaxation lifetime. The change in T2 is measured at the RF coil. The system is highly sensitive to these clusters, enabling a quantitative measurement of the concentration of analyte, without the need for purification.
Another example of a quantitative, rapid, sensitive diagnostic platform for multiple analytes is BioScale’s ViBETM Bioanalyzer and ViBE Workstation. These technologies achieve ELISA-like sensitivity in only 10 minutes. This platform has multiplexing capability and encompasses a broad range of analytes including proteins, small molecules, cells, viruses, bacteria, and DNA detection, and a range of potential sample types with minimal to no prep (tissue, whole blood, serum, plasma, urine). The technology is also compact and has been developed into benchtop systems as well as a hand held unit. The device currently requires standard power (100 V, 15 A) but the company has done research on battery powered devices.

The technology basis of the ViBE Systems is AMMP™, which stands for acoustic membrane and microparticle platform. The ViBE Workstation (a fully automated 96-well plate assay stations) and smaller ViBE Bioanalyzer (8-sample manual system) enable quantitative detection in complex sample types without extensive sample preparation. A microfluidic cartridge with a MEMS (microelectromechanical sensor) chip, functionalized universal surface, and magnetic microparticles with bound detection antibody form the components of this system. The MEMS device is a piezoelectric material functionalized with a capture antibody. Rapid changes in applied voltage cause the MEMS device to vibrate at a characteristic frequency. When a magnetic field is applied, the magnetic microparticles adhere to the surface of the MEMS device. This increased mass causes a quantitative change in the oscillation frequencies of the MEMS device. When the magnetic field is turned off, the microparticles disperse into solution. However, if analyte is present, an antibody sandwich is formed by the antibody on the surface of the MEMS device and the antibody on the microparticle. The amount of microparticle retained when the magnetic field is turned off gives a direct measure of the concentration of analyte in the test sample. The change in oscillation frequency versus analyte concentration results in a dose response curve. The method is essentially a modified immunoassay sandwich format with a read out that is insensitive to interfering substances in the sample.

Figure 18: BioScale’s ViBE Bioanalyzer
The Charles Stark Draper Laboratory is developing an intriguing approach to diagnosing infectious disease using its Differential Ion Mobility Spectrometer (DMS). The premise of this technology is that different species of bacteria create a unique signature of volatile organic compounds, which can be detected using relatively straightforward gas chromatography. The technology currently has high sensitivity and a time to result of 60 – 120 minutes. The company plans to improve the time to result to between 10 and 20 minutes. The institution also hopes to move towards direct analysis of patient breath in the near future. This could be a breakthrough in the early diagnosis of respiratory diseases.

Testing in the most rural areas of the developing world will likely require diagnostic solutions that minimize the size and bulk of required instrumentation. Minimizing equipment reduces training and maintenance and broadens the reach to clinics, health posts, and villages and homes accessed by community health workers. An area of high medical need is diagnostics for monitoring patients on anti-retroviral therapy for HIV. Therapeutic monitoring can take two forms: 1) CD4 counts; or 2) viral load measurements. CD4 counts are most likely to make a difference in the short term because quantitative measurement of viral load will require breakthroughs in molecular detection, both in terms of cost, ease of use, and portability.

The World Health Organization currently recommends that HIV-positive individuals in the developing world begin receiving antiretroviral therapy when CD4 T-lymphocyte counts drop below 350 per microliter (the range in most healthy volunteers is 500-1500 per microliter). Once patients begin therapy, guidelines suggest performing CD4 counts up to 4 times per year in order to monitor disease status and determine the effectiveness of the drug regimen. The problem throughout the developing world is that CD4 counting relies on flow cytometry, which is limited due to the expense and complexity of equipment, requirement for user training, dependence on electrical power, and use of expensive reagents. Even when samples can be transported to central labs, turn-around times can be days or sometimes weeks, and the reliability of CD4 counting results from stored blood is problematic. The need is for an inexpensive, portable, and robust solution to CD4 counting so that HIV therapy can be extended to patients in the rural areas of the developing world.
An example of a POC instrument for HIV monitoring is Daktari’s CD4 count microfluidic technology. It is a handheld instrument with a novel method for determining CD4 counts that overcomes two barriers to POC testing: complex sample preparation and the need for optical sensors. The handheld, cost-effective device captures the CD4+ T cells with an antibody and simultaneously removes adsorbed monocytes under high shear forces. A MEMS-based detector determines the number of captured CD4 cells by measuring electrical resistance. The instrument delivers a CD4 count within six minutes, which is accurate to within 15%. While this is less accurate than the reference standard equipment, flow cytometers, CD4 T cell counts in individual patients are known to vary day-to-day by significantly greater amounts. From a clinical perspective, the variance of 15% is much less important than knowing whether a patient’s count is near normal or critically below normal. Values significantly below a target may indicate the emergence of drug resistant HIV or poor compliance with a prescribed drug regimen. Thus, a CD4 count within 15% of the true number will provide clinically important information. This situation is a good example of appropriate trade-offs in designing diagnostics for the developed world that will benefit patients in minimal infrastructure settings.

Another approach to CD4 T cell counting has been developed by Zyomyx, Inc. through the Imperial College CD4 Initiative, funded by the Bill and Melinda Gates Foundation. The Initiative was established with the goal of developing a simple, affordable, rapid, and robust test to measure CD4+ cells to impact the availability of HIV treatment in the developing world. The Initiative defined a market-based product specification which broadly outlined the requirements such as cost, sensitivity, quality, throughput, training requirements, and absence of instrumentation, without constraining what technology could be used to meet the specification. Individual companies and academic groups were invited to submit product or technology ideas that would meet the defined criteria. Zyomyx, along with four other groups, was awarded funding for development and clinical tests in a milestone-based scheme.

The CD4 POC device developed by Zyomyx is based on the use of heavy particles that have been functionalized with a CD4 specific antibody. A fingerprick volume of whole blood is mixed with the particles, and the antibody binds selectively CD4 cells. Once bound, CD4 cells sediment into a high precision capillary tube in less than 10 minutes due to the high density of the particles. The CD4 cells form a stack in the approximately 100 micron wide capillary. The stack of cells can be seen by the naked eye due to light reflectance by the particle column; the height of the stack provides a quantitative read-out of the number of CD4 cells in the blood. The device performance has been compared to flow cytometry in clinical studies in HIV patients. Zyomyx product has a C.V. of 10% or less, which is comparable to flow measurements. The dynamic range of the assay is 30-1600 cells per microliter. The company is preparing to scale up manufacturing and is looking to establish distribution relationships in the developing world. Other cell-counting applications are under consideration in the developed world.
Eliminating the need for equipment altogether addresses several unmet needs within rural developing world settings. Requirements for water purity, control of dust and temperature, and adequate ventilation are often driven by concerns regarding instrumentation, not the underlying assay. Innovations allowing assays to be simplified to the point where they do not require equipment can, in many cases, address these other needs as well. Conventional lateral flow tests are an example of assays that have been simplified to allow sophisticated performance without laboratory equipment. The ability to quantify and to multiplex assays on a single disposable will be the next advancement in lateral flow.

Portability of diagnostics also increases dramatically when assays can be performed without an instrument. Furthermore, simplification of an assay to the point where no equipment is required removes the need for maintenance and essentially ensures that the read-out is visually based, which currently requires the least amount of training to use and interpret.

One of the most innovative POC platforms emerging is paper-based diagnostics. Paper-based platforms eliminate the need for bulky plastic disposables, which can dramatically reduce the size and weight, as well as the cost, of a novel POC test.

**Diagnostics For All (DFA)** is a non-profit organization working to design diagnostics specifically for POC use in the developing world. Using innovative technology to create patterns on paper, DFA is developing a fingernail-sized paper chip that changes color when exposed to bodily fluids (single drop of blood, urine, sweat). The paper itself is patterned with hydrophobic polymers that form a series of channels that wick a sample by capillary action throughout the device. The channels are loaded with reagent for specific tests, and use conventional colorimetric, enzyme-based endpoints to detect analytes.
Samples applied to the paper are wicked through the channels without external power supplies, and react with various reagents in preformed wells, leading to color changes that can be compared to a reference scale for visual interpretation. Proof of concept experiments have been carried out with clinically relevant concentrations of glucose (range of 2.5-50 mM) and protein (bovine serum albumin, range of 0.38-7.5 µM). Devices have been tested in the presence of contaminating dirt, pollen, and graphite with robust results. Due to the multiple channels, the technology can perform several tests on a single chip with a turnaround time of minutes. The first DFA application will be for liver function tests and the cost target is currently under $1. In the future, DFA plans to develop diagnostics for kidney disease, TB, malaria, HIV/AIDS, and diabetes.

Seventh Sense Biosystems, an early stage company based in Cambridge, MA, is also working with the concept of POC diagnostics that do not require an instrument. The company is at an early stage of developing diagnostics that can be worn directly on the skin (“on-skin”) or temporarily imprinted into the skin (“in-skin”). Seventh Sense’s “in-skin” diagnostics are compatible with a qualitative yes-no, or threshold, response, whereas the “on-skin” diagnostics are expected to be fully quantitative. The most significant potential of both types of diagnostics, once developed, is that users will be able to self-monitor various conditions outside of the traditional healthcare system. Although early in development, this concept has the ability to be applied in multiple indications in both developed and developing world applications with no need for lab infrastructure or lab personnel. The Bill & Melinda Gates Foundation recently awarded Seventh Sense a Grand Challenges Explorations grant to fund early research toward developing “in-skin” and “on-skin” diagnostics for malaria.

The “on-skin” diagnostic patch is adhesively applied to the skin, and the patch functions by drawing a small amount of interstitial fluid or blood from the patient for analysis. The “in-skin” diagnostic is enabled by a biocompatible assay reagent that can be temporarily imprinted into the skin and then sloughed off over several weeks. The reagent is a polymer bead composed of two distinct hemispheric phases, each with a different encapsulated dye. One of the hemispheres is surface functionalized with a binding moiety, such as an antibody. In the absence of an analyte, the polymer beads orient randomly. If the analyte of interest is present in the sample, the binding of the antibody causes reorientation of particles so that all particles are oriented in the same direction. This is detected as a change in color.

Figure 21: Seventh Sense Biosystems Technology
Discovering New Biomarkers

A critical need, albeit with the least predictable development timeframe, is the discovery and validation of biomarkers that specifically address developing world diseases and are accessible through sample types that are easy to acquire and process.

The discovery of relevant biomarkers for diseases of the developing world has been difficult. The basic science that leads to biomarker discovery often falls under the purview of academia because the extended timeline, high investment, and lack of financial return do not provide sufficient inducement to industry. Yet the validation of biomarkers, a laborious and time consuming task, often does not provide the rewards of near-term publication required for academic institutions.

Despite these impediments, a number of institutions have created or are in the process of instituting biomarker discovery programs and are making strides in identifying new biomarkers for developing and developed world diseases. These institutions use techniques to discover biomarkers that can be applied to a range of diseases, including infectious diseases of the developing world. For example:

- The Plasma Proteome Institute (PPI), a Washington, D.C., non-profit organization, is engaged in an effort to expand the repertoire of diagnostic proteins measurable in human plasma, changes in which may be used as biomarkers for a variety of disease states. Using clinically-implementable mass spectrometer platforms, PPI is working (through the hPDQ project) to produce a complete library of assays for all human proteins, focusing initially on the verification/validation of more than 1,000 existing biomarker candidates for use in both developed and developing world.

- Caprion Proteomics’ proprietary proteomics discovery technology – CellCarta® – employs mass spectrometry to produce quantitative and robust measurements of the protein expression differences across large sets of biological samples with the goal of discovering new drug targets as well as biomarkers. While multiple pharmaceutical companies have entered into collaborations with Caprion to look for markers of early efficacy and toxicity as they pursue clinical development of therapeutics, these same techniques are being employed to look for targets to be used in developing diagnostics for a range of diseases, including infectious diseases of the developing world. Funding sources and objectives determine, in large part, where Caprion directs its efforts. A five-year, $13 million grant from the United States National Institute of Allergy and Infectious Diseases (NIAID) resulted in discoveries for Brucella, an intracellular bacterial pathogen similar to TB in many regards, and Caprion has now received additional funding to begin looking for biomarkers for TB in human clinical samples, where it hopes to be able to identify biomarkers that distinguish between latent and active infection. Caprion is also interested in applying its technology to malaria, again looking to understand the different signatures between subclinical and clinical disease.

- SomaLogic has developed a highly sensitive detection assay to simultaneously measure large numbers of proteins (currently ~1,000) in small quantities of blood with sensitivity (LLOQ <1pM) and reproducibility (CV’s <5%). The assay is applied for biomarker discovery to develop and validate diagnostic signatures capable of distinguishing between stages of disease or susceptibility to therapeutic regimens. SomaLogic’s microarray platform uses chemically-modified aptamers – oligonucleic acids – which are developed through the company’s proprietary SELEX methods to optimize reagents which bind in a highly specific fashion to target proteins. The company is developing its first diagnostic products in lung cancer, with a discovery pipeline that ranges from oncology to cardiovascular disease, neurodegenerative disease, renal disease, and autoimmune disease.
Many other companies in the biotech, pharmaceutical, and diagnostics space have novel biomarker programs utilizing the latest genomics, proteomics and cellomics technologies, including Novartis, Roche, AstraZeneca, Pfizer, and Thermo Fisher Scientific. However, an important consideration is that even with the best tools available for biomarker discovery, the results still require translation into a functional diagnostic. While technologies such as mass spectrometry allow for relatively rapid identification and validation of proteins and/or peptides that may serve as appropriate markers for disease or response to therapy, diagnostic development and validation requires an additional set of expertise. Therefore, collaborations between these institutions and innovative diagnostic developers, like those profiled in this chapter, may allow for an effective funneling of relevant biomarkers into relevant assays for platforms suited for the developing world.
**Summary**

The technologies featured in this chapter are arrayed on the following chart with their potential attributes highlighted. The chart vividly demonstrates that while meaningful pieces of the diagnostics puzzle are being tackled, the ultimate solution will require a portfolio of products and intense collaborations.

**Figure 22: Summary of Promising Technologies in Development**

<table>
<thead>
<tr>
<th>Capability Goals</th>
<th>Abaxis</th>
<th>Alverix</th>
<th>Biohelix</th>
<th>Claros Diagnostics</th>
<th>Cepheid</th>
<th>DxBox</th>
<th>Gen-Probe</th>
<th>Inverness Medical</th>
<th>Ionian</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small Sample Volume</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No/Auto Sample Preparation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Optical Detection</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High Sensitivity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rapid Result</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Simple to Use</td>
<td>?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low Cost</td>
<td>?</td>
<td>?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimal Power Required</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No Running Water Required</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reagents Stable at Room Temp</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* N/A: Not applicable; *: No data available for this technology
The chart also has notable numbers of question marks, which represent either areas where data was not available or where product development is at an early stage and an answer is not known (for example, likely cost and ease of use).

<table>
<thead>
<tr>
<th>New Detection Technologies</th>
<th>Axela</th>
<th>Bioscale</th>
<th>Daktari</th>
<th>Diagnostics for All</th>
<th>Draper Labs</th>
<th>Quanterix</th>
<th>Seventh Sense</th>
<th>T2 Biosystems</th>
<th>Vivacta</th>
<th>Zyomyx</th>
</tr>
</thead>
<tbody>
<tr>
<td>N/A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Ionian Technologies device does not require sample preparation for most sample types, with the exception of whole blood, for which preparation is required.
The likely availability of the types of technologies described in this chapter is estimated in the following chart.

**Figure 23: Technology Timelines**

<table>
<thead>
<tr>
<th>Small or Central Lab-Based</th>
<th>Point of Care</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Long Term</strong></td>
<td><strong>Short / Medium Term</strong></td>
</tr>
<tr>
<td>• Development of assays for multiple sample types</td>
<td>• Development of assays for multiple sample types</td>
</tr>
<tr>
<td>• Portable benchtop instruments using novel detection technologies</td>
<td>• Portable benchtop instruments using existing detection technologies</td>
</tr>
<tr>
<td>• Novel sample preservation technologies</td>
<td>• Novel sample preservation technologies</td>
</tr>
<tr>
<td>• Simplified sample collection and sample preparation</td>
<td>• Simplified sample collection and sample preparation</td>
</tr>
<tr>
<td>• Increase installed base of central lab instruments</td>
<td>• Increase installed base of central lab instruments</td>
</tr>
<tr>
<td>• Biomarkers available for major neglected diseases in easily accessible samples and incorporated into validated tests</td>
<td>• Availability of point-of-care molecular diagnostics</td>
</tr>
<tr>
<td>• Breath analysis for infectious disease</td>
<td>• Point-of-care CD4 T cell counting</td>
</tr>
<tr>
<td>• “Open” point-of-care platform for proteins, nucleic acids, &amp; microbes that minimize sample preparation</td>
<td>• Improved lateral flow handheld devices</td>
</tr>
<tr>
<td>• Diagnostics without instrumentation</td>
<td></td>
</tr>
</tbody>
</table>
Chapter 5
Incentives for Diagnostics Innovation in the Developing World

The rapid evolution of in vitro diagnostic technology offers realistic hope that products being created by both large and small companies could be adapted for developing world use. As we have shown, breakthroughs in sample preparation, analyte detection, miniaturization, and signal generation could enable the creation of new products that meet the very challenging conditions present in developing world settings.

Developed world markets are driving many of these improvements, as the need for lower costs as well as ease of use, simpler procedures, and more robust instruments benefit developed as well as developing world applications. The developing world also represents a potentially large market for diagnostics. Non-governmental organizations and government bodies are increasingly aware of the need for and value of diagnostic products. Funding to purchase existing diagnostics and improve training and laboratory facilities has increased dramatically through programs such as the Clinton Global Initiative, PEPFAR, and the Fondation Mérieux. Yet the funding to support needed innovation for ensuring broader access throughout the developing world has not received comparable backing.

The most successful examples of introducing cutting-edge diagnostics into the developing world are situations where parallel markets also exist in the developed world. HIV is the best example of this alignment of need: the U.N. recently reported that the number of HIV diagnostic tests used world-wide doubled in 2008.

Diagnostics for TB are also being introduced into centralized laboratories in the developing world.

There is a growing trend for the developing world to play a lead role in the adoption of new diagnostics technologies. For instance, South Africa has taken the lead internationally in blood screening by adopting the most sensitive approach available for HIV detection: single donor nucleic acid testing (NAT). South Africa wants to provide the highest assurance of safety for its blood supply in response to the high prevalence of the virus among its population. Similarly, in the field of viral load testing in HIV patients, South Africa and Brazil are the second and third largest markets in the world behind the United States. This trend will become increasingly apparent as the governments of India, China, South Africa, and Brazil strive to extend health care coverage to their burgeoning populations.

Outside of the HIV market, however, diagnostic needs in the developing world are not so well matched with those of the developed world. Many emerging market and developing world settings lack sophisticated centralized laboratories. Trained medical technicians, to say nothing of doctors and nurses, are in short supply. Basic infrastructure such as reliable electricity and a consistent source of clean water are lacking in rural and outlying areas.

There is a need to develop the next generation of point-of-care (POC) platforms to ensure the broadest access to diagnostic testing in the developing world. Without market forces to drive these developments, the global health community will need to create financial incentives that will attract companies to invest in diagnostics for resource-poor settings.

Important recent precedents have shown the value of “pull,” or “market-based” incentives in global health funding. For vaccines and therapeutics, two novel incentive programs have recently been implemented: the Advance Market Commitment (AMC) administered by GAVI, for the purchase of $1.5 billion worth of pneumococcal vaccine; and the Priority Review Voucher (PRV). The PRV is a transferable voucher awarded to a company that receives FDA approval for a new vaccine or drug that prevents or treats a neglected tropical disease, such as malaria, tuberculosis, and intestinal worms. A PRV entitles the bearer to priority review for a future new drug application that would not otherwise qualify for priority. The philosophical approach behind these programs is to create a large, long-term, success-based incentive that stimulates the development of vaccines and therapeutics for resource-poor nations.

The unique characteristics of diagnostics necessitate a distinct approach to incentivize product development. Development timelines for diagnostics are shorter than for either vaccines or therapeutics, and a smaller amount of capital is required to develop and commercialize a diagnostic. Traditionally, diagnostic products have been commercialized based on a lower profit margin than therapeutics. However, the risk to successful commercialization remains high due to the complexity of bringing together multiple technologies in a single diagnostic.

With respect to the developing world, there is an additional fundamental difference between the economics of diagnostic and drug development: Diagnostic platforms that address the unmet needs of the resource-poor countries—platforms that are portable, easy to use, resource-efficient, robust and have low costs per test—will likely have substantial applications in profitable private markets of middle-income countries and in the developed world.

Despite this potential to leverage investment in diagnostics, the hurdles to corporate involvement in meeting the needs of the developing world are still high. Importantly, the commercial prospects for the next generation of point-of-care (POC) platforms in the rich countries of the developed world and the emerging middle-income economies remain uncertain because defining commercially viable indications can be challenging. The commercial potential of any given test will depend on the medical impact of the diagnostic information. It’s not enough to know the result of a POC test in a rapid manner. The information must be actionable and lead to better patient outcomes; otherwise, the higher costs associated with point of care testing can’t be justified. These criteria for a successful POC product give rise to the uncertainty about the market potential for most POC platforms.

An effective incentive program for diagnostics for the developing world will leverage this understanding of the interplay between developing, emerging, and developed world markets and use the existing needs and market forces to stimulate development of novel platforms.

What would be the key elements of an effective incentive that induces industry to develop POC platforms for the developing world?

---

• A well-designed incentive will provide compensation to companies for the cost of developing POC diagnostic platforms. Such a program should fund companies on a pay-for-success basis to achieve clearly defined R&D and commercialization milestones.

• Incentives should be structured so that companies understand customer requirements from the outset, including issues of cost, portability, ruggedness, resource utilization, product stability, sensitivity, and specificity. The incentive should not constrain companies to particular solutions but should invite engineers, scientists, and manufacturers to employ their full creativity to the problem of developing innovative solutions to well-defined problems. Since the requirements in the developing world are not widely understood by companies today, an independent group comprising participants from industry, medicine, academia, and the developing world should come together to set these customer requirements in a transparent fashion.

• The funders of an incentive should use their purchasing power to encourage consortium-based approaches. This could include encouraging academic licensors to eliminate royalties in resource-poor markets. It could also include encouraging partnerships between innovative small- or medium-sized enterprises with large, established companies. Often, innovation does not thrive in the largest companies, but smaller enterprises lack the experience and capacity to get a product to market at reasonable cost. Partnerships make sense to develop the best technological approaches and assure that they can be commercialized.

• In exchange for the rights to sell and distribute in profitable markets in the developed world and private health care systems, companies must agree to principles that assure access to their products in the resource poor regions of the developing world at a reasonable cost. Above all else, donors want to ensure that a successful product will be available to poor patients in the developing world, even if the circumstances of a company change.

• To create the biggest impact, the incentive should specify that platforms be based on a transparent design that enables other groups to develop assays for neglected diseases that could be readily implemented on the platform. Assay developers should be granted non-exclusive royalty-free licenses to intellectual property necessary to develop such assays, but the licenses may be limited to developing world indications and public markets. Commercial deals would need to be forged for other markets.

• Finally, the incentive must empower companies by recognizing industry’s need to control intellectual property in profitable markets and give companies the full right to commercialize platforms in the private markets of middle-income countries and in the developed world.

The issues of access, ownership of intellectual property, and granting of licenses are important elements of an incentive. The right balance must be struck between the interests of donors and companies; we offer a few thoughts on these topics below.

The donor who pays for the incentive has good reason to insist on access to intellectual property, especially intellectual property that is funded by the incentive. However, it should not be the primary objective of donors to create a parallel competing business to serve the developing world. Instead, the mission of serving poor patients in the developing world should be met by aligning the interests and commitments of donors and companies. And companies, with their deep experience in manufacturing, quality control, supply chain logistics, product distribution, and after-market servicing are often in the best position to ensure the successful introduction and support of a diagnostic in the developing world.

Access to intellectual property (IP), including know-how, is undoubtedly a more complex discussion for diagnostics than for therapeutics. In the case of therapeutics, the most important single piece of IP is the composition of matter to the drug used in a treatment. Occasionally, manufacturing patents or method of use patents can be
important positions in an IP portfolio. In contrast, for diagnostics, there are often myriad patents supporting sample acquisition, sample processing, assay configuration and chemistry, and manufacturing, as well as materials, software, and instrument design. Moreover, some of the most important intellectual property is not the patents themselves but know-how, including manufacturing expertise. The know-how to make a high quality product at the lowest possible cost is often a bulwark in a company’s IP portfolio.

Thus, it stands to reason that requiring a company to transfer its enabling know-how and manufacturing expertise to a third party in the developing world can be perceived as a threat that may empower competitors around the globe. A company is likely to balk before agreeing to give broad access to know-how to a third party.

An alternative approach, grounded in the pragmatic idea that what donors most want is to make sure that product is available to poor patients, is for companies to enter into manufacturing commitments as a requisite for receiving financial support. The manufacturing commitment could be implemented as a supply agreement specifying unit cost at various volumes. Companies would need to meet product cost requirements as a prerequisite to getting incentive funds. These commitments would become increasingly specific as product development proceeds forward and the final design of the product comes into focus. For its part, a company receiving an incentive could choose to manufacture in-house using its existing facilities, or it could establish a lower cost manufacturing facility in the developing world. This would allow the company to control the organization, training, quality system, and management of the operation.

The opportunities for a company that successfully develops a POC diagnostic platform for the developing world are significant. The product volumes that will be required to meet the needs are staggering. The World Health Organization estimated that in 2008 the developing world experienced 241 million cases of malaria, 2.7 million new infections with HIV, 7.6 million cases of re-activated TB, and 217 million cases of bacterial pneumonia. A manufacturer that successfully addresses these markets will have enormous opportunities to reduce cost of goods through scale and automation. These products may have low margins in the poorest countries of the world, but may also be sold profitably in private-pay markets in middle-income countries.

Achieving the broadest utility of an incentive-funded platform would be achieved by requiring a manufacturer to make specifications available to assay developers so that they could develop additional compatible assays for the system. One issue with these “open platforms” is that a problem can arise whenever the supplier of reagents is distinct and separate from the manufacturer of the instrument. In the event of a difficulty encountered by a customer, the instrument manufacturer points to the reagent, and the reagent provider points to the instrument. One way to address this would be to create non-exclusive licenses to develop assays for neglected diseases for the instrument, with the proviso that manufacturing and quality control would be the responsibility of the company that manufactures and distributes the POC platform. To make this work, donors may need to be willing to commit funds for investment in manufacturing capacity and for purchase of product for markets that can’t otherwise support the investment.

Incentives will make a large difference in reducing the financial barriers to company participation in developing diagnostics for resource-poor countries and neglected disease indications. However, the ultimate force that will drive improvements in global health will come from sustainable markets with the potential for companies to make reasonable profits at each step in the value chain required to deliver product to the end user. Having a model which enables profitability will be the best way to ensure a sustainable system in the long run. Diagnostics represent a unique opportunity to develop this approach because of the confluence between the needs of the developing world and the needs of middle income and developed economies.
Conclusion
A Call to Action

The Need for New Diagnostics for Neglected Diseases

Much of the investment in global health research and development (R&D) has focused on prevention and cure, but there is an equally urgent need to improve the ability to diagnose patients suffering from neglected diseases. Health care providers in the developing world lack tools for disease detection, treatment selection, treatment monitoring, and disease surveillance that would be taken for granted in wealthy countries.

Over the past decade, donor and government institutions have invested hundreds of millions of dollars in R&D to prevent and treat many neglected diseases that primarily affect the developing world poor. Public-private partnerships have arisen among not-for-profit organizations, academic institutions, and industry to develop new drugs and vaccines. Unfortunately, diagnostics R&D has only attracted a fraction of this funding, despite the fact that new diagnostics have the potential to save hundreds of thousands of lives each year by detecting disease that can be treated if caught in the early stages—ensuring that the patient gets the right treatment as quickly as possible—and thwarting the over-use of medicines due to misdiagnosis, critical in the fight against drug-resistant bugs.

The list of unmet diagnostic needs for neglected diseases is daunting. The complexities of the diseases, health care settings, cultures, geographies, and test purposes in the developing world make it difficult to imagine that any single technology will work in all situations. Questions about the potential for commercial success in these markets make it challenging for companies with advanced diagnostics technology to invest in research and product development for these indications. It is critical to find avenues to spur increased investment in diagnostic development for the developing world.

The Importance of Point-of-Care Diagnostics

To date, the diagnostics industry has focused its developing world efforts on improving instruments for centralized labs because these instruments have the most in common with instruments used in the developed world. The shortage of trained technicians in the United States that is driving the development of simplified instruments and methodologies has also yielded benefits for centralized labs of the developing world. Moreover, because centralized labs perform more diagnostic tests than local clinics and are more easily reached to provide support and service, these labs have been more attractive markets to the private sector.

Private-sector innovation is driving improvements in central-lab testing in the developing world. Yet centralized labs will never address the needs of many of patients in resource-poor settings. For this reason, BIO Ventures for Global Health (BVGH) believes that global health organizations and donors should focus on encouraging the development of smaller scale instruments for decentralized health clinics and POC testing. This will ensure that the majority of individuals in the developing world—whether they live in cities or rural communities with basic health care—have access to needed diagnostic tests.

The Need for Incentives to Spur Diagnostic Development for Neglected Diseases

In our extensive interviews with diagnostic companies, we found a strong interest in exploring ways to assist in the fight against neglected diseases. At the same time, companies reinforced their position that they will be unable to commit their resources to this goal in the absence of a financial model that makes business sense. Some companies may pursue a model in which they make existing technologies or patents available for others to commercialize. These technologies may be licensed with no or low royalties for use in developing markets. Others may elect to more actively participate in formal collaborations with not-for-profits. Finally, select companies may see a market opportunity that will emerge over time, and, while they may still seek public-sector support in the near-term, are prepared to participate as full partners in product development, manufacturing, and distribution. These companies will see that products developed to meet the operational, environmental, and economic constraints of resource-poor settings may also be successfully deployed in developed world and emerging markets, thereby leveraging their overall investment across a global market.

One market trend that may help drive additional investment into R&D for diseases of poverty is the rapid expansion of potential private-pay health care markets in the emerging economies of Brazil, India, China, Russia, and South Africa. These countries have large populations demanding improved health care; rapid increases in test volumes could lead to lower costs for their poor populations, and larger markets for diagnostics to treat tropical diseases. Moreover, we have observed synergies in parallel development of products for developed and developing world markets that could enhance the applications of these technologies in the developed world.

Despite these trends, public and/or donor investment will be needed to initiate, and in some cases to sustain, private-sector product development efforts. To ensure ongoing commitment by companies, new market-based incentives...
that provide industry with the promise of attractive downstream financial returns must be implemented. Without the potential for achieving competitive market returns, companies will choose other avenues in which to invest their intellectual, human, and financial resources.

The Need for Additional Analysis to Optimize Funding Approaches

BVGH believes that donor support would ideally focus on developing a limited number of novel POC instruments that could run a wide variety of tests for infectious and chronic diseases. Experts identify two possible approaches: a) portable benchtop instruments that can run small volumes with shorter turn-around times and b) rapid POC tests that don’t require instrumentation. Due to resource constrained budgets and a lack of trained health care providers, this approach will benefit health systems in the developing world by enabling the purchase and maintenance of fewer types of equipment as well as reducing training costs. If fewer instruments share the market, it will also increase manufacturer confidence in achieving large sale volumes, which is the key to driving down production and service costs through economies of scale. Leveraging research, development, manufacturing, and service costs across a larger volume of instrument sales could translate to lower prices for developing world governments, donors, and patients. It is essential that more analysis be done to assess the number of novel diagnostics that can be absorbed in developing countries and that this analysis is used to inform the approach donors take to funding product development activities.

The Path Forward: Risk and Cost-Sharing Collaborations

A key to nurturing technical advances and ultimately commercializing new diagnostic products—particularly POC diagnostics—for the developing world will be risk- and cost-sharing collaborations between diagnostic companies and not-for-profit organizations and others, such as academics.

Addressing the broad and demanding range of unmet diagnostic needs of the developing world will require bold investment decisions, forward-thinking collaborations between for-profit and not-for-profit organizations as well as donors and governments, and numerous technical and scientific breakthroughs. Additional analysis must be done to identify financial models that will support industry investment in developing novel diagnostics for neglected diseases and to bring coherence to donor funding of these efforts.

BVGH is committed to working with the range of players involved in the discovery, development, and commercialization of novel diagnostics—from for-profit companies to not-for-profit development organizations to donors—to catalyze investments and partnerships that will lead to better and faster innovations to address the urgent unmet diagnostic needs within the developing world.

If you are interested in learning more about BVGH’s efforts to support the development of novel diagnostic products for developing world markets, please contact Priya Mehta, Director of Global Health Markets at BIO Ventures for Global Health, at pmehta@bvgh.org.
Appendix I:

Organizations interviewed

- Abaxis
- Alverix, Inc.
- Axela, Inc.
- Beckman Coulter, Inc.
- Becton, Dickinson and Company
- BD Medical Center
- Bill & Melinda Gates Foundation
- BioHelix Corporation
- BioScale, Inc.
- Caprion Proteomics, Inc.
- Cellabs Pty Ltd.
- Cepheid
- Claros Diagnostics, Inc.
- Cleveland Clinic
- Dako North America
- Daktari Diagnostics, Inc.
- Diagnostics for All
- Duke University Medical Center
- Foundation for Innovative New Diagnostics (FIND)
- Gen-Probe, Inc.
- Genzyme Corporation
- Genzyme Diagnostics
- Global Health Initiative, Kellogg School of Management, Northwestern University
- Global Scientific Solutions for Health, Inc.
- Handylab, Inc.
- Harvard Vanguard Medical Associates
- Idaho Technology, Inc.
- InBios International, Inc.
- Inverness Medical Innovations, Inc.
- Ionian Technologies Inc.
- IQium, Inc.
- Iris Diagnostics, division of IRIS International, Inc.
- J. Craig Venter Institute
- Laboratory Corporation of America (LabCorp)
- Life Technologies Corporation
- Luminex Corporation
- Marafiki Foundation, Inc.
- McLaughlin-Rotman Centre for Global Health
- MicroPhage, Inc.
- Microsens Biotechnologies
- Mindray Medical International Ltd.
- Nanosphere, Inc.
- Novartis Vaccines and Diagnostics, Inc.
- Omega Diagnostics Group PLC
- OraSure Technologies, Inc.
- Ortho Clinical Diagnostics, Inc., Johnson & Johnson
- Oxford Immunotec Ltd.
- Peregrine Pharmaceuticals, Inc.
- Phthisis Diagnostics, LLC
- Quanterix Corporation
- Quidel Corporation
- Scott Development Group, Inc.
- Sequella Inc.
- Seventh Sense Biosystems, Inc.
- Siemens Healthcare Diagnostics, Inc.
- Sierra Molecular Corporation
- SomaLogic, Inc.
- T2 Biosystems, Inc.
- Tamil Nadu R. M.G.R. Medical University
- Tethys Bioscience, Inc.
- The Charles Stark Draper Laboratory, Inc.
- Thermo Fisher Scientific, Inc.
- Trinity Biotech
- University of Victoria
- University of Massachusetts Medical School
- University of Washington
- William J. Clinton Foundation Health Access Initiative
- Vivacta Ltd.
- Zyomyx, Inc.
Appendix II:  
Examples of Specific Diagnostic Unmet needs for Neglected Diseases

The examples below highlight some of the biomarker and instrument innovations anticipated to be the most valuable for the developing world:

Figure A: High-Impact Innovations

<table>
<thead>
<tr>
<th><strong>High-Impact Biomarkers</strong></th>
<th><strong>High-Impact Diagnostics</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acute Lower Respiratory Infections (ALRI)</strong></td>
<td>A panel of biomarkers to distinguish between bacterial and viral infection</td>
</tr>
<tr>
<td><strong>HIV/AIDS</strong></td>
<td>A biomarker in an easily obtained sample for infant screening and diagnosis that is not confounded by maternal anti-HIV antibody</td>
</tr>
<tr>
<td><strong>Tuberculosis</strong></td>
<td>A biomarker for active TB that is easier to obtain than sputum: blood, saliva, urine</td>
</tr>
<tr>
<td><strong>Malaria</strong></td>
<td>A human host biomarker that confirms active malaria with parasitemia and distinguishes from latent infection</td>
</tr>
<tr>
<td><strong>Lymphatic filariasis</strong></td>
<td>An accessible marker offering fewer cross-reactions with other helminths compared to antibody detection</td>
</tr>
<tr>
<td><strong>Schistosomiasis</strong></td>
<td>A reliable biomarker to detect <em>S. mansoni</em> that avoids the confounding fluctuations of egg shedding; ideally in sample type other than stool</td>
</tr>
<tr>
<td><strong>Dengue</strong></td>
<td>Biomarker(s) to distinguish between dengue serotypes</td>
</tr>
</tbody>
</table>

---

25 Health Advances interviews, secondary research and analysis; BVGH.
For the purposes of this report, a subset of seven neglected diseases prevalent in the developing world was selected to highlight examples of specific diagnostic testing unmet needs. Basic information about these diseases is summarized below:

Since the primary focus of this study is to identify novel diagnostic technologies that could address a range of unmet needs in the developing world, the unmet needs identified for each of these diseases are not meant to be comprehensive, but rather to serve as examples to aid in the broader identification of diagnostic technologies. The examples will hopefully prove useful as broader indicators of the types of unmet needs that exist in other neglected diseases and the range of issues to consider in designing novel diagnostic tests to address them.

---

**Figure B: Global Burden of Select Neglected Diseases**

<table>
<thead>
<tr>
<th>Disease</th>
<th>DALYs</th>
<th>At Risk</th>
<th>2008 Global R&amp;D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute Lower Respiratory Tract Infections</td>
<td>94.5MM</td>
<td>Global</td>
<td>$91MM</td>
</tr>
<tr>
<td>HIV/AIDS</td>
<td>58.5MM</td>
<td>Global</td>
<td>$1,165MM</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>34.2MM</td>
<td>Global</td>
<td>$446MM</td>
</tr>
<tr>
<td>Malaria</td>
<td>34MM</td>
<td>3,300MM</td>
<td>$542MM</td>
</tr>
<tr>
<td>Lymphatic filariasis</td>
<td>5.9MM</td>
<td>1,300</td>
<td>$11MM</td>
</tr>
<tr>
<td>Schistosomiasis</td>
<td>1.7MM</td>
<td>779MM</td>
<td>$20MM</td>
</tr>
<tr>
<td>Dengue</td>
<td>0.7MM</td>
<td>2,500MM</td>
<td>$127MM</td>
</tr>
</tbody>
</table>

Pathogen focus: *Streptococcus pneumoniae* and *Haemophilus influenzae* type b

Pathogen: rapidly mutating intracellular retrovirus

Pathogen: *Mycobacterium tuberculosis* (MTB)

Pathogen: single-celled parasite *Plasmodium*

Pathogen: parasitic worms; 90% of infections are caused by *W. bancrofti*, most of the remainder by *B. malayi*

Pathogen: parasitic worms, most often *S. mansoni* and *S. haematobium* in humans

Pathogen: positive-strand RNA flavivirus, with four serotypes: DENV1-4

---

Acute Lower Respiratory Infections (ALRI)

Globally, ALRI is a major source of disease burden in the developing world; pneumonia is ranked as the number one cause of morbidity and mortality of any neglected disease. Diagnostics fulfilling the unmet needs listed below will have a major impact on the global burden of ALRI. A study published in Nature, in 2006, considered two different diagnostic testing options for ALRI and the expected health benefits in children under five years of age in Africa, Asia, and Latin America. The study predicted that a test to diagnose bacterial ALRI, but not discern the specific pathogen type, with 85% sensitivity and 70% specificity, could potentially save >117,000 children per year in Africa alone. A test with 95% sensitivity and 85% specificity could save up to 152,000 children per year, even if only 33% of children with ALRI symptoms in the three continents modeled sought care. It also estimated that an additional 253,000 lives could be saved indirectly by reducing over-treatment with antibiotics.

Figure C: Key Diagnostic Unmet Needs for Acute Lower Respiratory Infections (ALRI)

<table>
<thead>
<tr>
<th>Available Diagnostics</th>
<th>Diagnostic Overview</th>
<th>Current Diagnostic Limitations</th>
<th>Diagnostic Unmet Needs</th>
</tr>
</thead>
</table>
| • Clinical diagnosis  | • Clinical case management protocols developed by the World Health Organization  
|                       | • Health care workers note elevated respiratory rate and inward movement of the lower chest wall on breathing (90% sensitivity when performed by experts, 70% specificity)  | • Low specificity and inability to quickly and accurately differentiate cause of fever (pneumonia vs. malaria vs. dengue, etc.)  
|                       |                     | • Inability to differentiate viral vs. bacterial cause of pneumonia  
|                       |                     | • Indiscriminant use of antibiotics due to low specificity of clinical diagnosis contributes to increase of antibiotic resistance  | • Rapid, accurate, affordable, point of care pneumonia diagnostic to discern bacterial versus viral pneumonias  
|                       |                     | • Pathogen typing test to discern infectious agent by species or virus type  
|                       |                     | • Fever panel to differentiate cause of fever including regionally co-occurring agents  
|                       |                     | • Simpler methods to obtain and process sputum samples  |
**HIV/AIDS**

While adult diagnostic screening technologies for HIV are improving in developing world settings, there are major unmet needs in low-infrastructure monitoring technologies as well as infant diagnosis. In 2005, 570,000 AIDS-related deaths occurred in children under 15 years of age; an improved diagnostic would have a major impact on this subset of the population.32

**Figure D: Key Diagnostic Unmet Needs for HIV/AIDS**31,32,33,34

<table>
<thead>
<tr>
<th>Available Diagnostics</th>
<th>Diagnostic Overview</th>
<th>Current Diagnostic Limitations</th>
<th>Diagnostic Unmet Needs</th>
</tr>
</thead>
</table>
| **Rapid diagnostic tests (lateral flow)** | • Routine testing  
  • Antenatal screening as a part of prevention of mother-to-child transmission  
  • Whole blood, serum, or plasma | • Lack of rapid, low-complexity diagnostics in infant screening and monitoring that avoids interference by maternal antibodies  
  • Few unmet needs and limitations for testing adult population | |
| **ELISA** | • High-throughput screening of HIV Ab or HIV Ab/p24 using whole blood, serum, or plasma  
  • p24 Antigen detection for infant diagnosis, early acute phase HIV diagnosis, with plasma or dry plasma spots | | |
| **IFA, LIA, Western blots** | • Confirmatory tests using whole blood, sera, or plasma | | |
| **Molecular tests** | • HIV RNA or DNA measured to diagnose infant HIV, identify acute infection (pre-seroconversion), monitor response to therapy  
  • HIV gene sequence used to identify drug resistance  
  • Molecular NAAT HIV tests  
  • Isothermal amplification, PCR, RT PCR or branched DNA for viral load testing in conjunction with HIV therapy using plasma or dry plasma spot | • Molecular tests require extensive training  
  • Tests are expensive  
  • Reagents have limited shelf-life at ambient temperature  
  • Expensive instrumentation, dedicated facilities | • Molecular tests with lower training and intuitive read-outs  
  • Lower cost/test  
  • Reagents stable in higher temp and humidity  
  • Simple instrumentation or no device required |
| **Flow cytometry or dedicated cell counter** | • CD4 cell counting used to stage patients for antiretroviral therapy and to monitor response to therapy  
  • Fresh or stabilized whole blood sample | • Cost of flow cytometry equipment  
  • Extensive training and QC required | • Tests with less instrumentation that can be done in the field  
  • Lower cost/test  
  • Tests that don’t require extensive training |
| **Light microscopy (uncommon)** | | | |

---

31 Health Advances interviews and analysis.
32 UpToDate (2009).
Tuberculosis (TB)

As the fourth highest cause of global morbidity and mortality from neglected diseases in 2004, the importance of finding improved diagnostic and therapeutic solutions for TB is critical. Continued research to find additional biomarkers for latent and active TB, and detection methods beyond sputum smear microscopy (SSM) will be valuable in alleviating TB’s global disease burden. The World Health Organization (WHO) TDR/European Commission joint expert consultation group recently outlined a number of potentially promising biomarkers for different disease stages of TB, including: cytokines expression as biomarkers of immune status, immune cell surface molecules as biomarkers for inflammation, cell surface receptors and their shedding, genomic and proteomics-based biomarker discovery, and even TB serodiagnostics, which have been largely unsuccessful to date.

A Nature study published in 2006 found that a rapidly and widely available diagnostic for TB with 85% sensitivity for sputum smear positive and sputum smear negative cases, and 97% specificity could save ~400,000 lives annually.

Figure E: Key Unmet Diagnostic Needs for Tuberculosis

<table>
<thead>
<tr>
<th>Available Diagnostics</th>
<th>Diagnostic Overview</th>
<th>Current Diagnostic Limitations</th>
<th>Diagnostic Unmet Needs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sputum smear microscopy (SSM)</td>
<td>Traditional smear microscopy, targeting the most infectious cases; SSM is highly specific in high-prevalence settings; Key component of the WHO Directly Observed Treatment Short-Course strategy (DOTS) to control TB</td>
<td>Requires extensive training and throughput is slow; Lack of sensitivity: 70% sensitive in most TB patients; &lt;50% sensitive in HIV patients; insensitive for extrapolumary TB; High limit of detection: 10,000 bacilli per mL of sputum</td>
<td>Improved sample collection and processing methods for sputum; Tests with rapid turnaround time to reduce patients lost to follow up; Improved sensitivity, below 10,000 bacilli per mL in sputum; Identification of new biomarkers, particularly in blood, urine or saliva; Biomarkers that differentiate between latent and active infection</td>
</tr>
<tr>
<td>Tuberculin skin tests</td>
<td>Tuberculin is injected into the skin; strong reaction after extended time interval indicates potential exposure to TB</td>
<td>Highly subjective; Not useful in developing world due to cross-reaction with BCG vaccine and environmental mycobacterial species</td>
<td></td>
</tr>
<tr>
<td>Cellular</td>
<td>Assays measure presence of T cells specific for TB; Blood is incubated with TB antigens; Secretion of interferon-gamma is measured; positive indicates that patient has been infected with TB</td>
<td>Does not differentiate between latent and active infection; Requires fresh blood sample; storage inactivates T cells; Must be performed in a lab setting</td>
<td></td>
</tr>
<tr>
<td>Bacterial culture</td>
<td>Traditional culture testing; Gold standard; Also used to determine drug resistance profile</td>
<td>Sample processing; Requires 3-4 weeks due to the slow growth rate of M. tuberculosis; Only feasible in centralized lab settings</td>
<td>More rapid methods to detect antibiotic resistance</td>
</tr>
</tbody>
</table>

39 UpToDate (2009).
Malaria

Malaria is a major cause of morbidity and mortality in the developing world. When left untreated, *P. falciparum* is often fatal in children under five years of age and pregnant women. The less fatal *P. vivax* is estimated to account for 25-40% of the global burden. A 2006 study published in *Nature* found that a 95% sensitive and 95% specific diagnostic requiring minimal infrastructure would avert >100,000 malaria-related deaths and around 400 million unnecessary treatments. A 90% sensitive and 90% specific diagnostic requiring no infrastructure would avert >300,000 malaria-related deaths and around 450 million unnecessary treatments, assuming adherence to test results and prompt and effective treatment following diagnosis. In addition, the ability to detect placental infection by antigen detection could have a significant impact on maternal/fetal medicine.42

Figure F: Key Unmet Diagnostic Needs for Malaria43,44,45,46,47

<table>
<thead>
<tr>
<th>Available Diagnostics</th>
<th>Diagnostic Overview</th>
<th>Current Diagnostic Limitations</th>
<th>Diagnostic Unmet Needs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical diagnosis</td>
<td>• Use of clinical algorithms to diagnose based on fever • In sub-Saharan Africa, 70% of fever cases in children in endemic areas are diagnosed at home and presumptive anti-malarial treatment is given</td>
<td>• 30% specificity due to inability to differentiate between malaria and other diseases that present with febrile symptoms at onset: pneumonia, dengue, or influenza • Overuse of antimalarial drugs due to false positives greatly accentuates drug resistance</td>
<td>• Biomarker (from host or parasite) that confirms clinical malaria in conjunction with parasitemia • A multiplex test that provides a differential diagnosis for diseases with febrile symptoms • Ability to detect placental infection</td>
</tr>
<tr>
<td>Rapid diagnostic tests (RDTs)</td>
<td>• Typically based on lateral flow • Antibodies detect malaria antigens, such as <em>P. falciparum</em>-specific histidine-rich protein-2 (HRP2), species-specific <em>plasmodium</em> lactate dehydrogenase (pLDH), or pan-specific adolase • Inexpensive and fast</td>
<td>• Published field trials show high variability in performance • Documented problems with manufacturing, quality control, and lack of regulatory licensure requirements • HRP2 antigens may not indicate current infection due to long half-life in blood</td>
<td></td>
</tr>
<tr>
<td>Blood slide microscopy</td>
<td>• Traditional slide microscopy • Sensitive, specific and consistent when performed by experts with consistent resources: electricity and well maintained microscopes</td>
<td>• Limitations of microscopy in the developing world: training, laboratory resources • Adults and older children with parasitemia may be partially immune, so tests for circulating parasites may not indicate clinical disease • Placental sequestration of parasites can reduce sensitivity, placing both mother and fetus at risk</td>
<td></td>
</tr>
<tr>
<td>Polymerase chain reaction</td>
<td>• Highly sensitive when performed by experts • Can differentiate amongst species</td>
<td>• Complex and expensive test • Unavailable in resource-limited settings</td>
<td></td>
</tr>
</tbody>
</table>

43 UpToDate (2009).
Helminth infections, including lymphatic filariasis and schistosomiasis, carry a major global disease burden with high estimates of morbidity and mortality rates. Improved technologies to address the unmet needs of these diseases will lead to direct improvements in individual health, in addition to improved surveillance and epidemiology data that is currently lacking.

Figure G: Key Unmet Diagnostic Needs for Lymphatic Filariasis

<table>
<thead>
<tr>
<th>Available Diagnostics</th>
<th>Diagnostic Overview</th>
<th>Current Diagnostic Limitations</th>
<th>Diagnostic Unmet Needs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscopic detection of microfilariae</td>
<td>• Required for species differentiation</td>
<td>• Blood must be drawn between 10PM and 2AM during the peak of microfilariae concentration</td>
<td></td>
</tr>
<tr>
<td>Detection of circulating filarial antigen (CFA)</td>
<td>• Can be performed at any time because antigen levels remain stable over time • Two methods: ELISA (quantitative) and immunochromatographic card test (ICT – qualitative) • The WHO endorses this test for its global program on the elimination of lymphatic filariasis</td>
<td>• Test kits are expensive • Cannot be performed on whole blood; serum required • Test interpretation makes it difficult to implement in endemic, rural areas</td>
<td>• Tests that don’t require nocturnal samples, such as more sensitive tests for microfilariae • Detection of CFA in plasma or urine • Tests with higher sensitivity and reduced cross-reactivity with other helminthes • Tests that can be implemented in remote settings</td>
</tr>
<tr>
<td>Antifiliarial antibody tests</td>
<td>• Detects IgG and IgE to filarial antigens</td>
<td>• Cannot differentiate between types of filarial infections • Poor sensitivity due to cross-reaction with other helminthes</td>
<td></td>
</tr>
<tr>
<td>Radiology</td>
<td>• Ultrasound • Lymphoscintigraphic techniques</td>
<td>• Complex and expensive • Inaccessible to resource-limited settings</td>
<td></td>
</tr>
<tr>
<td>Nucleic acid amplification tests</td>
<td>• Ability to detect presence of filarial worms</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

48 UpToDate (2009).
Figure H: Key Unmet Diagnostic Needs for Schistosomiasis 55,56,57

<table>
<thead>
<tr>
<th>Available Diagnostics</th>
<th>Diagnostic Overview</th>
<th>Current Diagnostic Limitations</th>
<th>Diagnostic Unmet Needs</th>
</tr>
</thead>
</table>
| Microscopy            | Detection of eggs in stool or urine | • Due to fluctuation of egg shedding, sensitivity is poor, often leading to false negatives  
• Not ideal for infections with *S. haematobium*  
• Need to process samples within 48 hours  
• Requires multiple samples in order understand infection intensity | • Antigen-based diagnostics  
• Diagnostics that work on small volume of blood or urine  
• Improvements in fecal sample processing for microscopy |
| Serology              | Detection of antischistosomal antibodies in serum samples in order to rule out infection in endemic populations | • Negative tests rule out infection, but positive tests do not distinguish between past infection, and current, active disease  
• Lack of standardization among different techniques leads to large variations in sensitivity and specificity, depending on reagents used, infection intensity, and species being evaluated | |
| Antigen-based detection | In development at this time  
• Initial studies have shown that these tests become negative 5-10 days following successful treatment | • Currently in development | |
| Radiology             | Imaging techniques (ultrasound, CT scans, and MRIs) allow for the detection of complications based on liver, urinary tract, or neurologic involvement | • Complex and expensive  
• Inaccessible to resource-limited settings | |
| Nucleic acid amplification | Ability to detect presence of pathogens  
• Highly sensitive, specific, and can be valuable for epidemiological studies | | |

55 Health Advances interviews and analysis.
56 UpToDate (2009).
Dengue Fever

Dengue fever shares many of the unmet needs with neglected diseases presenting febrile symptoms, but has a unique commercial market due to travelers, military, and prevalence in emerging markets such as Southeast Asia. Echoing the unmet needs in malaria, a multiplexable diagnostic platform that could address several fevers would be a valuable diagnostic contribution.

Figure I: Key Unmet Diagnostic Needs for Dengue

<table>
<thead>
<tr>
<th>Available Diagnostics</th>
<th>Diagnostic Overview</th>
<th>Current Diagnostic Limitations</th>
<th>Diagnostic Unmet Needs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical diagnosis</td>
<td>• Use of clinical algorithms based on fever</td>
<td>• Cannot differentiate between dengue and other diseases that present with febrile symptoms at onset: pneumonia, malaria, or influenza</td>
<td></td>
</tr>
<tr>
<td>Immunoassay</td>
<td>• Use of recombinant viral envelope glycoproteins</td>
<td>• Inadequate sensitivity and specificity for diagnosis of acute primary and secondary infections</td>
<td>• Reduction of antibody cross-reactivity with other flaviruses</td>
</tr>
<tr>
<td>ELISA</td>
<td>• Sensitive method</td>
<td>• Expensive</td>
<td>• Heightened ability to distinguish between serotypes in order to limit secondary infection after treating the first</td>
</tr>
<tr>
<td>Nucleic acid amplification</td>
<td>• Detection of dengue viral RNA in plasma or serum during acute-phase</td>
<td>• Complex and expensive</td>
<td></td>
</tr>
<tr>
<td>Cell culture inoculation</td>
<td>• Gold standard: inoculation in cell cultures or mosquitoes, followed by antigen detection using direct immunofluorescence</td>
<td>• Time consuming</td>
<td></td>
</tr>
</tbody>
</table>

55 UpToDate (2009).
56 World Health Organization
Axela: dotLab System

Diagnostic Type:
• Immunoassay

Purpose:
• Rapid protein detection

Platform:
• Immunoassay with diffractive optics technology (dot)

Description:
• Consumable consists of affinity reagents immobilized to form a diffraction grating (antibodies, antigens, DNA, virus)
• Sample enters through a flow channel, analyte binding shifts the diffraction signal intensity
• Open platform that can be used for assay development and final diagnostic

• Turnaround time of 10 minutes to 2 hours depending on application
• Benchtop reader requiring 100-240V, 50-60HZ, 250 watts electrical power

Key Differentiating Features:
• Picomolar limit of detection
• Label-free or amplified
• Quantitative, measures real-time binding
• 8-plex or greater multiplexing
• Large flow-through volume possible

Innovation:
• Simple optics, ultrasensitive detection, extended dynamic range

Potential Developing World Application:
• Labs
Diagnostics for All: Lab-on-a-Chip

Diagnostic Type:
• Immunoassay

Purpose:
• Inexpensive, equipment-free diagnostics

Platform:
• Lateral flow on paper-based microfluidic chip

Description:
• Fingernail-sized paper chip, which is pretreated with dried reagents that change color when exposed to bodily fluids (single drop of blood, urine, sweat)
• Paper is patterned with hydrophobic polymers forming a series of channels that guide sample throughout chip
• Sample binding resulting in a color change that can then be visually read and translated into a diagnosis
• Proof of concept completed with glucose (mM concentration, 2.5-50 mM clinical range) and protein (BSA, µM concentration .38-7.5 µM clinical range)
• First application will be for liver function tests

Key Differentiating Features:
• Requires no equipment to process and read samples
• No pumps or power sources are required
• Tested with dirt, pollen, and graphite with robust results
• Multiple tests can be performed on one chip
• TAT is within minutes
• Cost target is currently less than $1

Innovation:
• Extremely low-cost paper-based lateral flow

Potential Developing World Application:
• Point of care
InBios, Inc: InBios RDT

Diagnostic Type:
• Immunoassay

Purpose:
• Robust, rapid point-of-care (POC) detection of infectious diseases

Platform:
• ELISA and lateral flow

Description:
• Currently available are lateral flow, rapid diagnostic tests (RDTs) for Chagas (research and ex-US only) and Visceral Leishmaniasis (FDA cleared)
• Lateral flow test using standard antibodies and proprietary antigens
• Described as a RDT with a “different twist,” technology specifics undisclosed
• Currently cleared tests require 20ul sample of serum and 3 drops of buffer
• Results are readable in 10 minutes
• Sensitivity is advertised as greater than 95%

Key Differentiating Features:
• 10-15 minute TAT

Potential Developing World Application:
• Point of care
• Note: Serum is required for some assays. For these assays, centrifuge equipment is required to first spin the samples, limiting POC applications

Iris: NADIA

Diagnostic Type:
• Immunoassay

Purpose:
• Ultra-sensitive immunoassay

Platform:
• Immuno-PCR

Description:
• Nucleic Acid Detection Immunoassay (NADIA)
• Reagents consist of antibody pairs attached to nucleic acid linkers
• Binding of an antibody pair in close proximity enables hybridization and priming for PCR reaction
• Requires a traditional PCR platform
• PSA in serum for recurrence monitoring is the first assay under development
• Proof of concept demonstrated for direct detection of E. coli

Key Differentiating Features:
• Sensitivity superior to core lab immunoassay instruments

Innovation
• Ultra-sensitive protein detection

Potential Developing World Application:
• Labs

OraSure Technologies: OraQuick ADVANCE® Rapid HIV 1/2 Antibody Test

Diagnostic Type:
- Immunoassay

Purpose:
- Rapid HIV screening

Platform:
- Lateral flow immunoassay

Description:
- Highly accurate – greater than 99% agreement with Western Blot
- Rapid – reliable results in 20 minutes
- Flexible – the only test approved by U.S. FDA for oral fluid, whole blood, and plasma. Also has received the CE Mark for sale in the European Union.
- Ideal for both clinical and non-clinical settings.

Key Differentiating Features:
- Needle-free sample collection
- Rapid and easy to use

Innovation
- Detection of HIV from saliva

Potential Developing World Application:
- Point of care

OraSure Technologies: OraQuick ADVANCE® Rapid HCV Antibody Test

Diagnostic Type:
Immunoassay

Purpose:
Rapid HCV screening

Platform:
Lateral flow immunoassay

Description:
Currently in development
Under review for U.S. FDA approval and CE Mark for use in Europe.

Key Differentiating Features:
Needle-free sample collection
Rapid and easy to use

Innovation
Detection of HCV from saliva

Potential Developing World Application:
Point of care
Quanterix: SiMoA (Single Molecule Array™) Technology

Diagnostic Type:
• Immunoassay

Purpose:
• Detection of proteins in blood or other body fluids using minimally invasive, low volume samples

Platform:
• Novel, ultra-sensitive immunoassay technology utilizing an optical system

Description:
• Based on optical fiber arrays and microfluidics to perform ELISAs in very small volumes
• Individual glass fibers are preferentially etched; resulting femtoliter-sized micro-wells capture and retain desired analytes
• Simple detection technology: fluorescent labels and light source imaging combined with software that analyzes the images to determine analyte concentrations based on number of positive wells
• Automated sample handling system
• Time to result is currently several hours; the company is developing a consumable that will enable a 30-60 minute turn-around time
• The instrument costs in the low thousands of dollars, and consumables are roughly 10 cents each

Key Differentiating Features:
• Minimal sample volumes of 1-2 ml
• Measures analytes in urine, saliva, and other body fluids obtained through noninvasive measures
• Measures multiple analytes simultaneously

Potential Developing World Application:
• Labs

Seventh Sense Biosystems: Switchable Materials Platform

Diagnostic Type:
• Immunoassay

Purpose:
• “Ultimate” point-of-care diagnostic – ability to diagnose and monitor with diagnostics that are applied to the skin (“on-skin”) or that are imprinted into the skin (“in-skin”)

Platform:
• Bioresponsive switchable materials
• Fluid access technologies

Description:
• Biphasic particles consist of distinct hemispheres
• Each phase is differentially loaded with material and independently surface-functionalized
• Capture groups that bind analyte of interest are loaded onto one side
• Binding of analyte causes orientation of particles and color change
• Fluid access technologies for painless access to blood or interstitial fluid

Key Differentiating Features:
• Diagnosis through skin patches or imprints
• Equipment-free
• No sample preparation required

Innovation
• “On-skin” or “in-skin” diagnostics

Potential Developing World Application:
• Point of care
Vivacta: Near-Patient Rapid Diagnostics

Diagnostic Type:
• Immunoassay

Purpose:
• Near-patient, rapid immunoassays with no sample preparation and minimal sample volume

Platform:
• Kinetic assay with piezofilm detection system

Description:
• 30ul of blood is applied to a disposable cartridge and inserted into reader
• Pumps mix the sample into dried reagents consisting of specific antibodies bound to carbon-based labels
• Target analytes bind their specific antibodies and are diverted into wells where they are simultaneously bound by capture antibodies on the film surface, forming a sandwich
• Dual binding of the analyte brings more and more carbon labels into close proximity with the film surface over time
• LED excitation; label absorbs light and converts it to heat, thermal perturbation to the piezofilm causes a voltage proportional to the amount of label bound, and the reader quantifies the signal and calculates the analyte concentration

• Internal positive, negative, and calibration controls are included
• Results displayed on a touch-screen
• TSH for thyroid function is first assay in development; infectious disease applications considered

Key Differentiating Features:
• Requires no sample prep or fluorescent labels
• Low sample volume: 30 ul
• Analyte detection below the limit of detection of ELISA
• Quantitative results
• Rapid turn-around time of 10 minutes or less
• Runs on 25V electrical power, can also be run on rechargeable batteries
• Does not need running water or temperature control

Innovation
• Simple optics, ultrasensitive rapid immunoassays

Potential Developing World Application:
• Labs
Claros Diagnostics: Benchtop and Handheld Analyzer

Diagnostic Type:
- Immunoassay

Purpose:
- Point-of-care diagnostics suitable for use anywhere in the world

Platform:
- Microfluidics

Description:
- Fingerstick of whole blood is inserted onto credit card sized disposable cartridge
- Multiple LEDs of different colors monitor both endpoints and process steps
- Quantitative results available in 15 minutes
- The target cost point is $100 per device
- Claros is working to develop a panel for maternal health, combining HIV, syphilis and anemia testing in a single disposable

Key Differentiating Features:
- Proprietary method injection molding that meets exacting specifications of a microfluidic device
- Inorganic redox chemistry detection to deal with high heat/humidity
- "Open source" – proprietary but available to other developers
- Quality assurance and control built into the system
- Low sample volume; no sample prep – fingerstick of whole blood
- Rapid - time to result is 15 minutes

Innovation
- If cost target is achieved, will be a cost-effective and robust microfluidics platform

Potential Developing World Application:
- Labs, clinics (benchtop analyzer)
- Point of care (handheld analyzer)
**Abaxis: Piccolo® xpress**

**Diagnostic Type:**
- Chemistry

**Purpose:**
- Health screening and treatment monitoring

**Platform:**
- Enzymatic reagent discs and spectrophotometer/centrifuge

**Description:**
- 100uL of whole blood, serum, or plasma is collected and transferred to a self-contained, single use reagent disc that contains all reagents and diluents necessary to perform a complete, fixed multi-test panel
- Following touch-screen commands, user places disc into the analyzer, enters requested information
- Instrument produces results, including patient demographics, chemistry absorptions, reference ranges, sample integrity indices, and iQC on the display, as well as a print-out
- Up to 5,000 patient records can be stored in memory
- Uses a series of concentric channels in a spinning disposable to reflect chemical reactions typical of a large chemistry analyzer
- Originally designed for NASA, currently used routinely by military, physicians' offices, clinics, and small hospitals
- 3 simple steps, results in about 12 minutes

**Key Differentiating Features:**
- Largest test menu of any single point-of-care analyzer
- 11 of 13 available panels are CLIA waived
- Fast, lab-accurate results
- Multi-functional platform allows for future development of immunoassay, hematology and specialty tests

**Potential Developing World Application:**
- Labs, clinics

---

**Nanosphere: Verigene® and Verigene SP®**

**Diagnostic Type:**
- Immunoassay or molecular

**Purpose:**
- Benchtop workstation for rapid nucleic acid detection

**Platform:**
- DNA barcode-based molecular or immunoassay

**Description:**
- The Verigene system has three parts:
  - Reader tracks, monitors, and reports
  - Processor completes random access processing of four cartridges simultaneously
  - Each cartridge is a sample-ready consumable containing prepackaged reagents and array/solid substrate for target analysis
  - Extract genomic DNA, add to cartridge (shears to 300-500bp fragment), target fragment binds to probe and nanoparticle-bound barcode simultaneously, silver deposition amplifies signal
  - Turn-around time is less than 3 hours after sample preparation. The Verigene SP (not launched yet) will remove sample prep and combine the reader and the processor in one small benchtop machine, allowing for direct placement of flexible sample types in to the system; turn-around time less than 2 hours
  - Molecular tests in development: infectious disease, SNPs for genetic testing (cystic fibrosis), metabolic disorders and pharmacogenetics (warfarin)
  - Protein tests: troponin, PSA (recurrence), RA antibodies

**Key Differentiating Features:**
- Ultrasensitive detection (2-3 times more sensitive than ELISA)
- Robust cartridge/disposable
- Multiplexable

**Potential Developing World Application:**
- Labs
University of Washington, PATH, Epoch Biosciences, Micronics: DxBox

Diagnostic Type:
• Immunoassay or molecular

Purpose:
• Point-of-care detection with automated sample preparation

Platform:
• Microfluidics-based immunoassay and PCR

Description:
• Fingerstick blood sample diverted for immunoassay or PCR using on-cartridge microfluidic pumps/valves
• Can detect DNA/RNA in whole blood
• Self-contained sample processing
• Portable, battery-powered reader
• Throughput is one cartridge every 30 minutes
• Fever panel in development; assays for malaria, measles, dengue, typhoid, and Rickettsia have been verified with comparable performance to commercially available kits

Key Differentiating Features:
• Eliminates sample pre-processing
• 30 minutes sample to result

Innovation
• Rapid dual immunoassay and molecular platform designed for the field

Potential Developing World Application:
• Point of care

---

BioHelix: IsoAmp®

Diagnostic Type:
• Molecular

Purpose:
• Rapid, easy-to-use, nucleic acid detection

Platform:
• Isothermal, Helicase Dependent Amplification (HDA) technology and rapid test read-out

Description:
• Following sample prep for DNA extraction, HDA uses a helicase enzyme to unwind double-stranded DNA; exposure of the single-stranded target region allows primers to anneal
• DNA polymerase extends the 3’ ends of each primer using DNTPs to produce replicates, which independently enter the next cycle of HDA amplification
• The HDA reaction volume is between 10 µl and 100 µl, most commonly 50 µl per reaction tube. Between 1 to 10 µl sample is added per 50 µl reaction
• The HDA based nucleic acid test can be performed without external power and does not require running water; HDA amplification requires incubation temperature around 65°C which can be achieved by using heat packs
Cepheid: GeneXpert

Diagnostic Type:
• Molecular

Purpose:
• Rapid, easy-to-use, molecular diagnostics

Platform:
• RT-PCR (Real time PCR, not limited to Reverse Transcriptase PCR)

Description:
• Automated from sample prep through interpretation
• Ultrasonic lysis (integrated into cartridge) enables processing of difficult samples (e.g. sputum) and targets (bacterial spores, TB)
• Cartridges handle a range of sample volumes to obtain higher concentration of target
• Enclosed cartridge and fluid handling enables nested PCR, increasing sensitivity
• Can detect multiple target sequences in a single cartridge (6 colors for assays and controls)
• Does not require water supply
• Requires 110 or 220V electrical power; in theory could be powered by a car battery
• Assay shelf life is around 1 year at room temp, higher temperature for TB assay
• Turn-around time is between 30 min and 1 hour (depending on test)
• Tests available for MRSA/MSSA, C. difficile, Group B Strep, enterovirus, TB, BCR-ABL, Factor II/V

Key Differentiating Features:
• Isothermal amplification
• Low sample volume of 1-10 µl

Potential Developing World Application:
• Labs

• Following amplification, rapid detection by vertical flow occurs with an instrument-free disposable BioHelix Express Strip (or BESt™) cassette
• Kits are available for rapid staph and MRSA detection, for research only
• In development for C dif, HIV, Herpes, CT/NG, Factor V Leiden
• Turn-around time is 2 hours for factor V Leiden and MRSA
• The lyophilized HDA reagents are stable at room temperature for 12 months

Key Differentiating Features:
• Isothermal amplification
• Low sample volume of 1-10 µl

Potential Developing World Application:
• Labs

Innovation
• Fully automated and integrated nucleic acid extraction, amplification, and detection

Potential Developing World Application:
• Labs
• Microscopy centers
Gen-Probe: Tigris, Panther, CUDA (now owned by Roka Biosciences)

Diagnostic Type:
- Molecular

Purpose:
- Automated nucleic acid detection with minimal sample preparation

Platform:
- Direct tube sampling and nucleic acid amplification by TMA (transcription-mediated amplification)

Description:
- Tube-based nucleic acid detection: all steps of sample prep occur through detection
- RNA or DNA amplification 10⁹-fold in 15 to 30 minutes
- TIGRIS: high throughput (up to 1600 results in 8 hours)
- Panther: low- to mid-throughput (400+ results in 8 hours)
- CUDA (closed unit dose assay):
  - Handheld, 9 pounds, on-board sample processing for urine, plasma, swab; blood would require more processing
  - Disposable pouch ($5-7 cost of goods sold) where everything happens inside
  - Isothermal amplification and fluorescent chemistry with real-time reporting
  - First developed for water testing
- TIGRIS and Panther assays: CT, GC, GBS, Group A strep, HIV viral load using dried blood spots on TIGRIS

Key Differentiating Features:
- Nucleic acid detection with no sample prep
- Working on capability to use sputum samples

Innovation
- High-throughput sample to solution

Potential Developing World Application:
- Labs (TIGRIS and Panther)
- Point of care (CUDA)
Becton Dickinson (formerly Handylab): Jaguar, Lynx, Raider

Diagnostic Type:
• Molecular

Purpose:
• Rapid, automated, nucleic acid detection and quantification with minimal sample preparation

Platform:
• Microfluidic RT-PCR

Description:
• Jaguar: automated extraction and RT-PCR
• Lynx: extraction only
• Raider: RT-PCR only, desk-top sized instrument
• 4-5uL sample volume
• 45 cycles in 15 minutes
• Reagents stable at room temperature
• Detection by fluorescence

Key Differentiating Features:
• Reagents for Jaguar and Raider do not require refrigeration
• Multiple sample formats allowed on Jaguar

Innovation
• One of the first benchtop sample-to-solution molecular technologies
• Becton Dickinson has exclusive agreement to commercialize GeneOhm assays on Jaguar (to be called BD MAX platform)

Potential Developing World Application:
• Labs

Idaho Technologies: FilmArray™ Instrument

Diagnostic Type:
• Molecular

Purpose:
• Viral and bacterial pathogen detection

Platform:
• Automated, RT-PCR, PCR and detection

Description:
• Inject water and unprocessed sample into disposable pouch ($130 list price)
• Insert pouch into FilmArray instrument
• Simple loading: no pipetting or centrifugation
• Everything happens in the pouch: instrument extracts/purifies RNA/DNA, performs RT-PCR, multiplexed PCR (up to 120 tests/sample), dilution, singleplexed PCR, and then reports results for each targets
• Reagents are freeze-dried
• Current indication: respiratory panel (17 viral, 4 bacterial), for research only
• FDA clearance trials began in Q4 2009

Key Differentiating Features:
• Turnaround <1 hour
• Flexible sample type
• Multiplexable
• Sample to solution

Potential Developing World Application:
• Labs

Reprinted with permission from Gen-Probe, Inc., 2010.
Ionian Technologies: NEAR Assay

**Diagnostic Type:**
- Molecular

**Purpose:**
- Rapid, easy-to-use, nucleic acid detection

**Platform:**
- Isothermal nucleic acid amplification and detection

**Description:**
- Nucleic acid amplification combined with fluorescent of lateral flow read-out
- Commercially available polymerases amplifies short oligonucleotides (8-16 nt)
- Reiterative steps of DNA polymerase extension and the activity of a nicking enzyme produce rapid exponential amplification under isothermal conditions

**Key Differentiating Features:**
- Ultra-rapid nucleic acid detection – $10^{12}$ fold amplification in less than 5 minutes
- Stabilized reagents
- Lateral flow readout

**Innovation**
- Extremely rapid nucleic acid detection (does not require DNA extraction)

**Potential Developing World Application:**
- Labs

---

Iquum: Liat™ Analyzer

**Diagnostic Type:**
- Molecular

**Purpose:**
- Automated nucleic acid testing (NAT) and detection

**Platform:**
- Single step NAT, lab-in-a-tube

**Description:**
- A flexible tube is a sample vessel containing all assay reagents pre-packed in segments separated by peelable seals; actuators in the analyzer compress the tube to selectively release reagents from the tube segments, moving sample from one segment to another and controlling reaction conditions
- The small, portable device performs reagent preparation, target enrichment (magnetic beads), inhibitor removal (capture/wash), nucleic acid extraction, amplification, and detection
- Steps: collect raw sample (whole blood, plasma, urine, swabs) into a tube, scan barcode, and insert tube in analyzer
- Quantitative results on-screen: turn-around time is less than 1 hour
- The Liat FlowCycler™ is another available machine that has the capability to run PCR on multiple samples simultaneously

**Key Differentiating Features:**
- Sample to result in less than 1 hour
- Completely closed system prevents contamination
- Can accommodate several assays and samples

**Innovation**
- Single step nucleic acid testing

**Potential Developing World Application:**
- Labs
**ImmunoSite Technologies: CD4 Counter**

**Diagnostic Type:**
- Cell-based

**Purpose:**
- Simple, affordable point-of-care test for monitoring CD4 lymphocytes in patients with HIV/AIDS

**Platform:**
- N/A

**Description:**
- Binary measurement: e.g., less than 350 cells or greater than 250 cells
- Finger prick blood draw and reading of measurement through simple lines
- Part of the CD4 initiative funded by the Imperial College: Developed by the team from Beckman Coulter (now ImmunoSite Technologies, LLC)
- Sybil D’Costa, Ph.D., V.P. Research and Development, is the principal investigator, grant awarded in 2007

**Key Differentiating Features:**
- Elevated temperature - stable controls
- No outside power necessary
- No instrumentation, visual readout

**Potential Developing World Application:**
- Point of care

---

**Cellabs/Special Phage Services: Phage Diagnostics**

**Diagnostic Type:**
- Cell-based

**Purpose:**
- Detection of bacterial infections

**Platform:**
- Bacteriophage-based lateral flow technology

**Description:**
- Bacteriophage cocktail used to identify pathogen of interest

**Key Differentiating Features:**
- Short turnaround time
- Ability to use virtually any bodily excretion as a sample, including blood, stool, urine
- Highly specific: a couple of logs greater sensitivity than anything available except PCR
- Visual detection

**Potential Developing World Application:**
- Labs
Daktari: Handheld CD4 Cell Counter

Diagnostic Type:
• Cell-based

Purpose:
• CD4 count for HIV patient monitoring

Platform:
• Microfluidic cell chromatography

Description:
• Finger prick of blood (10ul) is added to a miniature chamber coated with anti-CD4 antibodies
• Rapid fluid flow elutes un-bound cells
• Electrochemical sensing using MEMS measures resistance, determines CD4 count
• $600 cost of goods sold for the instrument (which the company plans to give away); plastic assay cards sold at under $10 at low volume and under $5 at high volume
• Clinical trials to start in mid-2010
• Tuberculosis, HIV viral load tests in development
• Optimized for clinically relevant CD4 range of 80 to 1000. Reduced sensitivity below 80 enables lower cost instrument for developing world.

Key Differentiating Features:
• Turn-around time is 6 minutes
• Label-free
• Low sample volume
• Entirely automated system, operates like a glucose meter
• Not reliant upon optics

Innovation:
• Handheld, rapid CD4 count using stabilized reagents

Potential Developing World Application:
• Point of care (community health worker, villages, homes)

MicroPhage: Phage Diagnostics

Diagnostic Type:
• Cell-based

Purpose:
• Detection of bacterial infections and drug susceptibility in unprocessed samples

Platform:
• Phage amplification combined with lateral flow for detecting pathogens

Description:
• Sample (blood or nasal swab) is added to amplification broth containing a cocktail of pathogen-specific bacteriophage
• Bacteriophage infect the target pathogen, replicate, and lyse cells releasing thousands of progeny phage
• A bacteriophage-specific marker is detected using an equipment-free lateral flow test (10 minutes to result)
• For susceptibility testing, the process is performed in parallel in the presence of an antibiotic; resistant bacteria are able to grow and support phage amplification (giving a positive result on lateral flow), while susceptible bacteria do not (giving a negative result)
• Current turn-around time is 5-10 hours (10 hours for bacteria that take longer to grow or for resistant bacteria differentiation)
• Bacteriophage cocktail discovery for any bacterial pathogen believed to be a 1 to 2 year process, including for TB
• Initial indication in development is MRSA

Key Differentiating Features:
• Requires no equipment or sample preparation
• Likely to accommodate wide range of sample types

Innovation:
• Rapid identification and susceptibility (faster than culture)

Potential Developing World Application:
• Labs
**BioScale: ViBE Bioanalyzer and ViBE Workstation – Acoustic Membrane and Microparticle Platform**

**Diagnostic Type:**
- Emerging technology

**Purpose:**
- Quantitative, rapid, sensitive diagnostics for multiple analytes in complex samples

**Platform:**
- AMMP - Acoustic membrane and microparticle platform based on MEMS (microelectronic mechanical systems); built on premise that there is a membrane that is shaken, the oscillation frequency is measured and changes whenever mass is added or taken away.

**Description:**
- Fully automated 96-well plate assay stations (ViBE Workstation) for high throughput, or 8-sample manual system (ViBE Bioanalyzer)
- Three part system: 8-sensor MEMS chip, disposable microfluidic cartridge, magnetic microparticles
- Magnetic microparticles with antibody mix with sample and bind to analyte. Mixture flows over surface to engage magnet. Magnet pulls down complexes, which in turns changes the frequency.
- When the magnet is removed, only biologically bound beads remain attached to the sensor; beads without the analyte are washed away, causing another change in frequency; detection of frequency change vs. analyte concentration results in a dose response curve
- Potential analytes, some of which need minimal sample prep: whole cell, proteins, small molecules, viruses, bacteria, nucleic acids
- Currently requires standard power (100 V, 15 A) but the company has done research on battery powered devices
- Kept at 4°C, reagents are stable for 6 to 12 months. Reagents are stable for several weeks at room temperature, though further validation is required.

**Key Differentiating Features:**
- ELISA-like sensitivity with minimal sample time (10 minutes to 2 hours)
- Label-free detection
- Broad sample types with minimal to no prep

**Innovation:**
- Core lab sensitivity with rapid turn-around time and label-free detection

**Potential Developing World Application:**
- Labs
Draper Laboratory: Differential Ion Mobility Spectrometer

Diagnostic Type:
• Emerging technology

Purpose:
• Diagnosis through detection of pathogen or disease-related metabolites

Platform:
• Gas phase ion separation (differential mobility spectrometry)

Description:
• Disease diagnosis through quick analysis of clinical cultures, working towards patient breath analysis
• Micromachined differential mobility spectrometer, described as “mass spec, without machinery”
• Applied pattern discovery/recognition algorithms to analyze volatile metabolic compound signature generated by DMS to reliably discern multiple species of bacteria in vitro

Key Differentiating Features:
• Detection of metabolites in sputum (70-90% sensitivity)
• Inexpensive consumable ($2-4) and instrument ($1000-2000) costs
• Easy to use, wide range of samples
• No need for ambient temperature control

Innovation:
• Indirect detection of pathogen

Potential Developing World Application:
• Labs

T2 Biosystems: NanoDx

Diagnostic Type:
• Emerging technology

Purpose:
• Diagnostics without sample preparation

Platform:
• Use of magnetic resonance with nanotechnology

Description:
• Superparamagnetic iron oxide metal core is surrounded by dextran polymer layer with analyte-specific binding agents (nanoparticles)
• Presence of analytes results in change of characteristic signal, which is measured by MR detectors. The particles move into specific clusters, changing the T2 of the solution
• Sample is added to a cartridge and inserted in bench-top reader
• Cartridge has a 1 to 2 year shelf life, costs less than $1, and the reagents are dryable, removing need for refrigeration
• Analytes: protein, nucleic acid small molecules, virus, bacteria
• Samples: unprocessed whole blood, urine, water, saliva, nasal swabs in solution
Sierra Molecular’s AssayAssure®

Diagnostic Type:
• Sample preservation

Purpose:
• Stabilization of samples in poor environmental conditions

Platform:
• Sample stabilization chemistries

Description:
• Chemistries preserve cells, cell surface markers, intracellular proteins, and nucleic acids in a single tube for up to seven days without refrigeration.
• Sample stabilization chemistries for whole blood, genital tract swabs, and urine
• Stabilizes bacterial and viral targets as well as intracellular RNA.
• Because chemistries appear to stabilize cells and detoxify sample matrices rather than relying on lysis and precipitation, one sample can be used for multiple tests (e.g., molecular, flow cytometry, culture)

Key Differentiating Features:
• No need for sample manipulation, refrigeration, mixing, or handling
• Compatible with all molecular assay platforms

Innovation:
• Miniaturized MRI technology
• Point-of-care test expected in about 18 months

Potential Developing World Application:
• Labs
• Point of care

Key Differentiating Features:
• Label-free
• Non-optical
• Very robust: DOD has been interested due to “rugged/robust” nature of technology

Innovation:
• Miniaturized MRI technology
• Point-of-care test expected in about 18 months

Potential Developing World Application:
• Labs
• Point of care
APPENDIX IV

References


Acknowledgments

The Diagnostics Innovation Map: Medical Diagnostics for the Unmet Needs of the Developing World

Copyright © 2010 BIO Ventures for Global Health

All rights reserved

This report was written by Priya Mehta and David Cook, with the support of Health Advances, LLC.

Authors’ note:

We thank the Bill & Melinda Gates Foundation for its financial support. We are grateful to the team at Health Advances: Kristin Pothier, Sonia Gupta, Paula Ness Speers, Phalina Lee, Kimberly Howland, Mark Speers, and Donna Hochberg for their hard work and significant contributions to this document. We thank Christopher D. Earl for his continued contributions and valuable feedback. We also thank our Scientific Advisory Board for their feedback and support: N. Leigh Anderson, Deborah Burgess, David Kelso, Alan Magill, Francis Moussey, Rosanna Peeling, Mark Perkins, Mark Reynolds, William Rodriguez, Samuel Sta, John J. Sminsky, Amy Worng, and Paul Yager. We thank all those interviewed during the course of this project for their time and effort to ensure the accuracy of the report.


A young lady waits to be seen at a clinic in rural Liberia, West Africa.