

DIAGNOSTICS FOR THE DEVELOPING WORLD

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Although ‘diseases of affluence’, such as diabetes and cardiovascular disease, are increasing in developing countries, infectious diseases still impose the greatest health burden. Annually, just under 1 million people die from malaria, 4.3 million from acute respiratory infections, 2.9 million from enteric infections and 5 million from AIDS and tuberculosis. Other sexually transmitted infections and tropical parasitic infections are responsible for hundreds of thousands of deaths and an enormous burden of morbidity. More than 95% of these deaths occur in developing countries. Simple, accurate and stable diagnostic tests are essential to combat these diseases, but are usually unavailable or inaccessible to those who need them.

TROPICAL INFECTIOUS DISEASES

In developing countries, most deaths from infectious diseases occur in children or young adults, so each fatality results in the loss of many years of potentially healthy and productive life. As a tool for comparing the burdens of morbidity and mortality that are imposed by different diseases, the **World Health Organization (WHO)** and the World Bank have proposed the use of the disability-adjusted life-year (DALY)^{1–3}. The DALY not only takes into account the years of life that are lost as a result of premature death, but also includes a factor to allow for a reduction in the quality of life due to chronic disability. The global burden imposed by various diseases in terms of DALYs lost per year is shown in FIG. 1 (REF. 4). Most of the infectious diseases that are common in the developing world are treatable, and access to drugs has improved markedly over the past decade with the advent of drug-access campaigns, mass-treatment programmes and public resources, such as the **Global Drug Facility for Tuberculosis (TB)**. The need for accurate identification of patients requiring treatment, however, remains a major stumbling block to disease control, and the burden of infectious disease in the developing world could be substantially reduced if appropriate diagnostic tests were more widely available.

This review covers the role and application of diagnostics in the control of highly prevalent infectious diseases and in patient management in developing countries. We will describe the characteristics of an ideal diagnostic test, summarize the suitability of tests that are available at present for use in the developing world, and outline a scheme for the prioritization of diagnostics development. We will also consider barriers to the development of useful diagnostic tests, public-sector efforts to coordinate and accelerate test development, and the opportunities for innovation that are presented by advances in the understanding of host–pathogen interactions and by new technologies.

Disease-control strategies and diagnostics
Major initiatives that have been promoted to reduce the burden of disease due to infection in developing countries are shown in BOX 1. Better and more widely available diagnostic tests would facilitate the control of many important infectious diseases in developing countries. The role of available diagnostic tests in the control of selected highly prevalent infectious diseases in developing countries is summarized in TABLE 1. Depending on the recommended control strategy, better tests may be needed for two main reasons — to improve CASE-FINDING and case-management, and to improve disease surveillance.

CASE-FINDING

The identification of asymptomatic or poorly symptomatic cases of a disease or infection.

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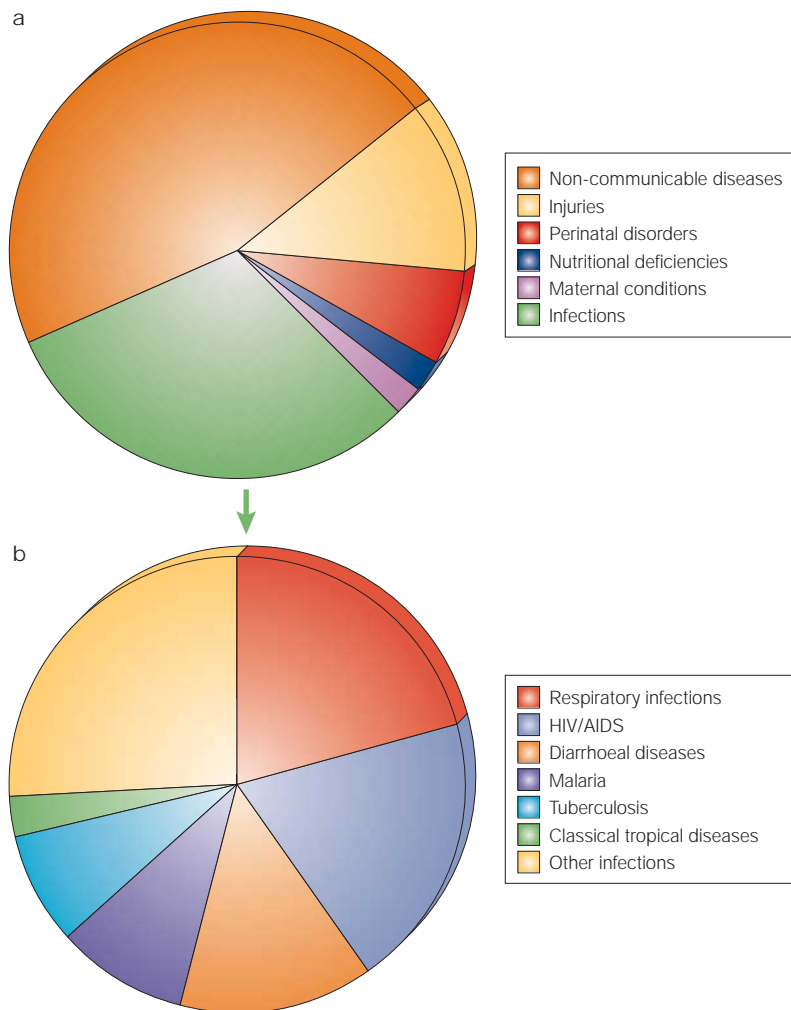


Figure 1 | **The burden of disease worldwide.** Disability-adjusted life-years (DALYs) lost worldwide. **a** | DALYs lost due to the main categories of disease. **b** | DALYs lost due to different infectious diseases, as a proportion of the total caused by infection. Data taken from REF. 4.

Improving case-finding and case-management. Diagnostic tests to guide the management of individual patients are familiar to all clinicians. They can be used to screen asymptomatic individuals who are at risk of disease, such as antenatal patients who are at risk from **syphilis**, or to confirm (or rule out) a clinical diagnosis in symptomatic patients — for example, by the use of sputum microscopy in patients presenting with a cough. Sound clinical management of common infectious diseases could greatly reduce the burden of disease in developing countries. 24% of the current burden of disease could be averted if 80% of the population of low-income countries received a minimum clinical package — comprising prenatal/delivery care, family planning, management of sick children, treatment of **TB** and case-management of sexually transmitted diseases — at a cost of only US \$8 per person per year³. However, clinical care is often severely compromised by the absence of diagnostics from healthcare facilities in developing countries.

Improving disease surveillance. Surveillance is the cornerstone of successful disease control and elimination programmes, as it enables programme managers to monitor the effectiveness of intervention strategies and identify which populations require continuing interventions. In the case of **smallpox**, the clinical features were sufficiently distinctive that surveillance could be based on clinical findings alone. The same is true of **measles** and **guinea worm**. In the case of **polio**, the clinical diagnosis of acute flaccid paralysis is strongly suggestive, but there are other causes of this syndrome and, as the incidence of polio decreases, the proportion of cases of flaccid paralysis due to polio also decreases. There is, therefore, a need to confirm suspected cases in the laboratory — for example, by electron microscopy of a faecal sample. The clinical features of many other important infectious diseases are insufficiently distinctive for surveillance purposes, especially in the early stages, and it is for these diseases that diagnostic tests are needed.

The rise of drug resistance threatens many disease-control programmes, including those for **malaria** and **TB**, and dramatically increases the cost and complexity of cure. Surveillance for drug resistance is fundamental to the refinement of treatment strategies and to the allocation of scarce resources. Periodic monitoring of susceptibility in clinically significant bacterial isolates would provide the necessary information. For example, the WHO and the International Union Against Tuberculosis and Lung Disease (IUATLD) have sponsored the Global Project on Anti-Tuberculosis Drug Resistance Surveillance since 1994. Through a supranational laboratory network, which helps national laboratories to conduct quality-assured drug-susceptibility testing on TB isolates, the project has now completed three rounds of global surveillance⁵. Unfortunately, funding is not available for conducting such surveys systematically for other diseases in most developing countries.

Characteristics of available tests

For most infectious diseases, laboratory-based tests with reasonable sensitivities and specificities exist, although they are not available in peripheral health centres, which serve most of the population. Microscopy is commonly used to diagnose parasitic and mycobacterial infections, but depends on the availability of well-trained and supervised technologists. Culture remains the mainstay of bacteriological diagnosis, and enables antimicrobial susceptibilities to be determined; however, it is time-consuming and expensive, and depends on stringent transport conditions to maintain specimen viability, a constant supply of reagents and electricity, well-maintained equipment and adequately trained and supervised technologists. In recent years, antigen or antibody detection tests, such as enzyme immunoassays (EIAs), have largely replaced culture for the diagnosis of many infectious diseases. EIAs do not require special specimen-transport conditions and can be performed in intermediate-level laboratories with relatively simple equipment. EIA

Box 1 | Initiatives to reduce disease burden in developing countries

- Vaccination for viral and bacterial diseases of childhood, such as smallpox, polio, measles, diphtheria, pertussis, tetanus, *Haemophilus influenzae* type b, *Streptococcus pneumoniae* and hepatitis B.
- Use of VECTOR CONTROL for diseases that are transmitted by arthropods, such as malaria^{33,34}, dengue³⁵, and American and African trypanosomiasis^{36,37}.
- Improved water supply and sanitation for the control of diarrhoeal diseases and intestinal helminths^{38,39}.
- Community-based mass treatment for infections that can be cured by single-dose treatments, such as endemic treponematoses²⁶, onchocerciasis⁴⁰, lymphatic filariasis⁴¹, schistosomiasis^{42,43}, intestinal helminth infections⁴⁴ and trachoma⁴⁵.
- Screening or case-finding, for example for tuberculosis, leprosy, syphilis and African trypanosomiasis.
- Intermittent preventive treatment of malaria in pregnant women and infants^{46,47}.
- Control of intermediate host populations for diseases such as schistosomiasis⁴⁸ and guinea worm⁴⁹.

VECTOR CONTROL

The control of insect vectors of infectious diseases, usually using insecticides or traps.

POINT-OF-CARE TESTS

(POC tests). Diagnostic tests performed in the clinic, with results available within a short time so that patients can be treated without a return visit.

IMMUNOCHROMATOGRAPHY

Test based on a strip of paper coated with an immobilized antibody specific for an antigen that is characteristic of a disease. Samples conjugated to a second antibody (which allows chromogenic detection) can be applied to the paper strip and results visualized in less than 10 minutes. By using multiple antigens at different positions on the strip, multiple diseases can be screened for in one sample.

PRIMARY HEALTHCARE SETTING

The first point of contact between sick people and the health service, such as a health centre or dispensary.

SYNDROMIC TREATMENT/MANAGEMENT

Treatment of a patient with a syndrome (a characteristic combination of symptoms and signs) for all the common infectious causes of that syndrome.

results are usually available in 3–4 hours and are not as expensive as culture, because tests can be processed in batches. Tests that are based on nucleic-acid amplification technology (NAAT), such as PCR, are available for many prevalent diseases and have excellent sensitivity and specificity. The increased sensitivity that is conferred by these tests allows the use of non-invasive specimens, such as urine, for the diagnosis of some infections — for example, *Chlamydia trachomatis* infection. However, NAAT tests are expensive and require technical expertise and equipment, with particular care needed to avoid false-positive results caused by contamination. They are therefore unavailable to most healthcare facilities in the developing world⁶. Although NAAT technology is not widely used in most patient-care settings in developing countries, it is being used as an adjunct to clinical care by hundreds of laboratories, often in a non-commercial or 'home-brew' format. Little is known about the quality or performance of such testing, or its clinical impact. The limited published work that has assessed the accuracy of such testing using proficiency panels indicates that the use of these tests without rigorous quality control is not justifiable^{7–9}.

Point-of-care tests. Simple, rapid POINT-OF-CARE (POC) TESTS that can be used to guide treatment are available for infectious diseases such as AIDS, malaria and syphilis¹⁰ (see links to the [WHO Sexually Transmitted Diseases Diagnostics Initiative \(SDI\)](#), [WHO Malaria Rapid Diagnostics Tests and Program for Appropriate Technology in Health](#) websites). Most POC tests use IMMUNOCHROMATOGRAPHY to detect antigens or antibodies in a dipstick or lateral-flow format. A few POC tests — such as the direct agglutination test (DAT) for visceral leishmaniasis and the rapid plasma reagin (RPR) test for syphilis — are based on the visualization of antigen-antibody lattice formation, either by coating red blood cells or latex particles with the antigen or antibody, or by entrapping charcoal or dye particles in the reaction to allow a positive result to be visualized^{11–14}.

These tests are cheap to produce, simple to perform, produce rapid visual readouts and often require no equipment. They are useful in PRIMARY HEALTHCARE SETTINGS where there may be no electricity for equipment or refrigerators (for the storage of reagents), and where patients often travel long distances and may therefore be unable to return for test results.

However, some POC tests still fail to meet the needs of disease-endemic areas. For example, once the reagents for DAT are reconstituted, the test must be refrigerated and results are only available after 18–24 hours of incubation. Although more than 20 companies are manufacturing rapid diagnostic tests for malaria and syphilis, most of these tests have not been carefully evaluated¹⁵. The performance of commercially available dipstick-type tests for malaria outside the research setting has frequently fallen below the expected level based on controlled clinical trials. POC tests are available for the diagnosis of sexually transmitted infections, such as *C. trachomatis*, but with reported sensitivities ranging from 32–74% compared with those of NAATs^{16,17}.

For the diagnosis of human immunodeficiency virus (HIV) infection, screening tests that are close to the ideal in terms of performance and simplicity are already commercially available (see the link to the [WHO HIV diagnostics website](#)). The development and production of these tests was driven by the private sector because of a demand from control programmes and blood banks in the developed world. However, as anti-retroviral treatment becomes more widely available, simple and affordable tests to measure HIV viral load and CD4 counts will also be needed in developing countries.

Priorities for investment in diagnostics

Given the limited resources that are available for the development of new diagnostics for infectious diseases in developing countries, there is a need for prioritization. Here, we consider several criteria that could be applied to prioritization, using examples where possible.

Disease burden. The global disease burden due to the most prevalent infectious diseases is shown in FIG. 1. In addition to considering the global burden of a particular disease, it is important to consider diseases that have a large, but localized, impact. For example, 300,000–500,000 people in equatorial Africa are infected with *Trypanosoma brucei gambiense*¹⁸, and 90% of the more than 500,000 new cases of visceral leishmaniasis that occur annually do so in just five countries: India, Bangladesh, Nepal, Sudan and Brazil¹⁹. For most of the infections that impose a heavy burden of disease in developing countries, there is no market in richer countries to attract private-sector investment into the development of diagnostic tests. This market failure is starting to be addressed by public-sector partnerships, as described later. All of the patients with these infections will die within a few years unless they are diagnosed and treated. So, the development and evaluation of better tests for these diseases should be a high priority for the public sector.

Table 1 | The role of diagnostics in the control of selected infectious diseases in developing countries

Disease	Control strategy	Diagnostic tests	Role of diagnostic tests		
			Case-management	Screening	Surveillance
ARIs	Vaccination and syndromic case-management	Blood/sputum culture	–	–	Blood culture useful at sentinel sites to inform treatment policy
Diarrhoeal diseases	Vaccination and syndromic case-management	Microscopy Stool culture	+ (for parasitic causes) –	– –	– Stool culture at sentinel sites to inform treatment policy, for example, for shigellosis; prediction of cholera outbreaks
Malaria	Vector control and case-management	Blood film	+	–	Identification of outbreaks outside hyperendemic areas
		Antigen detection (dipstick)	May be useful	–	–
		Antibody detection (other format)	Not indicated	Blood banks	–
HIV	Health promotion; STI control; voluntary counselling and testing; PMTCT	Serology (antibody detection)	+	Blood banks; screening of pregnant women for PMTCT	Sentinel surveillance in defined population groups
		CD4 ⁺ cell counts and viral load	+ (where ARVs are available)	–	–
TB	Case detection followed by DOTS	Sputum microscopy (and culture)	+	–	National reporting schemes
		Tuberculin skin test	Of debatable use	+	–
Visceral leishmaniasis	Case detection	DAT serological field test	+ (but does not distinguish between disease and sub-clinical or treated infection)	Possible role in blood banks	Of limited value
		Microscopy/culture of spleen or bone marrow	Required for confirmation of positive DAT	–	–
African trypanosomiasis	Case detection (vector control)	CATT serological field test	Useful for <i>T. b. gambiense</i> but not <i>T. b. rhodesiense</i>	+	To identify affected communities and monitor impact of control programmes
		Blood/CSF microscopy and examination	Required for confirmation of CATT and for <i>T. b. rhodesiense</i> diagnosis	–	–
Onchocerciasis	Mass treatment (vector control)	Skin-snip microscopy	Skin snips may be useful for individual case-management outside endemic areas	–	To monitor impact of control programmes
Syphilis	Screening of pregnant women; syndromic case-management of genital ulcers; case detection	Serology (antibody detection)	Useful for diagnosis of latent, secondary or tertiary syphilis	Screening of pregnant women prevents congenital syphilis	Screening of pregnant women to monitor impact of control programmes
Gonorrhoea	Case detection	Microscopy Culture	Useful in men Often not available	– –	– At sentinel sites to inform treatment policy
Leprosy	Case detection	Skin smears	Require expertise and are only useful in multibacillary cases	–	–
		Blood film	Blood film for MF, serology, or antigen detection may be useful for individual case-management outside endemic areas	–	Useful for monitoring impact of control programmes
Lymphatic filariasis	Mass treatment and vector control	Antigen detection	For <i>Wuchereria bancrofti</i> only	–	Useful for monitoring impact of control programmes
		Microscopy of stool/urine	+	–	To monitor impact of control programmes
Schistosomiasis	Mass treatment; control of intermediate host	Microscopy of stool/urine	+	–	To monitor impact of control programmes
Chagas' disease	Vector control; screening of donated blood	Serology (antibody detection)	Of limited value as treatment not effective	Blood banks	To monitor impact of vector control programmes
Dengue	Vector control	Serology (antibody detection)	+ (but often negative in acute illness, and of limited value as no specific therapy is available)	–	To monitor impact of control programmes

+, usually indicated; –, not indicated; ARIs, acute respiratory infections; ARV, anti-retroviral medications; CATT, card agglutination test for trypanosomiasis; CSF, cerebrospinal fluid; DAT, direct agglutination test; DOTS, directly observed therapy short course; HIV, human immunodeficiency virus; MF, microfilaria; PMTCT, prevention of mother-to-child transmission; STI, sexually transmitted infection; *T. b.*, *Trypanosoma brucei*; TB, tuberculosis.

Box 2 | African trypanosomiasis

More than 66 million women, men and children in 36 countries of sub-Saharan Africa suffer from African trypanosomiasis, and it is estimated that 250,000–300,000 people die every year owing to a lack of diagnosis and treatment. There are two forms of African sleeping sickness, which are caused by two different parasites: *Trypanosoma brucei gambiense* (*T. b. gambiense*) and *Trypanosoma brucei rhodesiense* (*T. b. rhodesiense*). The parasite that causes sleeping sickness is called the trypanosome and is transmitted to humans through the bite of a tsetse fly of the genus *Glossina*. Trypanosomiasis is always fatal unless treated. The clinical features are non-specific in the early stages, and detection and treatment of infected individuals is the main control strategy. Treatment is expensive, toxic and difficult to administer. Melarsoprol — which contains arsenic — was developed in 1932 to treat the disease, and has been used systematically since 1960 for cases in which there is involvement of the central nervous system. Melarsoprol exposes around 10% of treated individuals to serious risk, especially from arsenic encephalopathy, from which almost 1,000 people die each year. A rapid blood test is available for field diagnosis (the card agglutination test for trypanosomiasis (CATT)⁵⁰; the photograph shows this test being performed in rural Angola). CATT — which detects *T. b. gambiense* infections, but does not detect *T. b. rhodesiense* infections — is not specific enough to use as a test to direct therapy with the toxic agents available. There is, therefore, a great need for a more specific test that can be used by minimally trained individuals in remote settings. A more toxic treatment regimen is required when the central nervous system is involved, which is at present only detectable by lumbar puncture. Photograph provided by Dr August Stich. For more information about human African trypanosomiasis, see the [Disease Watch](#) focus article on p.186 of this issue.



Likely impact of a new test on disease burden. For diseases that are easily clinically recognized and can be treated SYNDROMICALLY, improved diagnostic tests would have little impact. For example, acute respiratory infections (ARIs) and diarrhoeal diseases are major killers, for which both immunization and effective case-management have a vital role. The WHO has developed guidelines for the syndromic management of ARIs and diarrhoea in children, in which treatment is given for the common aetiological agents, fluid losses are replaced and other supportive treatment is provided as required. As there is no clinical need to identify the causal agent, improved diagnostic tests are not required for the clinical management of these conditions. By contrast, some diseases cannot be managed syndromically because they have no symptoms, have symptoms that are common to diseases requiring different treatments, or require treatment that is complex or sufficiently expensive so that its use must be limited only to confirmed cases.

Some diseases have few, if any, symptoms, but may result in devastating consequences. For example, syphilis in pregnancy is usually asymptomatic, but it increases the risk of stillbirth or perinatal death by at least 20-fold²⁰. **Gonorrhoea** and chlamydial infection also cause few, if any, symptoms in a high proportion of infected women, but undetected and untreated infections can lead to pelvic inflammatory disease, ectopic pregnancy and tubal infertility. Such diseases can only be controlled by screening and treating populations that are at risk from them. Because of the serious morbidity and mortality of congenital syphilis, prenatal screening is cost-effective even when the prevalence of infection is low²¹.

Generally, diagnostic tests are more important for the case-management of parasitic than bacterial infections, as the latter can often be treated syndromically with broad-spectrum antibiotics or with combinations of antibiotics, whereas parasitic infections often require specific treatment. This does not apply to bacterial infections such as TB, **leprosy**, or **brucellosis** in which prolonged or specific treatment is needed. However, even for diseases such as **pneumonia**, diarrhoea and sexually transmitted infections that are often treated syndromically, there is a need for periodic diagnostic laboratory support, using conventional microbiological techniques on clinically relevant samples, to identify changes in disease spectrum and antimicrobial susceptibility.

Availability, expense and toxicity of treatment. Most deaths from malaria occur in African children. Because death may occur soon after the onset of symptoms, and because microscopic diagnosis may not be readily available, presumptive treatment with chloroquine has been recommended for all febrile children in malaria-endemic areas²². This was a feasible option in the past, as chloroquine is cheap and widely available. Unfortunately, in recent years, chloroquine-resistant strains of *Plasmodium falciparum* — the most virulent of the malaria parasites — have spread rapidly, and are now prevalent in most areas²³. As alternative treatment regimens are significantly more expensive, it could now be more cost-effective to use a simple dipstick test to identify children with malaria before treatment, rather than to treat all children presumptively. Clearly, this would have the added benefit of identifying febrile children who do not have malaria and may

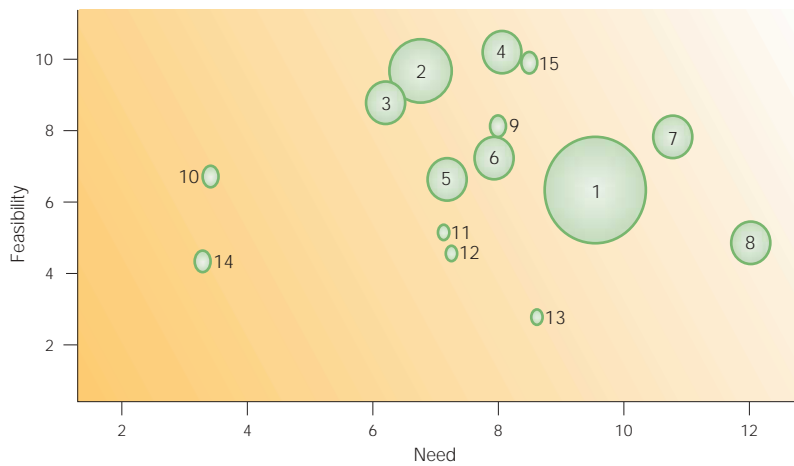


Figure 2 | Scheme to assess priorities for diagnostics development for selected diseases in the developing world. The horizontal axis shows the need for the test; the vertical axis shows the feasibility of developing such a test; and the size of the circle indicates the relative burden of disease in disability-adjusted life-years (DALYs) that could be prevented if it were widely used. Both scales are arbitrary. 1, tuberculosis case-management; 2, malaria case-management; 3, malaria test-of-cure; 4, syphilis case-management; 5, visceral leishmaniasis test-of-cure; 6, schistosomiasis case-management; 7, visceral leishmaniasis case-management; 8, African trypanosomiasis case-management; 9, lymphatic filariasis case-management; 10, dengue case-management; 11, leprosy case-management; 12, lymphatic filariasis test-of-cure; 13, leprosy — detection of latent disease; 14, Chagas disease (South American trypanosomiasis); 15, onchocerciasis case-management.

need treatment for other conditions. In regions that experience seasonal malaria — where multidrug resistance is common and microscopy services are rare — rapid diagnostic tests have already proved to be cost-effective^{24,25}.

In the case of visceral leishmaniasis and African trypanosomiasis, treatments are expensive and toxic, and must be given intravenously for prolonged periods. Presumptive treatment can never be justified, and diagnostic tests that are both sensitive and specific are a high priority (BOX 2).

Feasibility of global or local disease elimination. Several infectious diseases are now being targeted for global or regional elimination, including polio, guinea worm, leprosy, **trachoma**, **onchocerciasis**, syphilis, South American trypanosomiasis and lymphatic **filariasis**. Paradoxically, as the goal of elimination approaches, the need for surveillance to detect persistent foci of

infection increases. A decreasing disease burden, however, is often accompanied by decreasing resources, as the perceived importance of diagnostic testing diminishes and surveillance is no longer perceived to be cost-effective. The re-emergence of endemic treponemal infections (for example, yaws) after their virtual elimination in the 1960s resulted from a breakdown in surveillance²⁶. It is important that the lasting benefits that are conferred by the elimination of diseases from areas in which they have been endemic should be taken into account when deciding priorities.

Feasibility of developing an appropriate test. Some of the new diagnostic tests that are needed are already on the market, and seem likely to perform as required in the context of disease-control programmes, but have not been adequately evaluated. In other cases, diagnostic targets have been identified, and it seems likely that an adequate test could be developed using existing technology; in these cases, feasibility is high. In other cases, diagnostic targets have not yet been identified, or it does not seem likely that adequate sensitivity can be achieved with existing technology using the specimen that is available for testing in the field. In these cases, feasibility is lower.

FIGURE 2 shows the priorities for public-sector investment in diagnostics development for specific tropical-disease indications, taking into account need, feasibility and disease burden. It can be seen from the figure that a more sensitive test than sputum microscopy for the diagnosis of TB would alleviate the greatest burden of disease. The greatest medical need is for better tests to diagnose two neglected tropical parasitic diseases — African trypanosomiasis and visceral leishmaniasis. More widespread availability of simple and accurate dipstick tests for *P. falciparum* (malaria) would alleviate a great burden of disease in Africa.

Characteristics of the ideal test

For resource-limited settings, the WHO Sexually Transmitted Diseases Diagnostics Initiative has coined the term ‘ASSURED tests’ to describe the ideal characteristics of a diagnostic test (BOX 3). It will not be easy to develop an ASSURED test that is competitive with the best laboratory-based assays. However, striving for the best test should not be allowed to prevent the development of the most useful test. An ASSURED test that is less sensitive than a test carried out in the laboratory may result in more infected people receiving treatment, as not all patients return for the results of tests that are sent to the laboratory²⁷. So, the best test is not always the most useful test. What are the prospects for these sorely needed diagnostic tests being developed and made available to those who need them?

Barriers to the development of new tests
The process of developing a new diagnostic test and bringing it to the market is illustrated in FIG. 3. Although the overall cost is likely to be at least an order of magnitude lower than the cost of bringing a

Box 3 | Characteristics of the ideal diagnostic test — ASSURED

- Affordable by those at risk of infection.
- Sensitive (few false-negatives).
- Specific (few false-positives).
- User-friendly (simple to perform and requiring minimal training).
- Rapid (to enable treatment at first visit) and Robust (does not require refrigerated storage).
- Equipment-free.
- Delivered to those who need it.

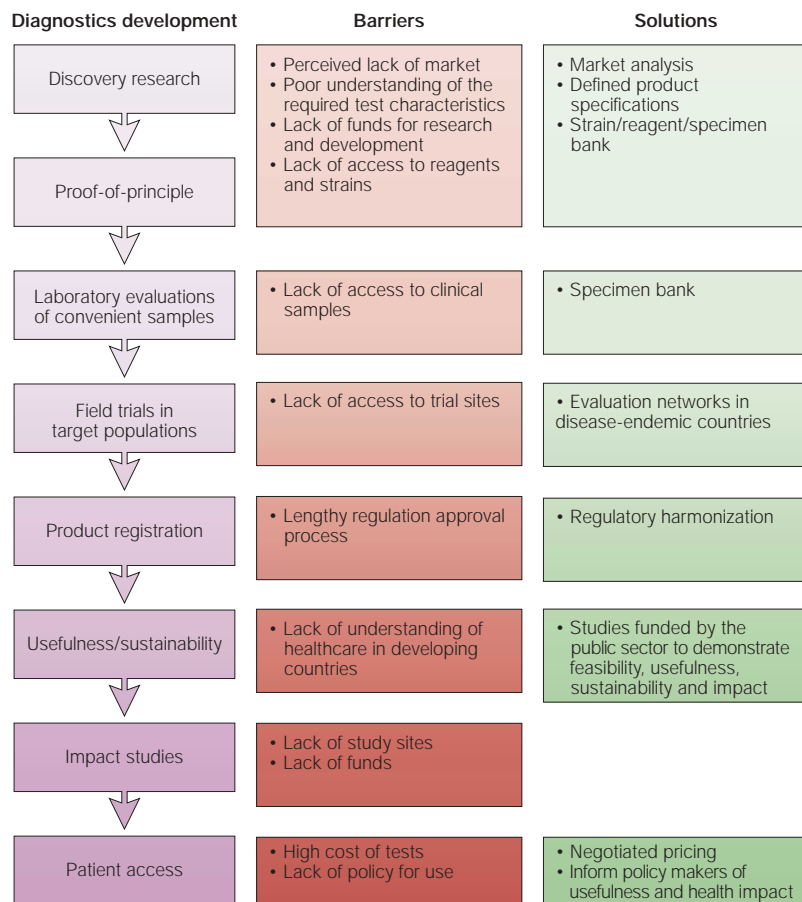


Figure 3 | **Diagnostics development: steps, barriers and solutions.** Development of a diagnostic test from discovery research to test use.

new drug to the market, the barriers remain formidable. However, in recent years new donors have invested large sums in health programmes for developing countries, creating new markets for vaccines and, potentially, drugs and diagnostics that meet the needs of developing countries (for example, [The Global Fund to Fight AIDS, Tuberculosis and Malaria](#), [The Medicines for Malaria Venture \(MMV\)](#) and [The Global Alliance for Vaccines and Immunisation \(GAVI\)](#)). Several public-sector initiatives aim to lower the barriers to test development and to provide solutions to some of the problems that are shown in FIG. 3.

New and ongoing initiatives

In the past decade, public-sector investment in diagnostic-test development has been largely investigator-driven. The United States National Institutes of Health funds test development through its grants to small businesses (see the [Small Business Innovation Research \(SBIR\) programme](#) website). The Wellcome Trust has also supported diagnostics research and development, as do the United States Centers for Disease Control and Prevention and the defence departments of some developed countries. Most

recently, the Bill and Melinda Gates Foundation announced a US \$200 million 'Grand Challenge' for addressing inequities in global health. One of its priority areas for funding is the development of technologies that would allow the assessment of individuals for multiple conditions or pathogens at the point of care. A number of initiatives for specific diseases have also focused on diagnostics development in recent years.

TDR. The UNICEF/United Nations Development Programme/World Bank/WHO [Special Programme for Research and Training in Tropical Diseases \(TDR\)](#) was established in 1975 to conduct research aimed at the development of new tools for the control of tropical diseases and to train researchers from disease-endemic countries. The tools, which include drugs, vaccines and diagnostics, are developed through public-private partnerships. Current TDR priorities for diagnostics include visceral leishmaniasis, human African trypanosomiasis, [schistosomiasis](#), malaria and TB. Its diagnostic-development activities for TB led to the creation of the [Foundation for Innovative New Diagnostics \(FIND\)](#) in 2003.

FIND. Recognizing that the biotechnology revolution of the past few decades has not resulted in a significant change in diagnostic practices in disease-endemic countries, a new, independent, not-for-profit entity — FIND — was recently created by the Bill and Melinda Gates Foundation to respond specifically to the need for better diagnostics in the developing world. FIND is unique as it is the only not-for-profit organization that is dedicated wholly to the development of diagnostic tests for infectious diseases. It was created to overcome the obstacles that have blocked academic, governmental and corporate entities by moving promising ideas through a developmental pipeline and ensuring their implementation by public health systems in order to decrease global health inequities. By harvesting the best biotechnologies that are available, and developing and validating new tests for use in patient management, disease control, and surveillance, FIND aims to create a model for public action that addresses the current failure of market forces to provide diagnostics for neglected diseases.

SDI. The SDI was founded in 1990 in response to a widely perceived need to improve care for patients with sexually transmitted infections (STIs) in resource-limited settings through improved diagnostics. The mandate of the SDI is to promote the development, evaluation and application of affordable, rapid, simple point-of-care STI diagnostics for resource-limited settings. The placement of the SDI in the Product Research and Development group of the TDR has allowed the initiative to benefit from the considerable expertise in product development, evaluation and implementation within the group and to exploit synergies in the development of diagnostics for other communicable diseases.

Box 4 | New technology and diagnostic-test development

Solid-phase separation techniques

Diagnostic targets in clinical specimens can be concentrated by using solid-phase separation techniques, such as magnetic beads and other specialized matrices, to bind to the targets of interest and concentrate them before testing.

Isothermal amplification technology

This could be adapted to a point-of-care format: the amplification of target nucleic-acid sequences using techniques such as the polymerase chain reaction (PCR) can improve test sensitivity up to 100-fold over antigen-detection tests, such as enzyme immunoassays. The first generation of nucleic-acid amplification technologies (NAATs) require complicated instrumentation for temperature cycling. Single-temperature or isothermal amplification has been developed and can now be packaged in a self-contained device, or can be detected through an inexpensive lateral-flow device in a single step, without wash stages⁵¹.

Real-time detection of NAAT products

Methods for real-time detection of NAAT products are now available that give quantitative results in as little as 20 minutes. Nucleic-acid sequence-based amplification (NASBA) technology has been used to develop a test that can give results for human immunodeficiency virus viral load in plasma in less than 2 hours. The entire reaction is contained in a single closed tube that requires minimal specimen handling and poses little risk of contamination⁵².

Improved efficiency of detection systems

In conventional POC lateral-flow assays, a positive test is indicated by a coloured line. This represents only the top 10 µm of a reaction that is contained in a matrix several hundred microns thick. The sensitivity of these assays can be enhanced 10–100-fold using silver-enhancement techniques and high-quality antibodies. Another means of enhancing test sensitivity involves the use of colloidal magnetic nanoparticles, called paramagnetic particles (PMPs). By linking or conjugating biological ligands to the PMPs, the target of interest can be captured and concentrated after being separated from the sample matrix. As PMPs are only magnetic when they are placed in a very strong magnetic field, the PMP technique allows the entire volume of labelled analyte to be quantified, thus increasing test sensitivity by several orders of magnitude over optically read lateral-flow assays⁵³. Another advantage is that, unlike optical assays, few biological substances have magnetic properties that would interfere with this assay. Magnetic-assay readers are being developed that are the size of a paperback book and can be handheld and battery-powered.

Opportunities for innovation in diagnostics
Apart from dramatic increases in public-sector investment in diagnostics development, there are also unprecedented opportunities for innovations in diagnostics development due to the convergence of advances in two important areas: first, the potential for the discovery of novel diagnostic targets or biomarkers through genomics, and an increased understanding of pathogen virulence factors and host gene expression using microarrays and other approaches; and second, rapid technological advances in areas such as material science, nanotechnology and microfluidics, which have been fuelled recently by the drive for the rapid detection of potential bioweapons.

Improved understanding of pathogens and host responses to infection. The sequences of the human genome and those of more than 1,000 viruses and 135 bacteria are now known^{28,29} (see the [Entrez Genome](#) database and the [Wellcome Trust Sanger Institute](#) web site) and comparative genomics has already yielded promising developments in diagnostics. For TB, for example, the identification of genomic deletions in vaccine strains of bacille Calmette–Guérin (BCG) has allowed the development of tests that can distinguish

cellular immune responses caused by latent TB infection from those that result from prior vaccination.

DNA microarrays are being used to study pathogen and host gene expression in response to infection and may result in the discovery of novel diagnostic targets or disease biomarkers³⁰. Microarrays allow the monitoring of thousands of biomolecular interactions using exquisitely small volumes of reagents dotted on a single glass slide. This technology has also been adapted to monitor pathogen mutations that give rise to drug resistance. For malaria, for example, segments of DNA from regions of interest — such as the dihydrofolate reductase and dihydropteroate synthetase genes (targets of pyrimethamine and sulfadoxine, respectively) — can be dotted onto a glass slide and hybridized to DNA from clinical isolates. Point mutations can be readily detected, as complementary hybridization targets give a stronger signal than those that contain mutations. With advances in materials science, it is now possible to make the microarrays on metal-oxide substrates, which will allow flow-through incubations instead of discrete single reactions (for example, see the Netherlands Royal Tropical Institute (KIT) microarrays web page).

Technological advances. The challenge of developing ASSURED tests is that POC tests are expected to give laboratory-quality test results but should be simple to perform, requiring minimal training of users. All the chemistry must occur in small volumes and must be packaged into a self-contained device that is portable and stable at room temperature. The cost can be lowered by multiplex testing for several infections within a syndrome, for example, by using a test that could diagnose syphilis, **chancroid** and herpes-simplex-virus infection in patients with genital-ulcer syndrome. Such multiplex tests would be more useful clinically, but may bring additional complexity to test development or compound royalty costs. Although the challenge of developing such tests requires long-term investment, there is reason to hope for near-term improvements in test performance by adapting existing technology. Some examples of how this can be achieved are shown in BOX 4.

Long-term innovations. The ultimate goal is to have a 'lab on a chip' that fulfils the ASSURED criteria (BOX 3). Nanotechnology has allowed the miniaturization of complex reaction processes into small, self-contained packages. Microelectromechanical systems (MEMS) have been exploited to develop personal computers and cell phones that operate from a microchip made from a silicon wafer. Biomedical applications of MEMS are called bioMEMS or microfluidics³¹. Such technology allows specimen processing, biochemical reactions and detection of the resulting product in a flow-through format with on-chip control of thermo-pneumatic pumps, micro-heaters and temperature sensors, miniaturized fluorescence detectors, sample/analyte concentrators and filters. Some of the novel sensor technologies, such as surface plasmon resonance (SPR) and mirror resonance technology, allow for the analysis of three-dimensional biomolecular interactions in real-time, without the need

for labels or purification. Changes in the SPR signal are monitored through a reduction in the reflected light intensity with regard to both angle and wavelength.

Fibre-optic technology that is based on long period grating (LPG) and optical-fibre sensors has generally been promoted as a breakthrough for telecommunications. LPG sensors with specially designed affinity coatings, such as monoclonal antibodies, can bind target molecules and can be adapted for multiplex diagnostics detection. Such technology is useful for screening combinatorial-chemistry libraries, examining large arrays of candidate drugs and discovering new pharmaceuticals. As wavelength is a direct measurement of the bound mass and is a characteristic of the individual fibre profile and grating period, this can be adapted for use in diagnostics where the difference in wavelengths between the control and the test sample is directly proportional to the concentration of bound analyte. This type of technology provides accurate analyte quantification to parts-per-trillion levels within 5 minutes. The technology can be developed into a compact, lightweight biochemical-detection platform that is suitable for both centralized (laboratory) and decentralized (point-of-care) clinical settings³².

With these innovations, there is the capacity to develop and manufacture new diagnostic tests in middle-income countries, such as China, Thailand, India and South Africa. Some of these manufacturing facilities are International Organization for Standardization (ISO)-certified. Prototype tests that have been shown to perform well in target populations can, hopefully, be manufactured in vast quantities at reasonable prices.

The convergence of knowledge of pathogen-host interactions with the remarkable advances made in biotechnology during the past few decades should offer a great opportunity for the development of 'leapfrog' technologies that overcome many of the diagnostic problems faced by patients and healthcare workers in developing countries. Putting sophisticated reagents and detection systems into easy-to-use formats that deliver patient-empowering point-of-care information is a goal that now seems achievable. Applying this technical capacity to the enormous medical needs of developing countries, despite the lack of obvious commercially attractive markets, is one of the grand challenges for the public sector. To be successful, this will require sustained investment, political will, partnership between the public and private sectors, innovative finance mechanisms and focused tool-development efforts driven by medical need.

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Competing interests statement

The authors declare that they have no competing financial interests.

 **Online links**

DATABASES

The following terms in this article are linked online to:

Infectious Disease Information:

<http://www.cdc.gov/ncidod/diseases/index.htm>
 AIDS | brucellosis | chancroid | *Chlamydia trachomatis* | filariasis | gonorrhoea | guinea worm | leishmaniasis | leprosy | malaria | measles | onchocerciasis | pneumonia | polio | schistosomiasis | smallpox | syphilis | trachoma | *Trypanosoma brucei gambiense* | tuberculosis |

FURTHER INFORMATION

Entrez Genome:
<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Genome>

Foundation for Innovative New Diagnostics (FIND):
<http://www.finddiagnostics.org/>

Medicines for Malaria Venture (MMV):
<http://www.mmv.org>

Netherlands Royal Tropical Institute (KIT) microarrays web page:
http://www.kit.nl/biomedical_research/html/microarrays.asp

Program for Appropriate Technology in Health:
<http://www.path.org/>

Small Business Innovation Research (SBIR) programme:
http://grants.nih.gov/grants/funding/sbirstr_programs.htm

Special Programme for Research and Training in Tropical Diseases (TDR): <http://www.who.int/TDR>

The Global Alliance for Vaccines and Immunisation (GAVI):
<http://www.vaccinealliance.org>

The Global Drug Facility for TB: <http://www.stoptb.org/GDF>

The Global Fund to Fight AIDS, Tuberculosis and Malaria:
www.theglobalfund.org

The World Health Organization (WHO): <http://www.who.int/>

Wellcome Trust Sanger Institute: <http://www.sanger.ac.uk/>

WHO HIV Diagnostics:
http://www.who.int/bct/Main_areas_of_work/BTS/HIV_Diagnostics/HIV_Test_Kit_Evaluation.htm

WHO Malaria Rapid Diagnostics Tests:
<http://www.wpro.who.int/rdt>

WHO Sexually Transmitted Diseases Diagnostics Initiative:
http://www.who.int/std_diagnostics

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