Requirements for high impact diagnostics in the developing world

Authors: **Mickey Urdea[1], Laura A. Penny[1], Stuart S. Olmsted[2], Maria Y. Giovanni[3], Peter Kaspar[4], Andrew Shepherd[5], Penny Wilson[6], Carol A. Dahl[7], Steven Buchsbaum[7], Gerry Moeller[8] & Deborah C. Hay Burgess[7]**

Author Affiliations: [1]Halteres Associates, 1900 Powell Street, Suite 1050, Emeryville, California 94608 USA
[2]RAND Corporation, 4570 Fifth Avenue, Suite 600, Pittsburgh, Pennsylvania 15213, USA
[3]National Institutes of Health, 6610 Rockledge Drive, Room 6007, Bethesda, Maryland 20892, USA
[4]Biomerieux, 5 Rue des Berges, 38024 Grenoble Cedex 1, France
[5]Omega Diagnostics Group PLC, Hillfoots Business Village, Alva, Clackmannanshire FK12 5DQ Scotland, UK
[6]Consultant to the Biotechnology, Life Science & Healthcare Industries, 4 Hawarden Road, Altrincham, Cheshire WA14 1NG, UK
[7]Bill & Melinda Gates Foundation, PO Box 23350, Seattle, Washington 98102, USA
[8]FIND Diagnostics, Scheffelstr 20, Heidelberg 69120 8, Germany

PREFACE

Infectious diseases continue to devastate the developing world. One crucial factor is the lack of diagnostic tests that can be performed at low-infrastructure sites, which serve most of the global population. Without these diagnostics, health-care workers do not know who should be treated and, just as importantly, who should not be treated. A detailed understanding of the resource limitations in testing locations will have a great impact on the design of diagnostic tests — sometimes in surprising ways.

INTRODUCTION

Despite recent advances in the availability of powerful drugs, infectious diseases that are largely treatable continue to take a massive toll on the populations of developing countries. Many previously published analyses have provided estimates of the burden posed by specific diseases. For instance, recent estimates reported by the World Health Organization (WHO) of the proportion of childhood deaths attributed to pneumonia were 21% in the Africa region, 21% in the eastern Mediterranean region and 19% in the southeast Asia region[1]. The evidence indicates that respiratory disease is one of the leading causes of infant death in all countries where there is still significant childhood mortality[1,2]. Other estimates indicate that between 1 million and 3 million deaths, and between 500 million and 5 billion episodes of clinical illness are caused by malaria, along with an enormous morbidity burden that is more challenging to calculate[3,4]. Despite advances in understanding malaria and the development of interventions, >50% of the global population, or ~3 billion people, remain exposed[4]. Several studies in Africa, which bears most of the burden of malaria, have indicated that in some regions, for every case of fever-related (febrile) illness that is seen in a health-care facility, another four or five remain untreated in outlying communities[3]. In many countries in sub-Saharan Africa, up to 20% of pregnant women are infected with human immunodeficiency virus (HIV)[5]. Without intervention, >25% of infants born to infected women will acquire HIV infection during the first year of life[5]. In 2005, an estimated 2.3 million children worldwide were living with HIV/acquired immunodeficiency syndrome (AIDS), 2 million of whom were in sub-Saharan Africa[6]. In the developing world, syphilis remains a significant health concern during pregnancy; its prevalence among pregnant women attending antenatal centres in Africa ranges from 3 to 18%[7]. Infections of *Neisseria gonorrhoeae* and *Chlamydia trachomatis*, which are the causative bacteria of gonorrhoea and chlamydia, respectively, remain significant health concerns. In Africa, the prevalence rates for these infections are up to 66% in high-risk populations (for example, commercial sex workers) and up to 40% even in low-risk populations[8]. Estimates of the impact of diarrhoeal diseases around the world range from 1 billion to 4 billion diarrhoea episodes every year among children aged <5 years in developing countries, causing ~2.5 million deaths, of which ~85% occur in the poorest parts of the world[9,10]. In some countries, diarrhoeal diseases account for >20% of all deaths in children aged <5 years; this is particularly disturbing given that, in the more economically developed world, diarrhoea is associated with <1% of deaths in young children[9].

Although this tremendous burden is often measured by the number of deaths, other more recent work has aimed to calculate the morbidity burden in the form of disability-adjusted life years (DALYs). This measure is an index of lost years of life, as well as the decreased quality of life for those who chronically suffer with the diseases. The infectious disease areas discussed in this supplement account for ~325 million DALYs per year, which is a staggering number[11].

In order to take advantage of the treatment options that are available today, it is essential to identify those individuals who require treatment. It is imperative not only to administer drugs for the appropriate diseases to those who need them, but also to prevent overtreatment that will eventually result in the predominance of resistant microorganisms, which is becoming

a major threat. For instance, the rapid acquisition of resistance in the malaria-causative organism *Plasmodium falciparum* has been partly due to overuse of the anti-malarial drug chloroquine, and has driven a shift towards a new and more expensive class of anti-malaria compounds derived from artemisinin. Similarly, it is typical for children examined in a health-care facility who have symptoms indicative of an acute respiratory infection (ARI) to be offered antimicrobials without any investigation into the aetiology of the condition. Because the proportion of children with viral acute lower respiratory infection (ALRI) can be greater than the proportion with bacterial ALRI, many infants who do not need, and will not benefit from, treatment are still receiving antibiotics[12]. Improved identification of the individuals who need to be treated, and distinguishing them from other individuals with non-specific symptoms who do not require treatment, could extend the lifespan of currently effective medicines in the treatment formulary. Another consideration in resource-limited settings is that the use of a treatment with a limited supply by individuals who do not need it might result in it being unavailable to those who do.

In 2004, the Global Health Diagnostics Forum of the Bill & Melinda Gates Foundation began to study the potential for diagnostic tests to save lives in the developing world. The other papers in this supplement present the work of forum members in developing mathematical models for the six disease areas that were selected for analysis (ALRIs, HIV/AIDS, diarrhoeal diseases, malaria, tuberculosis (TB) and sexually transmitted infections) and the specific clinical decisions (intervention points) at which diagnostic products are most urgently needed. An important output of these analyses was the quantification of the DALYs or lives saved that could result from specific diagnostic tests and their accompanying therapies being broadly available. The conclusions for the six disease areas are presented in Table 1.

Increased access to the diagnostic products that could achieve these results will require the development and deployment of tests that can be used in remote resource-limited settings by persons with little or no laboratory training. We consider the specific issues associated with the required tests below. Several barriers currently limit access to the diagnostic products that are needed. For some diseases and intervention points, extraordinary technical hurdles remain to be overcome if broad access is to be provided. Despite these challenges, many organizations have already made great strides towards overcoming these obstacles during the past few years.

The WHO has developed a list of general characteristics that make a diagnostic test appropriate for resource-limited sites, which are abbreviated using the acronym ASSURED (Box 1). These criteria have helped to frame

Box 1 | Characteristics of the ideal diagnostic test for the developing world: ASSURED

- **A**ffordable by those at risk of infection.
- **S**ensitive (few false-negative results).
- **S**pecific (few false-positive results).
- **U**ser-friendly (simple to perform by persons with little training).
- **R**apid treatment at the first visit and robust use without the need for special storage.
- **E**quipment-free (that is, no large electricity-dependent instruments needed to perform the test; note that portable handheld battery-operated devices are acceptable, which differs from the criterion of the original authors).
- **D**elivered to those who need it

Table 1 | Potential impact of improved access to diagnostics

Infectious disease area	Clinical decision points	Potential DALYs or lives saved per year
ALRI	Identification of children aged <5 years with bacterial ALRI among those presenting with ARI for antibiotic treatment or in severe cases for hospitalization	A new diagnostic test for bacterial ALRI with at least 95% sensitivity and 85% specificity accompanied by greater treatment access and minimal laboratory infrastructure requirements could save ≥405,000 adjusted lives. A new diagnostic for severe ALRI would also bring significant benefit provided access to effective hospital care is increased globally.
HIV/AIDS	Identification of HIV infection in infants aged <12 months	A test with 90% sensitivity, 90% specificity and minimal laboratory infrastructure requirements could save ~ 180,000 DALYs if 5% of the targeted population has access to ART, and ~2.5 million DALYs could be saved if 100% of the population has access to ART.
Diarrhoeal diseases	The detection of *G. lamblia, C. parvum* and enteroaggregative *E. coli* to reduce diarrhoea-related stunting in children	A test with 90% sensitivity, 90% specificity and minimal laboratory infrastructure requirements for each of the pathogens *G. lamblia, C. parvum* and enteroaggregative *E. coli* could reduce the prevalence of stunting by 12.5% and save 2.8 million DALYs. This result assumes that the cost of treatment is US$6 and the positive externalities associated with treatment are equal to 0.25 DALYs.
Malaria	Diagnosis in febrile children aged <5 years in sub-Saharan Africa	A test with 95% sensitivity, 95% specificity and minimal laboratory infrastructure requirements could save ~1.8 million adjusted lives and prevent 396 million unnecessary treatments per year. A new test with no infrastructure requirements and 90% sensitivity and specificity would save ~2.2 million adjusted lives and prevent ~447 million unnecessary treatments per year.
TB	Diagnosis of active infections in symptomatic individuals, with or without concomitant HIV infection	A rapid diagnostic requiring no laboratory infrastructure, with at least 85% sensitivity for smear-positive and smear-negative cases, and 97% specificity, could save ~400,000 lives annually.
Sexually transmitted infections	Syphilis screening of antenatal women	A new diagnostic test that is at least 86% sensitive, 72% specific, requires minimal laboratory infrastructure and has either a 100% rate of return for test results or a 100% treatment rate could save ≥138,000 adjusted lives and avert >148,000 stillbirths. A similar test requiring no laboratory infrastructure could save >201,000 adjusted lives and avert 215,000 stillbirths.
Sexually transmitted infections	Gonorhoea and chlamydia screening and diagnosis in female CSWs	A new diagnostic with 85% sensitivity and 90% specificity for both gonorrhoea and chlamydia that requires minimal laboratory infrastructure could save ~3 million DALYs, avert >12 million incident gonorrhoea and chlamydia infections, and prevent >161,000 HIV infections among female CSWs in sub-Saharan Africa, China and southeast Asia. A test that requires no laboratory infrastructure could save ~4 million DALYs, avert >16.5 million incident gonorrhoea and chlamydia infections, and prevent >212,000 HIV infections.

AIDS, acquired immunodeficiency syndrome; ALRI, acute lower respiratory infection; ARI, acute respiratory infection; ART, anti-retroviral therapy; *C. parvum, Cryptosporidium parvum*; CSWs, commercial sex workers; DALYs, disability-adjusted life years; *E. coli, Escherichia coli; G. lamblia, Giardia lamblia*; HIV, human immunodeficiency virus; TB, tuberculosis.

the issues considered in the current paper; however, as discussed below, we believe that there are technical options outside the scope of these guidelines that are worth pursuing. In this paper, we primarily focus on the technical criteria, rather than the cost and distribution issues.

METHODS & DISCUSSION

Assessment of infrastructure at testing sites

We developed a list of the resources and capabilities for three levels of laboratory infrastructure that are often found at diagnostic-testing sites in the developing world[13] (Table 2). The delineation of these resource levels was employed to develop the user requirements presented below. These user requirements are the minimum needs that must be met for a diagnostic test to be successfully implemented and to provide adequate performance. It will be important for test developers to consider the constraints imposed by the available infrastructure. This knowledge will help to drive the selection of acceptable specimen types and biomarkers, as well as the technology innovations required to create a diagnostic product that is useful in resource-limited settings.

The summary of health-care settings and capabilities presented in Table 2 was derived from several sources, including the WHO Service Availability Mapping (SAM) reports (http://www.who.int/health_mapping/about/services_SAM/en/index.html), the Service Provision Assessment (SPA) data produced as part of the Demographic and Health Surveys (DHS) project (http://www.measuredhs.com) and interviews with forum members. After analysing many of the countries in the developing world, three common clusters of resources and capabilities were identified, and were used to define three categories of infrastructure for testing sites: no laboratory infrastructure, minimal laboratory infrastructure and moderate-to-advanced laboratory infrastructure. Because our intention was to focus on the potential impact of diagnostic tests that could be used in the broadest range of settings, and therefore provide the greatest possible access, we focused our analysis on only the lowest two levels of laboratory infrastructure (Fig. 1a,b).

Settings with no laboratory infrastructure offer the greatest technical challenges for test developers. The onus is on the developer to create a test format that is both feasible and provides adequate performance, because these settings have few of the amenities found in the more-established urban diagnostic laboratories. The lack of clean water, dependable sources of electricity and cold storage (or shipment) facilities greatly limits the use of existing tests that are widely employed in the developed world. Purified water, which is necessary for most tests, will have to be supplied in some fashion. If electricity is required, it will have to be supplied in the form of batteries, preferably solar, as the distribution or recharging of expensive solid fuel batteries is not practical. The lack of cold storage presents at least two issues. First, most patient samples will have to be analysed immediately. Second, reagents must be extraordinarily stable to fluctuating temperature and other environmental challenges. As many parts of the developing world are in perpetually hot locations, the lack of laboratory temperature control poses a vexing problem. Tests are often run at ambient temperature, which can be much higher than the 'room temperature' conditions under which the performance and stability were evaluated by the manufacturer, and is often too high for the diagnostic to perform properly. For instance, the popular nucleic-acid detection method polymerase chain reaction (PCR) has a temperature-cycling process that requires repeated switching from low to high temperatures. Developers will have to be careful to ensure that the low temperature of the cycle is greater than the highest potential ambient temperature to be encountered. This has a direct impact on the design of assay components (for example, oligonucleotide primers needed for PCR). The alternative is to include a cooling capability in the PCR device, which could greatly increase manufacturing costs.

In settings with no laboratory infrastructure, which do not have trained personnel, the technical processing of a diagnostic test must be simple — that is, of comparable complexity to a home pregnancy test. Typically, the more complicated the currently-used laboratory procedure, the more difficult and costly it will be to develop an easy-to-use and inexpensive version for a resource-limited site. Immunoassays that are extremely simple to perform have become more common at resource-limited sites, particularly in the form of lateral flow immunochromatographic devices. Inexpensive assays that can be used to detect nucleic acids are not yet available in formats that can be performed at sites with no laboratory infrastructure, although some can be successfully performed in settings with minimal facilities.

User requirements for specific clinical decisions and their implications

Table 3 summarizes the user requirements developed by the disease and technology working groups of the forum (see the accompanying papers in this supplement for further details). Each group selected the level of infrastructure that test developers should strive to accommodate, based on the collective clinical judgment of members and using mathematical modelling approaches to estimate the potential health impact of improving access to a diagnostic. In some instances, significant improvement in outcomes would be obtained by deploying tests at sites with both minimal and no laboratory infrastructure. For instance, in the case of syphilis, the analysis indicated that a test that required a minimal laboratory infrastructure would provide a level of access that prevented ≥138,000 congenital syphilis cases and >148,000 stillbirths annually; by contrast, a test that could be performed with no laboratory infrastructure could prevent >201,000 congenital syphilis cases and 215,000 stillbirths annually. The desirable sensitivities and specificities were also determined using mathematical modelling.

Table 2 | Resources and capabilities at three infrastructure levels common in the developing world

	Health-care setting (personnel)	Summary of resources and capabilities
No laboratory infrastructure	In the community or home (possibly health-care worker, pharmacist or family member)	No electricity or clean water available; no trained personnel; no laboratory space; cold storage not available; room temperature not controlled; venipuncture impossible; sputum impossible to process; rapid answer required to prescribe treatment before patient leaves; no physician oversight.
Minimal laboratory infrastructure	Health clinics in Africa; rural health clinics in Latin America and Asia (nurse)	No reliable electricity and clean water; minimal trained personnel; no or minimal laboratory space; cold storage occasionally available; room temperature rarely controlled; venipuncture unlikely; sputum difficult to process; rapid answer required to prescribe treatment before patients leave; no physician oversight.
Moderate to advanced laboratory infrastructure	Urban health clinics in Asia and Latin America; hospitals in Africa, Latin America and Asia (nurses, technicians and physicians)	Dependable electricity and clean water available; trained personnel available; dedicated laboratory space; cold storage available; room temperature sometimes controlled; venipuncture routine; sputum acceptable (except children); time to answer usually less crucial with hospitalized patients, but still important for clinic patients; physician oversight routine.

The timeframe in which a test should be completed was determined by the working group members based on their experience at various diagnostic testing sites throughout the developing world. For sites with no laboratory infrastructure, we believe that timeframes ranging from a few minutes up to 1 h would be ideal to implement treatment during the same health-care encounter, although longer periods might be acceptable in some situations.

Although equipment-free tests are desirable, we consider approaches that require equipment to be useful in some instances, providing that the system is robust, simple to operate without training, self-powered and affordable (both to acquire and to maintain). The issues of affordability and delivery, which are not considered in this analysis, will be the topics of future publications. The delineation of the specific user requirements for each clinical decision point is essential to expedite the development of appropriate products.

Several important findings emerge from the information presented in Table 3. For ALRI, syphilis, gonorrhoea, chlamydia and TB, significant improvements in outcomes could be achieved if tests could be deployed to sites with both minimal and no laboratory infrastructure. An HIV test to identify infected infants was the only diagnostic identified for which it would be sufficient to deploy the test to sites with minimal laboratory infrastructure without the additional need to deliver it to sites with no laboratory infrastructure. This indicates that a two-step approach to test deployment — and possibly also to test development — would be the most rapid way to expand access. In such an approach, a test suitable for sites with minimal infrastructure is likely to be ready to deploy much sooner, and a second-generation test that is appropriate for sites with no laboratory infrastructure, but that might take much longer to develop, would be deployed later. This is not to say that we recommend a delay in the deployment of tests for the sake of the greatest access; rather, we suggest that one does not have to wait for the ultimate technical solutions to begin saving lives.

Another important observation from this analysis is that the practicality of obtaining a particular type and volume of specimen is a primary driver of the feasibility of diagnostic tests at sites with no and minimal laboratory infrastructure. Test development for moderate-resource and high-resource sites has often proceeded in the opposite direction; that is, the best biomarker in almost any bodily fluid or accessible tissue is selected, and then a technology to detect it is designed with limited regard for the resources required to obtain the specimen or to implement the test. For instance, the currently available HIV viral-load tests require 1 ml of blood obtained via venipuncture by a trained phlebotomist in order to get a sample that contains a sufficient quantity of biomarkers (viral genomes) to be reliably detected. This requirement is impossible to achieve in settings with no laboratory infrastructure. A switch to a more acceptable specimen type for resource-limited sites might create other challenges, particularly for detection limits. A blood sample from an infant obtained via a heel stick is a practical approach, but the amount of blood obtained is usually <5% of a venipuncture specimen. This greatly reduced sample size would necessitate the development of a test with a molecular limit of detection that is at least 20-fold lower to be equivalent to a test that uses a venipuncture specimen. This significant improvement in the detection limit would be difficult to achieve with even the best methods available today. This trade-off between specimen type and quantity, biomarker levels and limits of detection is also an issue for malaria and syphilis diagnosis. Chlamydia and gonorrhoea are easily detected in active infections through the use of vaginal swabs or urine; however, there are asymptomatic cases with low levels of organisms that will challenge the best of methods.

Due to the requirement for practical specimen types, some existing tests will need to be significantly redesigned. This is a particularly acute issue for respiratory diseases. ALRI is difficult to distinguish from upper respiratory tract infections using any specimen that must

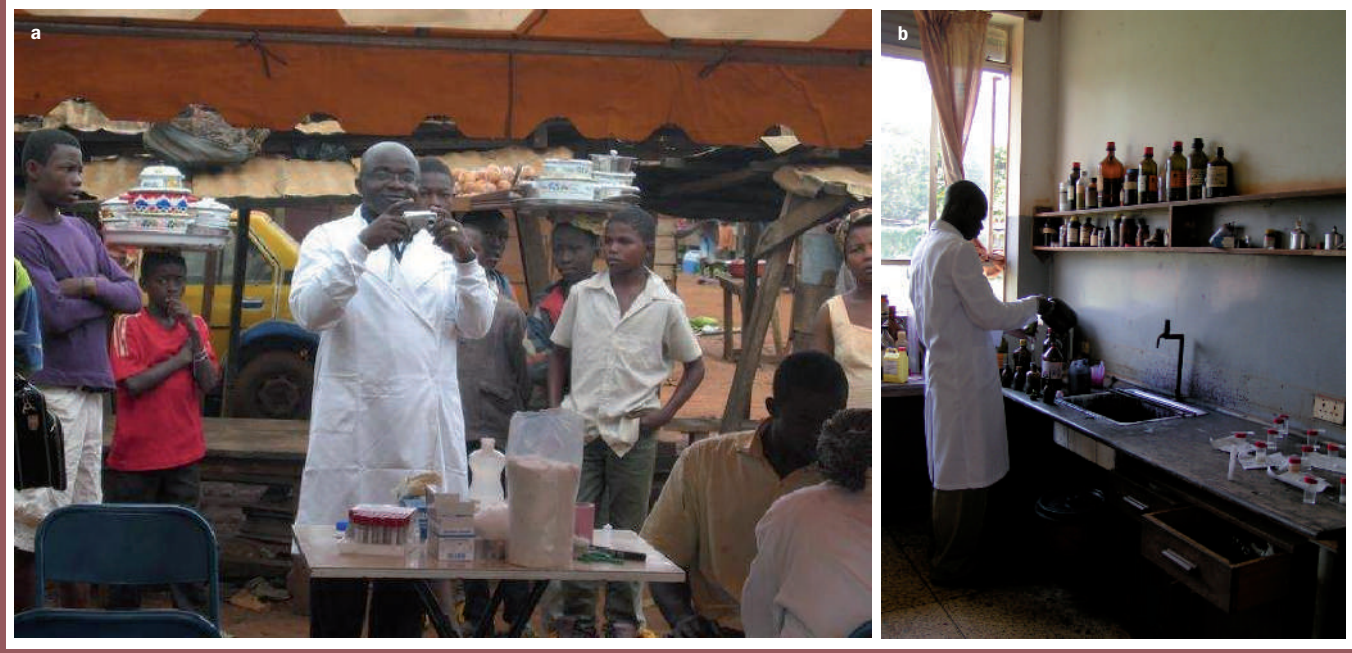

Figure 1 | Diagnostic testing sites with minimal and no laboratory infrastructure. (**a**) Diagnostic testing site in Cameroon with no laboratory infrastructure. Reagents, devices and/or equipment must be able to function at ambient temperature, without electricity, running water or a sterile environment. Tests should be completed rapidly so that results can be provided to the patient before leaving the site. Photo provided by J-P. Bonn (BBT Partners, Paris, France). (**b**) Diagnostic testing site in Uganda with minimal laboratory infrastructure. The site might have running water, has electricity and has limited equipment, but is at ambient temperature. Photo provided by D. C. Hay Burgess courtesy of the Bill & Melinda Gates Foundation, Washington, USA.

pass through the mouth. Sputum is not simple to collect from adults, and is extremely difficult to collect from children at any resource level. In sophisticated testing settings, many attempts at self-induced coughing fail to produce a useful sputum specimen and atomized chemical sputum induction must be used; however, this approach is expensive and requires intensive training, and is therefore not feasible for resource-limited settings. The diagnosis of TB faces similar issues because of the impracticality of using sputum; therefore, blood or another novel sample type is needed as a replacement.

Switching to a more practical specimen type could mean that novel biomarkers will be needed to provide adequate clinical performance. This might be necessary for ALRI, TB, diarrhoeal diseases and possibly even malaria. Bacterial ALRI diagnosis is probably the most difficult case among those evaluated. Some reports have indicated that the most important bacterial causes of pneumonia in developing countries are *Streptococcus pneumoniae* and *Haemophilus influenzae*[12,14]; however, other studies have implied that a wide range of other infectious agents play significant roles[15–18]. This suggests that a diagnostic test would ideally detect at least half a dozen bacterial species. Basic studies demonstrating the prevalence of the suspect organisms, coupled with the development of methods to simultaneously detect a biomarker for each of the predominant species, are probably needed. An alternative biomarker could be a host-derived non-specific biomarker of bacterial infection. The human immune-response protein, triggering receptor expressed on myeloid cells-1 (TREM-1), is a candidate as its concentration appears to be locally elevated at the site of bacterial infections[19]. However, given the overall prevalence of bacterial infections in the developing world, it could prove difficult to use a non-specific test to inform specific treatment decisions. Histidine-rich protein 2 (HRP2), which is used today in most rapid diagnostic tests for *Plasmodium falciparum*, varies in its primary sequence from one strain to another; this raises concerns about how well the antibodies that are used to detect HRP2 in immunoassays function for the diverse malaria strains found throughout the world[20]. Similar concerns have been raised regarding whether the antibodies used in commercially available tests to detect the pan-*Plasmodium* species antigens lactate dehydrogenase and aldolase work equally well to detect all the species[21,22]. It might therefore be worth considering whether other less-variable antigens should be selected as biomarkers, or whether antibodies could be raised against less-variable epitopes of the existing biomarkers. For HIV, syphilis, chlamydia and gonorrhoea, the currently-used biomarkers might be sufficient to determine whether a patient should be treated. However, other technical issues might necessitate the consideration of potential changes (for example, syphilis test-reagent stability and the ability to distinguish past from present infection).

Table 3 | User requirements for infectious disease tests at sites with minimal and no laboratory infrastructure

Clinical decisions	Infrastructure level*	Possible sample types	Biomarker possibilities	Sensitivity/specificity	Time requirement
Diagnosis of bacterial ALRI in children, to initiate antibiotic therapy	No laboratory infrastructure	Blood (finger prick), urine, saliva, breath	Bacterial antigens, host factors (for example, possibly TREM-1), possibly volatile organics in breath	>95%/85%	<1 h
Diagnosis of severe ALRI requiring hospitalization	No laboratory infrastructure	None (for example, pulse oximetry), blood (finger prick), urine, breath	SaO_2, blood chemistry (for example, pH, CO_2); metabolites from breath	>85%/>90%; at least 50% of the population must have access to hospital care	
Detection of HIV infection in infants aged <12 months	Minimal infrastructure	Blood (heel stick, fresh or dried on filter paper), saliva	HIV RNA, HIV antigens (for example, p24), host factors	>90%/>90%	<1 h†
Diarrhoeal symptoms and detection of *G. lamblia*, *C. parvum*, and enteroaggregative *E. coli*	Minimal infrastructure	Faeces, vapours	Organism antigens, host factors, adhesion assays (*E. coli*), volatile organics	>90%/>90%	<1 h
Diagnosis of malaria in febrile children aged <5 years in sub-Saharan Africa	Minimal infrastructure	Blood (finger prick) urine, saliva	Parasite antigens (for example, HRP2); new antigens	95%/95% minimum down to at least 500 parasites per ml	<5 min
	No laboratory infrastructure	Blood (finger prick), urine, saliva	Parasite antigens (for example, HRP2); new antigens	90%/90% minimum down to at least 500 parasites per ml	< 5 min
Case detection of active TB in symptomatic HIV positive and negative individuals	Minimal infrastructure (for example, TB clinic)	Sputum (adults), blood (venipuncture), urine	Nucleic acids; bacterial antigens (many examples, but not well studied); metabolites in breath	85%/97% for smear negative/positive	<1 h
	No laboratory infrastructure	Sputum (adults), blood (finger prick), urine	Nucleic acids; bacterial antigens	>85%/97% for smear negative/positive	<1 h
Syphilis screening in antenatal women	Minimal infrastructure	Blood (finger prick), saliva, urine	Cardiolipin in RPR (currently used); marker that correlates with transmission to infant would be ideal	86%/72% (RPR)	<1 h
	No laboratory infrastructure	Blood (finger prick), saliva, urine		Same or better than RPR	<1 h
Chlamydia and gonorrhoea diagnosis in female CSWs	Minimal infrastructure	Urine or vaginal swab	Bacterial antigens (for example, MOMP for *C. trachomatis*; ribosomal protein for *N. gonorrhoeae*); nucleic acids	85%/90%	<1 h
	No laboratory infrastructure				
	No laboratory infrastructure	Blood (finger prick), urine, breath			<1 h

*Data from Table 2. †In some of the papers in this series, <2 h is proposed. Here, we recommend the more aggressive goal of <1 h as a stronger safeguard against patients leaving the site of testing prior to a treatment decision. ALRI, acute lower respiratory infection; CO_2, carbon dioxide; CSWs, commercial sex workers; *C. parvum, Cryptosporidium parvum; C. trachomatis, Chlamydia trachomatis; E. coli, Escherichia coli; G. lamblia, Giardia lamblia*; HIV, human immunodeficiency virus; HRP2, histidine-rich protein 2; MOMP, major outer membrane protein; *N. gonorrhoae, Neisseria gonorrhoae*; RPR, rapid plasma reagin; SaO_2, oxygen saturation; TB, tuberculosis; TREM-1, triggering receptor expressed on myeloid cells-1.

A final set of observations that emerged from our analyses relates to the test-performance goals. The sensitivity and specificity requirements presented in Table 3, which are based upon the mathematical modelling analyses, indicate that the minimum sensitivity for all tests varies from 85 to 95%, whereas the required specificity has a broader range from 76 to 97%. Increases in both measures would improve treatment effectiveness and save more lives. Under typical Gaussian-noise assumptions, one can show that as performance approaches 100%, increases in either sensitivity or specificity become progressively more difficult to achieve. For example, in the 1990s, the development of the assays currently used to detect infectious organisms in blood-bank samples, which must have specificities >99.99%, required larger development teams, longer timelines and higher costs than the development of the commonly used clinical diagnostic tests for the same organisms, for which specificities of <95% are acceptable.

Achieving the new user requirements

In order to meet the user requirements for each clinical decision point, a variety of strategies might be employed. The most successful strategy might differ in each case depending upon the deficiencies in the available diagnostic approaches and the degree of difficulty in implementing the approach, given the unique disease biology and capabilities of the available technologies. Where the sample will be limited in volume but can be used in a low-infrastructure setting (for example, finger-prick blood instead of venipuncture), possible strategies include adjusting the molecular cut-off limits assuming that the test still provides adequate clinical performance (for example, a limit of detection of 1,000 HIV viral genomes instead of 50) or improving the molecular detection limits for low-volume sample types, which is often a difficult task. Where the sample type used in current diagnostic products will be impractical at a low-infrastructure site (for example, sputum), test developers might change the sample type (for example, by switching to blood instead of sputum), but only if the biomarkers are present, or they might switch to an alternative biomarker that is more easily detected in the new sample (for example, bacteria, antigens or antibodies in blood instead of *Mycobacterium tuberculosis* in sputum), but only if the biomarkers can be shown to provide clinical utility. If none of these approaches is feasible, it is probably time to re-think the technical approach. This process is most likely to be successful if it starts by considering the practical specimen types.

New approaches

Perhaps the best solutions for the challenges faced in some disease areas will be derived from novel approaches. For example, volatile organic compounds (VOCs), such as 1-methyl-naphthalene and 1,4-dimethyl-cyclohexane in the breath of TB patients[23], have shown promise as biomarkers for infectious pathogens in a number of disease areas. VOCs can be detected using either mass spectrometry or so-called electronic-nose instruments. Researchers have shown that cultured samples of a number of the bacteria associated with ALRI have distinguishable VOC signature patterns[24]. In addition, a pattern of VOCs in the breath of TB patients was shown, in a small study, to detect *Mycobacterium tuberculosis* infection with 83% sensitivity and 100% specificity compared with the culture of sputum samples[23]. The VOC patterns of vapours from the faeces of diarrhoea patients have shown differences associated with various aetiologies, and can be distinguished from those emanating from normal faeces[25]. If such approaches are clinically validated, the detection of VOCs using an electronic nose could provide a user-friendly approach to diagnosis. Another interesting approach uses unprocessed whole blood to detect viruses (such as HIV), bacteria and proteins, using an ultrasensitive acoustic sensor[26]. These are just two of a large and growing number of novel approaches that are being explored, and are reviewed elsewhere[27]. It should be noted, however, that some of these technologies could take substantially longer to develop and implement than other options, as neither the methods nor the biomarkers are fully validated for the diseases under consideration.

Other considerations for diagnostic-test developers

So far, we have considered only tests that detect a single infectious organism; however, combination tests, in which multiple infectious pathogens could be detected in the same assay, could be extremely useful in some situations. There are at least two strategies for devising the combinations of biomarkers that would be detected using a single assay or technology platform: a technology-based strategy and a clinical decision-based strategy.

A technology-based strategy would utilize a diagnostic test platform to perform diagnoses in all disease areas where a single sample and/or technology is advantageous, regardless of whether the disease areas are clinically related. For instance, a finger-prick blood sample that is used to detect the antigens of infectious organisms might be useful for ALRI, malaria, syphilis and TB. If VOCs could be employed, ALRI, diarrhoeal diseases and TB would all be candidates. Similarly, a single device might be used to detect DNA biomarkers for HIV, chlamydia, gonorrhoea and TB infections.

The alternative strategy for grouping tests aims to assist clinicians in the differential diagnosis of diseases that produce common symptoms or that are often found as co-infections. Discussions with members of the forum have highlighted a few useful combinations. If a child with a fever presents at a clinic, the possibilities of ALRI, malaria and TB must all be considered, along with whether the child has dehydration, hypoxaemia and hypoglycaemia. Whenever TB is suspected, the HIV status needs to be considered simultaneously. HIV patients will require testing for CD4, viral load and opportunistic infections. It is possible that all sexually transmitted diseases (including syphilis, chlamydia, gonorrhoea and HIV) might be tested in a single assay. This second strategy for combining tests based upon diseases that are clinically related might require multiple sample and biomarker types to be analysed in the same device. Although technically more demanding, a number of organizations are working to combine immunoassays, nucleic-acid assays and cell counts into single analytical systems[28]. These combinations could significantly increase the usefulness of new tests.

From our review of performance, we find that many of the biomarkers currently in use have not been properly validated in clinical settings. Because the clinical utility of a biomarker is dependent upon the prevalence of the disease within the population, it is essential that existing biomarkers and new candidates are tested in clinical studies that are large enough to be statistically significant, using samples collected from the populations that will ultimately be tested in the developing world. The right biomarkers will be those that deliver the necessary clinical specificity and sensitivity in the target populations.

When considering product designs for the developing world, it is important to consider social and cultural issues. In some locations, blood sampling is not easily accepted and could inhibit the local use of tests. The name given to a device in English can have a different and unintended meaning in another language. Even the colour of the materials used can be an issue. One well-known example that highlights the importance of addressing social and cultural issues is the unsuccessful introduction, in some populations, of anti-malaria bed nets that were white, due to the local cultural association of this colour with the dead[29].

The introduction of new testing products at remote sites has the potential to greatly increase local problems for the disposal of products, reagents and biohazardous waste. If

fully implemented, many millions of new tests will be run per year. Developers should consider the implications and either institute recycling programmes (a difficult process anywhere) or use biodegradable materials for product components whenever possible. Finding a biodegradable alternative to existing plastics is a major focus of the chemical industry at this time.

Intellectual property is a significant issue for many of the disease areas considered by the forum. Issued patents covering specific biomarkers and even entire organisms currently exist. It is important for product developers to factor this into their plans. In a significant fraction of the developed world, patents cover "making, using and selling" potential products. Attempts to manufacture products in a location where the biomarker is protected would infringe such a patent. Many millions of US$ have passed from one organization to another due to the infringement of patents in the infectious disease diagnostics arena.

In addition, substantial intellectual property exists around diagnostic testing platforms, and specific components of both tools and platforms. Licensing of this intellectual property can create a substantive impediment (for example, high royalties or a lack of license availability) to the development of newer, more effective and cost-efficient tools. A rational patenting and licensing strategy should be developed to ensure global access to these technologies for the poorest populations worldwide.

The use of these new types of test will necessitate new ways of thinking. In order to prevent future deaths from antibiotic resistance, patients who want drugs despite the fact that there is no chance that they will benefit from them must either be refused treatment or provided with alternatives. This will be a difficult transition; however, it is an essential component of the changes in medical practice that are needed to optimize the number of lives and DALYs saved. Test developers will have to work closely with local health-care officials and agencies, such as the WHO, to ensure that the impact of the new diagnostic tests can be maximized.

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Acknowledgements

The authors thank B. Manian (Reametrix, San Carlos, California, USA,) S. Lwanga (Kampala, Uganda) and D. W. Kitimbo (District Director of Medical Services, Jinja District, Uganda).

Correspondence and requests for materials should be addressed to M.U. (email: murdea@halteresassociates.com)