Critical Reviews in Clinical Laboratory Sciences, 2010; 47(3): 139–169

informa
healthcare

REVIEW ARTICLE

Biosensors as rapid diagnostic tests for tropical diseases

Fernando Sérgio Rodrigues Ribeiro Teles[1], Luís Alfredo Pires de Távora Tavira[1], and Luís Joaquim Pina da Fonseca[2]

[1]Centre for Malaria and Tropical Diseases, Instituto de Higiene e Medicina Tropical, Lisboa, Portugal, and [2]Institute for Biotechnology and Bioengineering, Centro de Engenharia Biológica e Química, Instituto Superior Técnico, Lisboa, Portugal

Abstract

Effective diagnosis of infectious pathogens is essential for disease identification and subsequent adequate treatment, to prevent drug resistance and to adopt suitable public health interventions for the prevention and control of epidemic outbreaks. Particular situations under which medical diagnostics operate in tropical environments make the use of new easy-to-use diagnostic tools the preferred (or even unique) option. These diagnostic tests and devices, usually based on biosensing methods, are being increasingly exploited as promising alternatives to classical, "heavy" lab instrumentation for clinical diagnosis, allowing simple, inexpensive and point-of-care testing. However, in many developing countries the lack of accessibility and affordability for many commercial diagnostic tests remains a major cause of high disease burden in such regions. We present a comprehensive overview about the problems of conventional medical diagnosis of infectious pathologies in tropical regions, while pointing out new methods and analytical tools for in-the-field and decentralized diagnosis of current major infectious tropical diseases. The review includes not only biosensor-based rapid diagnostic tests approved by regulatory entities and already commercialized, but also those at the early stages of research.

Keywords: *Bioanalytical; biochip; biomolecular; diagnosis; infection; lab-on-a-chip; microfabrication; nanobiotechnology; point-of-care; surveillance*

Abbreviations: **AIDS**, acquired immunodeficiency syndrome; **ASSURED**, *a*ffordable, *s*ensitive, *s*pecific, *u*ser-friendly, *r*apid, robust, *e*quipment-free, *d*eliverable, ideal characteristics of a diagnostic test; **bioMEMS**, biomicromechanical systems; **BSA**, bovine serum albumin; **CAA**, circulating anodic antigen; **CCD**, charge-coupled device; **CLIA**, Clinical Laboratory Improvement Amendments; **CNT**, carbon nanotube; **DAT**, direct agglutination test; **DNA**, deoxyribonucleic acid; **EIA**, enzyme immunoassay; **EIS**, electrochemical impedance spectroscopy; **FDA**, Food and Drug Administration; **FGT**, formol-gel (aldehyde) test; **FIND**, Foundation for Innovative New Diagnostics; **FRET**, fluorescence resonance energy transfer; **HAT**, human African trypanosomiasis; **HI**, hemagglutination inhibition; **HIV**, human immunodeficiency virus; **HRP**, horseradish peroxidase; **HRP-2**, histidine-rich protein-2; **IgG**, immunoglobulin G; **IgM**, immunoglobulin M; **ITO**, indium-tin oxide; **LAMP**, loop-mediated isothermal amplification; **LCA**, latent class analysis; **LED**, light-emitting diodes; **MAC**, monoclonal antibody capture; **mAECT**, mini anion exchange centrifugation technique; **MS**, mass spectrometry; **μTAS**, micro total analytical systems; **NASBA**, nucleic-acid sequence-based amplification; **NS1**, nonstructural 1; **OC**, oligochromatography; **PCR**, polymerase chain reaction; **PDMS**, polydimethylsiloxane; **PGL-1**, phenolic glycolipid-1; **pLDH**, lactate dehydrogenase; **PNA**, peptide nucleic acid; **QD**, quantum dot; **RNA**, ribonucleic acid; **rRNA**, ribosomal ribonucleic acid; **RT**, reverse-transcription; **SAM**, self-assembled monolayer; **SARS**, severe acute respiratory syndrome; **S/N**, signal-to-noise; **SPR**, surface plasmon resonance; **Tat**, trans-activator of transcription; **TDR**, *t*ropical *d*iseases *r*esearch; **TB**, tuberculosis; **UN**, United Nations; **UNICEF**, United Nations Children's Fund; **UPT**, up-converting phosphor technology; **VL**, visceral leishmaniasis; **VSG**, variant surface glycoproteins; **WHO**, World Health Organization

Referee: Dr. Robert S. Marks, Department of Biotechnology Engineering, National Institute for Biotechnology in the Negev, Ben-Gurion University of the Negev, Beer-Sheva, Israel

Address for Correspondence: Dr. Fernando Teles, Center for Malaria and Tropical Diseases, Instituto de Higiene e Medicina Tropical, Rua da Junqueira 100, 1349-008 Lisboa, Portugal. E-mail: fteles@ihmt.unl.pt

(Received 09 March 2010; revised 09 August 2010; accepted 23 July 2010)

ISSN 1040-8363 print/ISSN 1549-781X online
DOI: 10.3109/10408363.2010.518405

Introduction

Infectious diseases cause millions of human deaths every year,[1] with more than 95% occurring in poor and developing countries,[2] generally in the tropical and sub-tropical regions of the world. The emergence of many such diseases over past decades can be mainly attributed to the demographic explosion in those countries and the failure of control and eradication programs.[3] In parallel, many of these afflictions, especially vector-borne diseases, have also become serious threats to more temperate and developed countries. This is due to increased international traveling and subsequent exposure of susceptible individuals to deadly pathogens, as well as to global warming in such countries.

In the absence of specific treatments and prophylaxis for many of these diseases, definite diagnosis may be of decisive importance. However, in the early phase the symptoms of many tropical infectious diseases are usually nonspecific; they cannot be clinically distinguished from those of other infections, therefore requiring definite laboratory confirmation. Improved diagnostic tests are necessary for asymptomatic diseases, diseases with misleading symptoms, diseases requiring different treatments, or for diseases that by their complex or costly treatment require previous case confirmation. Even for infections indicated for syndromic treatment for which there is no clinical need to identify the causal agent, new diagnostic tools may be required for surveillance and control purposes in order to deal with the risk of major epidemics. Diagnostic strategies need to be effective not only in resource-limited areas of endemicity, but also in developed countries where expertise in diagnosis of tropical diseases is lacking.[4]

In general, isolation of infectious microorganisms is the most direct and conclusive approach to medical diagnosis despite being highly undesirable as a first-line approach in endemic situations. Moreover, isolation and classification of the etiological agent can take days, and the demand for high-level equipment, technical skills and manpower may be limiting.

Serological techniques are simpler than isolation and direct detection of the pathogen, but usually require several days before a sufficient amount of antibodies is produced. Serology is able to yield straightforward interpretations even after the first exposure to the pathogen and after prolonged exposure to a tropical climate. However, in practice serological diagnosis is less specific than diagnosis by culture.

Polymerase-chain reaction (PCR) and other PCR-based automated techniques make use of the nucleic-acid hybridization process, but require only electrophoresis of the amplified product, whereas conventional hybridization requires labeling, purification and standardization of probes. Despite its ability to potentially deliver unlimited sensitivity and amplification, PCR-based methods are usually too complex, prone to easy contamination, require skilled manpower and bulky equipment for in-the-field applications, and sample storage at very low temperatures; a difficult task in more remote regions.[5]

The enormous amount of genetic information brought by extensive genome sequencing has led to a need for simple, fast, cheap, high-throughput miniaturized and mass-producible analytical devices for the growing market of molecular diagnostics, thus accomplishing the basic criteria for large-scale and decentralized biomolecular testing. In this regard, microchips (also called microarrays or biochips) constitute one of the major trends towards the research of novel diagnostic systems allowing the monitoring of thousands of biomolecular interactions using very small reagent volumes dotted on a single glass slide. However, their efficiency is usually hampered by the large size of biological samples and by their complex treatment (which also makes it difficult to obtain real-time outputs), difficulty of scaling down the array density, limited resolution and strong sample concentration dependence.[6] Microarray technology is still too expensive to become, in the short term, valuable for point-of-care diagnosis.

Despite the fact that some technological improvements have met the needs for application in the field, the available techniques often fail to accomplish the basic requirements for a successful diagnosis in tropical sub-developed countries, namely simplicity, economy, rapidity and accuracy.[7] Such new diagnostic tools must also meet the challenges of the evolving regulatory, payer and patient requirements, as well as the new business and economic realities.[8]

Unlike bioassays or conventional bioanalytical systems, biosensors do not require additional processing steps (*e.g.* reagent addition), and are characterized by permanent fixing of the assay design in the construction of the device.[9] Biosensors are usually designed to be highly selective, sensitive, fast, portable, easy to operate, reusable, cheap, and in general, response-proportional. In this sense, they differ considerably from microarrays. Many commercial biosensors rely on dipstick or immunochromatographic formats, very suitable for single-case diagnosis in field applications.

The ultimate goal of decentralized testing may be the fabrication of lab-on-a-chip devices, essentially an adaptation of microchips with channels and chambers for biorecognition in liquid rather than solid phase, similar to the *in vivo* conditions. They integrate, in a single platform, modules for specimen processing,

amplification, biochemical reactions and product detection. Disposability is also an advantage, especially when dealing with infectious agents; it not only eliminates the problem of the biohazardous nature of infected body fluid wastes, but also avoids the need for washing steps between sample preparations, a serious burden in many tropical regions poorly supplied with clean water.[10] Other advantages include lower contamination, reagent consumption, time of detection and cost, as well as enhanced rapidity, performance and automation ability. Nevertheless, many lab-on-a-chip devices still require off-line sample preparation and reagent handling, and are therefore unsuitable for routine home testing.[11]

Nanobiotechnology has been an emergent theme for development and production of new bioanalytical procedures, structures and systems. It is possible to view, in technological and industrial terms, the fabrication of future nanochips and nanofluidic systems as an extension of current mechanical methods for production of microsensing devices, but the need for high-throughput analysis is creating a demand for a broader range of low-cost and easy fabrication methods. This may correspond essentially to shifting from top-down to bottom-up methods, which are prone to cheap and easy production of small nanostructures.

On the other hand, mass spectrometry (MS) techniques have been used for a long time for pathogen detection and quantification using bulky laboratory apparatus. Unlike rapid and simple biosensing devices, MS is able to identify pathogens in samples of unknown composition, which constitutes a different paradigm in biodetection. MS techniques allow rapid biodetection, a high degree of automation and software control and no need for specialized manpower. However, they are handicapped by difficult device miniaturization, high complexity and high-power instrumentation. As such, it will be a long time before MS-based platforms can fulfill the necessary requirements for application in point-of-care diagnosis in the field.

One of the most important characteristics of rapid diagnostic tests is the ability to provide quick results, an essential feature to avoid delays in starting the appropriate therapy, thus leading to reduced mortality and hospitalization costs.[12] Delayed diagnosis can ultimately result in longer patient suffering, increased pathogen toxicity and drug misuse, higher probability for sudden of more severe symptoms and complications and increased risk for acquisition of drug resistance.

The unprecedented opportunities for innovations in new diagnostic tools have benefited from the increased potential for the discovery of novel diagnostic biomarkers through genomics and proteomics, better understanding of pathogen virulence factors and host gene expression, and developments in cutting-edge materials science and nanotechnology. The application of this wide technical capacity to the enormous medical needs of developing countries is a major challenge for the public sector, given the lack of obvious attractive commercial markets for private entrepreneurs. The following text will attempt to cover the general context of diagnosing tropical infectious diseases, especially in developing countries. Old diagnostic techniques and new rapid tests based on biosensor technology will be reviewed, as well as the status of their application for the medical diagnosis of some of the main infectious tropical diseases.

The threat of tropical infectious diseases

Overall, communicable and non-communicable diseases account for several dozen million individual deaths each year, for which infectious and parasitic diseases correspond to approximately 35%.[1] From these, around 1 million die annually from malaria, 4.3 million from acute respiratory infections, 2.9 million from enteric infections and 5 million from AIDS and tuberculosis (TB). Other parasitic and sexually transmitted infections account for hundreds of thousands of deaths. More than 95% of resulting deaths occur in poor and developing countries mainly located in the tropical and sub-tropical regions of the world.[2] In addition, increasing human populations, human migrations and the collapse of vector control programs (frequently the most effective measure for preventing disease) in many countries, have led to outbreaks of many of such pathologies.[3] Among the most serious emergency health care drawbacks in endemic countries is transfusion with contaminated blood, since screening of donors does not include monitoring of many infectious agents able to infect either whole blood or some blood components.[13]

At the same time, many endemic diseases of tropical and sub-tropical regions are also potential threats for more temperate regions. The threat of their expansion comes mainly from an increase in air travel, which transports travelers to endemic areas, as well as conveying pathogens, their vectors and patients themselves to previously disease-free regions, thus putting susceptible (non-immunized) humans in close and prolonged contact with tropical pathogens. Among travelers, special attention and care must be paid in the case of vector-borne diseases, since they may result not only from severe infections brought from endemic countries (where, very often, they occur in the form of epidemical outbreaks),[14] but also from infections acquired post-traveling, *i.e.* in the returning

countries, as a result of vector importation (*e.g.* in airline-shipped luggage). Most of the infectious diseases in the tropics are treatable, but accurate identification of patients requiring treatment remains a difficult task for disease control. Improved diagnostic tests will likely have little or no impact for diseases which are easily recognized by their clinical symptoms, and for which syndromic treatment is recommended. Moreover, there is no clinical need to identify the causal agent of these diseases. By contrast, these tests are necessary for asymptomatic diseases, for diseases with misleading symptoms, for diseases requiring different treatments, or for diseases that by their complex or costly treatment require previous case confirmation. However, even for easily recognizable diseases, surveillance and ongoing control measures must be maintained in order to deal with the risk of major epidemics. Depending on the control strategy, better diagnostic tests may be needed for case-finding (identification of nearly asymptomatic infection cases) and case management, and for improvement of disease surveillance. On the other hand, surveillance enables program managers to monitor the effectiveness of intervention strategies and to identify which populations require continuing interventions.

The importance of diagnosis

Many disease control programs around the world, including those for malaria and tuberculosis (TB), have been threatened by the rise of drug resistance from pathogens and vectors, with a subsequent increase in treatment costs, complexity and effectiveness.[2] In the absence of specific treatments and prophylaxis for many of those diseases (for instance, the development of safe and effective vaccines requires laboratory testing, production and validation), diagnosis may be of decisive importance. However, specifically in the early phase, the symptoms of many tropical infectious diseases are usually nonspecific; they cannot be clinically distinguished from those of other infections and therefore require definite laboratory confirmation. For many diseases such as malaria the high accuracy of clinical diagnosis is hindered, even in regions with high incidence rates, by indistinct clinical symptoms and also by co-infections.[15] The importance of differential diagnosis is even greater in cases where at least one of the cross-symptomatic infections requires specific treatment.

For many tropical disorders, the best diagnostic tools currently available require laboratory staff and equipment. Simpler tests are urgently needed for diagnosis of most tropical afflictions especially in primary health care centers in order to reduce errors both in sample preparation and in result interpretation. The increasing number of reported cases of tropical infections amongst European and US tourists reflects both the preference for holidays in endemic areas and the reluctance to take prophylactic medication.[16] The lack of acquired immunity of these travelers too often leads to rapid fatal outcomes without early diagnosis. This situation is about to worsen as global warming is rapidly spreading vectors and pathogens of tropical infections to non-endemic regions, including southern Europe and the US. Thus, diagnostic strategies need to be effective not only in resource-limited areas of endemicity but also in developed countries where expertise in diagnosis of tropical diseases is frequently lacking,[4] a serious drawback for disease management in travelers returning home.

The importance of simple and affordable diagnostic tests for infectious diseases, based on modified molecular technologies, has been rated ahead of that of vaccines or medicines.[17]

Classical methods of laboratory diagnosis

In general, isolation of infectious microorganisms is the most direct and conclusive approach to medical diagnosis. In fact, since the nineteenth century clinical microbiology has basically evolved around culture, microscopic analysis and staining, a paradigm that only recently has changed with the advent of molecular biology tools, which have the additional advantage of assessing bacterial resistance.

Microscopic examination is the "gold-standard" test for confirmatory diagnosis of many tropical infectious pathologies, despite being highly undesirable as a first-line approach in endemic situations. Moreover, some days are often required for isolation and classification of the etiological agent, and usual demand for high-level equipment, technical skills and manpower may be limiting. As such, the highest sensitivities are rarely obtained outside specialized laboratories.[18] The whole process of preparing specific enrichment media to separate, identify and count bacterial cells may take a few days after collecting the test sample.[19]

Culture enables determination of antimicrobial susceptibilities, but in tropical environments is hampered by the need for stringent transport conditions to maintain specimen viability and a constant supply of reagents and electricity.[2]

Serological techniques are simpler than isolation and direct detection of the pathogen, but usually require some days until a sufficient amount of antibodies is produced. Serology is able to yield straightforward interpretations even after the first exposure to the pathogen (unlike in cases of previous infections)

and suitable for testing even after prolonged exposure to a tropical climate because antibodies are not easily inactivated by harsh treatment of specimens. In addition, serological techniques are relatively simple and some reagents are commercially available. However, in practice, serological diagnosis is, in general, less specific than diagnosis by culture, and usually requires paired specimens (as is the case of the well-known hemagglutination-inhibition (HI) test), which delays diagnosis.

Alternatively, conventional methods for specific genomic sequence analysis include nucleic acid sequencing and hybridization between a known DNA sequence (probe) and the complementary chain (target). Among them, nucleic-acid hybridization is the more routinely used in clinical laboratories, due to its higher simplicity.[20]

Meanwhile, new biomolecular approaches for diagnostics have been fueled by strong societal and governmental demands. They offer the advantage of assay running in a highly parallel format on a cost-effective basis and with considerable time savings which may be important for point-of-care testing. Criticism about molecular diagnostics usually relies on the fact that positive-tested samples do not always correspond to current infections and that, among these, not all lead to disease. However, techniques for molecular diagnosis are singularly useful for detection of microorganisms that are not easily cultivated *in vitro* or when methods based in growing cultures and microscopy have low sensitivity, or when they are too expensive, laborious, unreliable or unavailable.[21]

PCR and other PCR-based automated techniques make use of the nucleic-acid hybridization process, but require only electrophoresis of the amplified product, whereas conventional hybridization requires labeling, purification and standardization of probes. Despite its ability in principle to render unlimited sensitivity and amplification, PCR-based methods are usually too complex (requiring meticulous manipulation for optimal preparation of specimens), prone to easy contamination (leading to false-positive results) and require skilled manpower and bulky equipment for applications in the field.[5] The storage of serum samples at very low temperatures may be essential, for example, for maintenance of nucleic-acid samples in optimal conditions, a hard task in many remote regions. While the development of real-time TaqMan® PCR provides some improvements over conventional PCR, it does not avoid the somewhat time-consuming two-step amplification, composed of a reverse-transcription (RT) step (for DNA production from RNA genomic material, *e.g.* in the case of RNA virus) and an expensive second thermal cycling PCR step (RT-PCR). Ultrafast PCR can already achieve more than 30 cycles in less than 15 min. This has been mainly achieved by reducing the solution volume to be cycled or by pumping fluid through serpentine or spiral flowing channels with alternating temperature zones. Other DNA amplification techniques are rolling-circle amplification, strand-displacement amplification, RT loop-mediated amplification and helicase-mediated amplification. These methods operate isothermally, which greatly simplifies instrument design while being generally less efficient than PCR.[8]

Compared to immunoassays (in which antibody raising usually requires a long time), genetic testing can be detected in real time as soon as the patient has been infected. As molecular techniques become increasingly efficient and more widely used, resultant lower costs per test will bring greater applicability and implications of molecular diagnosis for the clinics.[21]

Microarrays

The enormous amount of genetic information provided by extensive genome sequencing has raised the need for simple, fast, cheap and high-throughput miniaturized and mass-producible analytical devices for the growing market of molecular diagnostics, thus accomplishing the basic criteria for large-scale and decentralized biomolecular testing. Genome sequencing has allowed the detection of inherited disease-causing point mutations and human pathogens through their particular specific nucleic acid sequences. Drug screening, monitoring of differential gene expression and forensic analysis have also benefited from the ongoing research in microarray technology. In this regard, biochips constitute one of the major trends towards the research of novel diagnostic systems usually associated with microfabrication of diagnostic kits by screen-printing techniques (which allows highly selective immobilization of their recognition elements[22]), inspired by planar, silicon-based technologies. Biochips are essentially very dense microband sensor arrays coated with different probes for simultaneous detection of multiple targets (with or without a label) printed on the chip by conventional photolithography.[20] They allow monitoring of thousands of biomolecular interactions using very small reagent volumes dotted on a single glass slide.

Instead of single-cased biosensors, miniaturized sensor arrays can offer multi-component information, dynamic compensation for sample matrix effects, detection of malfunction/deterioration and improved selectivity through the interpretation of response patters obtained from the array.[23] DNA microarrays for multiple and simultaneous target detection make

use of sequence-specific DNA detection, and have been extensively used for studying genomic structure and gene expression. They achieve high efficiency of analytical processing, as a high density of individual hybridization spots is a major characteristic of microchip-based genetic analysis. The high frequency of optical signals – compared, for instance, with electrical ones – is an undeniable advantage considering the enormous amount of information that can be carried by optical devices. This has been decisive for the superior commercial success, in the last few years, of many optical sensing platforms, compared to those based on other transduction mechanisms. Some of the most commercially successful platforms include, the pioneer GeneChip® high-density (high spatial resolution of individual probes) microarray from Affymetrix (Santa Clara, CA), with fluorescence-based detection coupled to a confocal readout; the fluorescence-detecting microbead-based BeadXpress® array system from Illumina (San Diego, CA); the pioneer BIAcore (real-time biospecific interaction analysis) surface plasmon resonance (SPR)-based platforms of Pharmacia Biosensor (Uppsala, Sweden);[24] and the NanoLantern from Lighthouse Biosciences (West Henrietta, NY) that reaches exquisite sequence specificity by using DNA hairpin probes, which is further enhanced by the conversion of raw genomic sequences into effective hairpin probes through a DNA folding computational program that predicts genomic DNA portions with naturally occurring hairpin structures.[8] It is envisaged that many of these systems may be applied to several biochemical assays, including DNA and protein arrays, and may eventually replace the well-known enzyme immunoassay (EIA). The difficulty of implementing EIA in routine clinical settings arises from the lack of robustness when performed occasionally and for single-case identification. Conventional EIA requires long assays and significant amounts of expensive reagents and equipment, thus being unsuitable for efficient use in resource-limited settings.

Microarrays have also been adapted to monitor pathogen mutations that give rise to drug resistance, *e.g.* DNA segments from regions of interest can be dotted onto a glass slide and hybridized with DNA from clinical isolates. The signal of the complementary sequences is stronger compared to that of the hybrids containing point mutations. The efficiency of microarrays is usually hampered by the typically large size of biological samples and by their complex treatment, which also makes it difficult to obtain real-time outputs. Other drawbacks include the difficulty of scaling down the array density, limited resolution and strong sample concentration-dependence.[6] Moreover, in spite of the ability of DNA biochips to detect many genes in a single assay, detection at the cellular level without previous genomic sequence amplifications remains limited.[25] Microarray technology is still too expensive to become valuable for point-of-care diagnosis in the short term. It seems clear that implementing sensor arrays with multiple-sample delivery, while being simple in concept, will require improved technological development fueled by strong commercial demands.[26]

The "ideal" diagnostic test

An "ideal" diagnostic test should overcome the main limitations of the existing techniques. Despite the fact that some technological improvements have met the needs for application in the field, the available techniques often fail to accomplish the basic requirements for a successful diagnosis in tropical sub-developed countries namely, simplicity, economy, rapidity and accuracy.[7] Under tropical environments, successful employment of diagnostic devices must take into account the lack of (good quality) water, irregular electricity supply and high temperatures and humidity.[27] In this regard, analytical *in vitro* diagnostic equipment must comply with the needs of a larger and more decentralized target market. Ideally, the test should be able to detect recrudescence or relapse and blood clearance of the pathogen if therapeutic monitoring is required.[28] Such new diagnostic tools must also meet the challenges of the evolving regulatory, payer and patient requirements, as well as the new business and economic realities.[8] These rapid diagnostic tests are useful in primary health care settings where there may be no electricity for diagnostic equipment or refrigerators for reagent storage. The World Health Organization (WHO) has proposed the acronym "ASSURED tests" to describe the ideal characteristics of a diagnostic test for resource-limited settings, namely *a*ffordable, *s*ensitive (yielding few false-negative results), *s*pecific (yielding few false-positive results), *u*ser-friendly (simply to perform and requiring minimal training), *r*apid (enabling treatment at first visit) and robust (not requiring refrigerated storage), *e*quipment-free, *d*eliverable (to those who need it).[2]

Many walk-in health care environments are located in remote and poor regions worldwide. In such conditions, tests and their results must be supplied at once while the patient is still in the clinical facility, as the return of the patient (some days after), who often has to travel long distances for test results is not always an easy task. Thus, even when competing with the highest sensitive laboratory-based assays, ASSURED tests may allow more infected people to receive treatment. Consequently, the best test is not always the most useful one.

Biosensors: new diagnostic tools

Recent advances in bioelectronics have generated many diagnostic kits and new techniques based on biosensor technology which have been commercially exploited. Biosensors are compact and portable analytical devices that convert a biochemical reaction or interaction into an analytical signal that can be further amplified, processed and recorded. Biorecognition occurs directly on the surface of a physical transducer, unlike in conventional solid-state formats. Biosensors consist of integrated modules where the biochemical components, the transducer (*e.g.* electrochemical, piezoelectric, acoustic or optic) and the detector are packaged in a single unit. Unlike bioassays or conventional bioanalytical systems, biosensors do not require additional processing steps (*e.g.* reagent addition), and are characterized by permanent fixing of the assay design in the construction of the device.[9] Unlike individual biosensors, biochip surfaces must be scanned for acquisition of full information about the bioaffinity reaction profile. The ongoing progress from microarrays to biosensors will certainly be mediated by the production of disposable, easy-to-use devices, thereby obviating the typical limitations of current microarray technology.

The most important application of biosensors is in the rapid and early detection of pathogens in blood stored in donor blood banks,[29] but also for detection of virulence in vaccines, of dangerous pathogens in the environment, and of septic wounds (bandage-like devices), as well as disease in prevalence surveys.[28] Biosensors should allow easier, faster and cheaper results than traditional bioaffinity assays, while keeping high sensitivity and specificity of detection. Ongoing research and increased sensitivity have pointed towards the development and optimization of devices to operate in an *in vivo* or non-invasive mode (*e.g.* using urine specimens), precluding the need to draw and handle blood.[18] Due to the complexity of some pathogen's life cycles, molecular diagnosis with new and rapid tests often remains too invasive and challenging. As such, *in vivo* measurements frequently make use of implantable devices, a process significantly less invasive and damaging for body's structure and function when miniaturized devices are used. Under prolonged use, however, the efficacy of implanted biosensors may be limited by the need for sterilization, the leaching of toxic components from the biosensor, eventual lack of device's biocompatibility, and difficulties in calibration.

Biosensors for glucose detection still represent by far the greatest group of developed and commercialized biosensors for rapid and *in vitro* clinical diagnosis. The commercial driving force may, in part, be due to the conjugation of two facts not easily achievable with other diseases, namely a potentially very broad target public and the need for multiple daily screenings. Many glucose and other commercial biosensors rely on dipstick or immunochromatographic (lateral flow) formats, very suitable for single-case diagnosis in field applications. In these formats, which are similar to those of cheap, rapid and common immunoassay pregnancy tests, the clinical liquid sample migrates across the surface of a nitrocellulose membrane by capillary action.[30] However, some diseases (including diabetes) cause significant visual disabilities, which may lead to misinterpretation of results by end-user patients. As a consequence, devices with digital output-based displays have opened the door to other forms of signal generation.

Classification and characterization of biosensors for medical diagnosis

Biosensors can be tailored to the detection of nucleic-acids (genosensors), antigens or antibodies (immunosensors), enzymes, whole-cells, non-enzymatic receptors and carbohydrates (among which glucose is the most well-known). Amongst them, immunosensors and genosensors are the most well studied for their clinical relevance. Immunosensors exhibit the advantages of sensitivity and selectivity inherent in the use of immunochemical interactions, although cross-reactivity may occur. A major drawback of genosensors when compared to immunosensors is the fact that, *in vivo*, microorganism genomes are tightly packaged inside the cell biomembrane, thus, their concentration may be too low for a successful diagnosis without prior amplification by PCR. In general, microorganism proteins are also more abundant than nucleic acids. Despite the ultimate goal of directly determining DNA traces in clinical samples, only very low levels of nucleic acids exist in biological fluids, otherwise undetectable, if previous PCR amplification is not performed.[31] In the case of blood infections the amount of human genomic DNA can be 10^{14} times higher than pathogen target DNA; an important challenge in terms of sensitivity and selectivity.[32] Of note, a truly high performance biosensor with an immobilized DNA-probe should be able to discriminate a single base-pair mismatch between different target DNA sequences. Biosensor schemes for single copy detection have already been developed, but amplification has the benefit of increasing the signal-to-noise (S/N) ratio of an assay, thus lowering the probability of interferences by sample contaminants. In this regard, a key challenge has been obtaining sufficiently pure nucleic acid templates to allow efficient amplification.[8]

Moreover, salt-dependent electrostatic effects greatly influence the stability, structure, reactivity and binding of nucleic acids in solution or at an interface.[33]

DNA, on the other hand, is the only biomolecule that can be easily copied and amplified by PCR and other related techniques. Careful choice of a suitable DNA sequence conserved within the genomes of pathogens of the same species is usually the basis for DNA hybridization detection in such cases. DNA also has superior ability to distinguish different strains of the same organism, especially when isolated from different geographical locations, by simple choice of strain-specific DNA probes. Moreover, when compared to immunological assays, nucleic acid testing allows earlier diagnosis.[34] Unlike antibodies, DNA forms biological recognition layers easily synthesizable and reusable after simple thermal melting of the DNA duplex.[35] Nucleic acid arrays are also more suitable than protein counterparts for direct synthesis onto a chip surface, without the need to produce and purify the ligands.[36] Concerning the transduction mechanism, electrochemical and optical biosensors are by far the most promising and readily available in biosensor research and development. Most described catalytic (*e.g.* enzyme-based) biosensors rely on electrochemical transduction, whereas affinity (*e.g.* protein- and nucleic acid-based) biosensors are usually more suitable for optical methods.[37]

In general, electrochemical biosensors are relatively simple, rapid, less costly and amenable to miniaturization and mass-production when compared to optical biosensors. Together with the adaptability, compatibility with microfabrication techniques, low power demands and the portable nature, electrochemical transduction seems appropriate for decentralized diagnosis of many infectious and inherited diseases.[38] These biosensors, however, have problems of interference from electrochemically active substances and troublesome electron-transfer pathways between the biosensing layer and the surface of the bare electrode.

Concerning optical transduction, fiber-optic sensors seem the most suitable for decentralized testing, because of the absence of electrical interferences, inherent small size, high transmission efficiency and chemical stability and flexibility, thus opening the way to miniaturization and remote sensing. Furthermore, the harmless nature of optical signals (compared with electrical ones), biocompatibility and the easy sterilization make them attractive for *in vivo* measurements.[39] Nonetheless, their propensity to interference from biological samples and the intrinsic complexity and expense of most optical transducers and components has hindered wider applications for use in the field.

Mass-sensitive piezoelectric biosensors constitute another important class of biosensors. However, regardless of their wide use in on-the-bench systems, they seem quite limited for field use since their performance and sensitivity strongly depend on perturbing external influences, *e.g.* even very small fluctuations in the surrounding air flow. In general, appropriate performance of piezoelectric biosensors requires extensive washing and drying steps. Obviously, this severely restricts real applications to well-controlled, in-house laboratory conditions.

Lab-on-a-chip and microfluidics

There has been a growing interest over the past years for microfluidic analysis schemes and devices (also called micro total analytical systems (μTAS), lab-on-a-chip or biomicromechanical systems (bioMEMS)),[40] essentially an adaptation of DNA chips, containing channels and chambers for flowing liquids. Such flow-through systems, which are often built on metal oxide substrates, have emerged with advances in materials sciences, and possess obvious advantages compared to simple discrete single reaction vessels. Microfluidic devices integrate, in a single chip, modules for specimen processing, biochemical reactions and product detection, with on-chip control of thermo-pneumatic pumps, micro-heaters and temperature sensors, miniaturized fluorescence detectors, sample/analyte concentrators and filters. The advantages include lowering contamination, reagent consumption and time of detection, while enhancing rapidity, performance and automation ability. In these systems the biorecognition event occurs in a liquid instead of a solid phase, a similar condition to the *in vivo* microenvironment. The ability of microfluidic platforms to manipulate very small volumes of fluid (thus minimizing the sample volume needed for analysis) has great interest for faster and more accurate molecular diagnosis.

Ultimately, the goal in decentralized testing is achieving a suitable lab-on-a-chip device fulfilling the ASSURED criteria.[2] Detection sensitivity in particular can be improved by surpassing some geometrical constraints of on-chip devices. This can be accomplished by decreasing the complexity of flowing channel configurations, for example, by simply shaping the detection region in the format of an expanded blister.[41] Consequently, the fluid sample speed decreases in the detection region, but the detection throughput remains the same since the flow rate also does not change. In this way detection sensitivity can be significantly enhanced compared to normal channels. Ongoing efforts with DNA chips aim the production

of PCR-free DNA detection systems, but this has not been fully achieved yet in commercially available devices.[42]

As efforts for improving the amplification and detection of nucleic acids have been a major concern, the sample preparation and the nucleic acid extraction steps remain relatively underestimated with regard to the development of a true point-of-care diagnostic device. In addition, many of these current systems also require off-line sample preparation and reagent handling, being therefore unavailable for routine home testing.[11] These pretreatments for cell lysis and subsequent extraction (with eventual labeling) of the target nucleic acid sequence significantly burden these assays, the reason why many efforts have been made to develop rapid and reliable methods to break the hard coating that packages genetic material within many bacterial and viral cells,[8] and ultimately for whole-organism detection.

The concept of μTAS is rapidly becoming an independent area of analytical chemistry. This is due to the fact that the combination of sample analysis and rapid analysis offered by microfluidic devices is blurring the traditional distinction between sensors and instruments as these new systems exhibit some characteristics usually associated with biosensors (*e.g.* real-time outputs and small size).[23] The high reliability of optical microscopy for detection and imaging of infectious pathogens (at both cellular and molecular levels) has been combined with microfluidic technologies to develop point-of-care diagnostic devices.[43] These systems usually face problems of lack of portability and expensive optics, but new platforms based on lensless and portable charge-coupled devices (CCD) have emerged as promising alternatives for cell detection.[44] Instead of using typical fluorescent labeling, these systems may operate by simpler shadow imaging processes, thus opening up opportunities for incorporation into portable self-powered platforms for use in the field. The trend for miniaturization of conventional flow cytometers has been propelled in part by convenient lensless imaging techniques.[45]

Minimal expensive and complex optics, label-free detection and simple channel geometry are essential requirements for cytometer miniaturization. An additional requirement for this goal is sheathless focusing of the flowing sample, aiming to prevent direct contact between the sample and the microchannel inner walls, thus avoiding nonspecific cell or molecule adsorption.[46] Technological improvements have recently paved the way for further miniaturization of microfluidic devices down to the nanofluidic domain, basically an extension of microfluidics to fluid flow and manipulation around nanoscaled structures.[47] The goal is to surpass limitations of microfluidics, namely spatial and temporal control of nanosized biological entities such as viral particles.[48] Lab-on-a-chip devices promise to lower the cost of point-of-care diagnosis, for which the cost of reagents and mass-production, the quality control and the ability for miniaturization must be taken into account.[49]

Nanobiotechnology

An emergent topic in the development of new bioanalytical procedures, structures and systems is nanobiotechnology. A brand new range of electronic devices and biosensor nanoplatforms has emerged as a consequence of the inherent small size and unusual physicochemical properties of nanoparticles, unlike those of bulk materials. As the system decreases below 10 nm, many physicochemical properties such as plasticity, thermal and optical parameters, reactivity, catalytic ability, electron/ion transport and quantum mechanical properties become more pronounced.[50] Moreover, with an appropriate transducing method the selectivity of nanobiosensors may be tuned as a result of signal dependence on nanoparticle morphology.[51] By avoiding the use of complex and expensive instrumentation, many nanoparticle-based biosensors seem quite promising for point-of-care diagnosis.

Traditionally, nanostructures involved in capillary-driven microflow within plastic supports have been used to scale-up disposable microfluidic devices for mass production through known molding techniques.[52] Inversely, it is possible to see, by technological and industrial innovation, mass fabrication of future nanochips and nanofluidic systems as an extension of current mechanical methods for production of microsensing devices most often based on organic polymers and gels, especially polydimethylsiloxane (PDMS) frames. However, the need for high-throughput analysis is creating a demand for a broader range of less costly and easier fabrication methods. Paper, for example, has been assayed as a potential and suitable material for high-throughput production of disposable chip devices through lithography-based processes that avoid the requirement for complex microfabrication infrastructures.[53] Even so, complying with growing cost-effectiveness and simplicity may correspond essentially to shifting from top-down methods (which begin with a patterned, larger-scale layout and reducing its dimensions) to bottom-up methods, by building up nanostructures from atoms or molecules. Such bottom-up methods are conducive to cheap and easy production of small nanostructures. The sudden rise in the expected cost/benefit of miniaturizing photolithographically produced microsystems has led to the assembly of

micromechanical systems and functional biomimetic structures in the 5–100 nm range.[54]

Carbon nanotubes (CNTs) are among the most recently developed and promising bottom-up nanotechnological platforms for electrochemical biosensing. This is due to a notable range of unique electronic properties, including excellent electrical conductivity, and enlarged surface area for DNA immobilization, which makes them excellent elements for chemical sensing. In addition, they are physically robust and inert towards most chemicals.[55] As part of a loosely packed nanostructure network, each nanotube may act as an individual nanoelectrode, with sufficient free space between neighboring nanotubes preventing the overlap of their diffusion layer, therefore yielding high S/N ratios and improved detection limits.[50] In view of the unavailability of fast, sensitive, selective, inexpensive and easy-to-use methods for detection and quantification of pathogenic bacteria cells, CNTs were used as efficient ion-to-electron transducers in potentiometric analysis for specific electrochemical detection of ultra-low levels of such targets.[19] Traditionally this is difficult without chemical labeling, as the receptor–bacteria interaction does not yield a measurable electrochemical signal; however, CNT-coupled aptamers can be used as highly selective receptors to detect such living cells as easily as measuring the pH value. Aptamers are specific nucleic acids that mimic antibodies in many applications, being comparable in affinity and specificity, while not requiring animal systems for their production, occurring rather *in vitro*.[56] In this sensor, the presence of target bacteria probably induces a conformational change in the aptamer which, in turn, promotes a charge change to the CNT (leading to a change in potential). Simultaneously or alternatively, the bacteria can also lean towards the CNT, thus establishing charge transfer between them and the highly concentrated H^+ ions that surround the cell wall. Highly accurate linear response and good reproducibility were obtained without any pretreatment. CNT-aptamer hybrids have been successfully used for nanobiosensing of a wide range of molecular targets.[57,58]

Nanowires are an interesting nanostructure of metallic or semiconducting materials, with potential use for the interconnections of biochip circuits.[50]

Another important bottom-up nanostructure is the quantum dot (QD), a type of nanoparticle for fluorescence tagging of probe biomolecules. It differs from a conventional organic fluorophore by its brightness (due to the higher quantum yields) and its photostability. In addition, its color can be directly correlated with its size, with emission of a single, well-defined wavelength after excitation (a higher size corresponding to a higher emitted wavelength). QDs have broad absorption spectra and narrow emission spectra with large emission shifts, allowing excitation at wavelengths far-removed from their emission peaks.[59] Nearly all

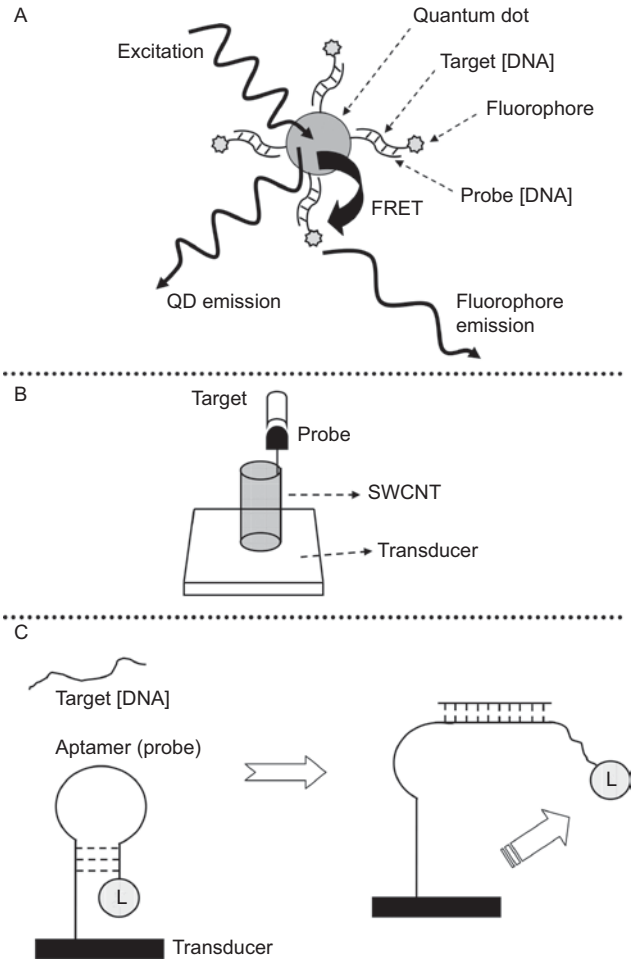

Figure 1. Schemes of novel nanostructures for biosensing. (A) QD. Specific binding occurs between a fluorophore-tagged target-biomolecule (*e.g.* a DNA strand from a clinical sample) to a specific probe-molecule (*e.g.* the complimentary DNA strand) immobilized on the surface of a QD. Afterwards, light excitation of the assembly with a particular wavelength results in QD-originated emission. In addition, through the cascade phenomenon of fluorescence resonance energy transfer (FRET), which occurs between the donor QD and the acceptor fluorophore, specific emission of the fluorophore at a given wavelength also takes place. (B) Single-walled CNT. In the biorecognition event, the target biomolecule is specifically recognized by the probe attached (through a linker) to the transducer surface-immobilized CNT. (C) Aptamers. The DNA aptamer probe can be tagged, in one extremity, with an organic label (*L*), *e.g.* fluorescent or redox, which is in close proximity with the transducer surface. In this layout, electronic flow between the transducer surface and a closely located electroactive label or absence of fluorescence (due to tight binding of a fluorescent label to a quencher located near the aptamer/transducer binding site) may be observed, among other possible changes. Specific hybridization with the complimentary DNA chain (target) causes linearization of most of the aptamer linear sequence. As a result, *L* moves away from the transducer surface, thus rendering a specific signaling response (*e.g.* electroflow vanishing or arising of fluorescence).

QDs of different emission peaks can be excited using a single short-wave excitation source, making it a powerful tool for monitoring several components in complex biological systems.[60] These properties make dots of different sizes suitable as distinguishable labels for different targets.[61]

Nanostructures for biological detection (some of them are schematically depicted in Figure 1) may be built in the form of nanoarrays, which are intuitive extensions of microarrays to nanoscale dimensions. These arrays sometimes may include millions of unique peptide or oligonucleotide sequences, allowing testing replicates for each probe, and S/N ratio enhancement in turn. Ultra-high density nanoarrays constitute promising powerful tools for genomic- and proteomic-based assays and screening, as millions of minimal biomolecular variations can be scanned concomitantly using very small sample volumes.[62]

Implantable biosensors for *in vivo* diagnosis

Nanotechnology has accompanied recent developments in implantable biosensors for *in vivo* diagnosis. Implantable biosensors are especially useful for continuous monitoring of biomarkers in the body, while not requiring patient intervention. Unlike *in vitro* diagnostics (*e.g.* in the form of test strips), this approach allows earlier and more reliable indication of disorder and disease. Implantable biosensors will allow real-time detection of a pathogen in the organism in a way that not only accounts for the patterns and trends associated with patient daily habits, but also enables the triggering of fast responses to changes in pathogen changes. Understandably, most developed implantable biosensors have been tailored for glucose measurements in diabetic patients. One major handicap of implantable devices is their propensity after implantation to fouling from proteins and living cells. This may hinder biotarget mass transfer and significant changes in local O_2 concentrations, leading to significant errors in the sensor's response (as O_2 is a cofactor in most enzymatic biosensors) and compromising its long-term stability.[63,64]

Another drawback is the generation of tissue inflammation due to foreign body response, the significance of which depends on the extent of the tissue damage that occurred during implantation. Foreign body response was initially countered with the use of biocompatible antifouling coatings. However, despite not rendering toxic effects to the body, biocompatible materials still stimulate immune responses associated with the implantation event.[65] Biodegradable materials have also been applied for construction of implantable biosensors, aiming to facilitate explantation at the end of their useful life. Unfortunately, combination of biodegradable and biocompatible materials within implantable devices has been considered a difficult task.[50] These obstacles have led to device miniaturization as a way to minimize body tissue damage, and thus patient invasiveness, *e.g.* by using implanted ultra-fine needles.[66] This is the challenging task for miniaturization of all biosensor components, including sensing elements, power sources and signal-processing elements. In this context nanotechnological approaches have been introduced in order to improve an implantable biosensor's performance, namely in terms of sensitivity and selectivity. "Smart" nanoporous membranes are particularly useful because they change permeability as a response to changes in pH, temperature, saline concentration and electromagnetic fields, usually by reversible expansion of pore-incorporated polymers.[67]

In an attempt to reduce biofouling, nanocomposite coatings based on CNTs[68] and silica nanoparticles[69] have also been used. The use of polymeric nanocomposites in device manufacture allows enhancement of antifouling properties through changes in polymer surface charge, hydrophilicity and tensile strength. Furthermore, the softness and flexibility of polymer structures can be used to diminish tissue damage during implantation and to enhance adaptation of the device to the body's anatomy. In addition, nanotechnology can also be used to incorporate new imaging approaches (*e.g.* through the use of fluorescent nanoparticles) into implantable devices for kinetic studies and to help estimate the exact time for explantation after the lifetime of the device has expired, as well as visualizing nanomaterial leaching.

As for *in vitro* biosensors, implantable diagnostics will greatly benefit from multi-target detection abilities, although physiologically based interdependence between different biotargets may be a serious obstacle. Ultimately, suitable multiplexed arrays for point-of-care detection will not only render efficient monitoring of body disorders, but will also to determine if and which daily habits influence sensor responses.[50] The measurement of critical physiological parameters (*e.g.* seroconversion) may provide reliable indication of current infectious disease.[70]

Mass spectrometry

MS techniques have been used for pathogen detection and quantification using bulky laboratory apparatus. Unlike rapid and simple biosensing devices, MS is able to identify pathogens in samples of unknown composition, which constitutes a different paradigm in biodetection. The mass spectrum represents a specific

fingerprint, or molecular profile, of the microorganism analyzed. Essentially, the software system compares the mass spectrum with that of an existing infectious agent database, being also able to describe how the analyzed organism relates with others in the database. Commercially available MS-based universal platforms for nucleic acid detection usually employ sets of broad-range primers to amplify PCR products from a large number of closely related organisms without prior knowledge of their specific genomic sequences. This strategy successfully led to the inclusion of the human severe acute respiratory syndrome (SARS) virus in the coronavirus family.[71] One such commercial device was also tested as a rapid and inexpensive method for global surveillance of emerging influenza virus genotypes.[72]

The main advantages of MS-based detection are high resolution speed (above one sample per min), high degree of automation and software control, no need for specialized manpower, and possibility of performing strain typing and antibiotic resistance studies. The main disadvantages are the intrinsic difficulty of MS device miniaturization, the need for continuous enrichment of databases with new genomic sequences, and the requirement for high-power instrumentation. As such, it will be some time until MS-based platforms can fulfill the necessary requirements for application in point-of-care diagnosis in the field.

Conventional and new laboratory diagnostic tools for the main tropical diseases

The following subsections give a short overview of some of the major current tropical diseases encompassing conventional and new laboratory diagnostic tests. For the reasons stated above, many traditional tropical infections are now threatening the developed world. Conversely, some of these diseases such as acquired immunodeficiency syndrome (AIDS) or TB, are not exclusive to developing countries, but rather affect the entire world (as will be discussed below) for diverse reasons and circumstances. However, in general, most of the burden of such diseases occurs in developing countries, usually for the same reasons pointed out for classical tropical infections. This has much to do with harsh life conditions, poverty, difficult or nonexistent access to basic education and health care and failure of many disease control and eradication programs, among other reasons.

This overall picture has led recently to a convenient shift in the paradigm of "tropical diseases" to that of "tropical health". In this regard, and bearing in mind the crucial importance and burden of such diseases in the developing world, some of them will be included in the following list of the main tropical infections. We decided not to include *Ebolavirus* in this list due to the specific conditions of this infection, especially the very high propagation (through bodily fluids of contaminated patients) and lethality, and to the usual occurrence in sparsely populated remote regions. Direct exposure to infected patients poses great risk of contamination and even death to health care workers. The use of complex and expensive protective vestments is thus strongly advised for these professionals. Understandably, this may be more limiting for patient and disease management than the availability of point-of-care diagnostics itself. On the other hand, proper isolation and quarantine measures are usually undertaken in typical outbreaks, thus limiting the risk of infection to no more than a few hundred cases. This is a very unattractive driving-force for commercial developers and suppliers of rapid diagnostic devices. Taken together, these facts have hindered the development of biosensors for diagnosis of *Ebolavirus* infections, where immediate and presumptive isolation/quarantine and supportive treatment may be more urgent and efficient. Thus, not only may symptoms be clear enough to preclude the need for rapid diagnosis, but also other circumstances (*e.g.* the occurrence of epidemic outbreaks in usual Ebola-occurring regions, the high severity of symptoms and even the high mortality rate), including the presumed risk of easy contamination of healthcare professionals close to infected individuals, may discourage wider implementation of diagnostic supportive programs in affected regions. This may result in a lack of driving-force to develop such rapid diagnostic tests. The list below includes the majority of diseases to which the UN Tropical Diseases Research (TDR) Program for diagnostics has been devoted.[14] In general, this list excludes infectious diseases for which approved and effective vaccines are available (*e.g.* yellow fever, typhus and hepatitis B).

Malaria

This is the most important and well-known tropical disease, and the main cause of child deaths in sub-Saharan Africa. It is vertically transmitted to humans by female *Anopheles* spp. mosquitoes infected by parasitic *Plasmodium* spp. It affects all the tropical and sub-tropical regions worldwide, especially the poorest ones, and is a potential threat to half of the world's population. Apart from clinical criteria, high-power microscopy of Giemsa-stained thick blood smears is the "gold-standard" method for its diagnosis,[73]

although being time-consuming, of variable application and often unreliable.[18]

Cost/benefit issues have hindered a more worldwide availability of rapid diagnostic tests for malaria. In fact, presumptive treatment for all febrile children in malaria endemic areas was applied in the past, due to the lack of available microscopy diagnosis and to the occurrence of many deaths soon after the onset of malarial symptoms. This was feasible because chloroquine for malaria treatment was widely available, effective and cheap. Thus, definite diagnosis was unnecessary. However, several chloroquine-resistant strains of the malaria parasite have emerged in most endemic areas. Given the paucity and expense of alternative drugs, it may be now more economically advantageous to confirm malaria occurrences with rapid diagnostics rather than treating all children presumptively, with the additional benefit of allowing the identification of febrile children without malaria, but most likely requiring treatment for other afflictions.[2]

Sensitivity analysis studies regarding rapid tests for malaria have shown that cost-effectiveness towards conventional diagnostic techniques becomes favorable above a given number of diseased patients.[74] Many companies are already producing rapid diagnostic tests for malaria, usually set up in the form of dipstick or lateral flow devices to detect malarial antigens, in accordance with WHO recommendations for malaria case management through parasite-based diagnosis.[75] Lactate dehydrogenase (pLDH) and aldolase enzymes have already been identified as valuable diagnostic targets among all human malaria[76] in peripheral blood during infection. Such tests usually generate results within 15 min and require only minimal operator training, but when compared to microscopy, they are somewhat expensive, only render qualitative results, and are of limited usefulness in detecting low-level parasitemia.[28] Nevertheless, not many of them have been carefully evaluated.[77] Many of these tests suffer from excessive variation on quality control parameters in manufacture and product stability.[73]

WHO has recently undertaken a malaria evaluation program for commercial rapid diagnostic tests to improve their overall impact,[78] including an online list of manufacturers and distributors. In this multipartner, collaborative study which involved WHO, TDR, the Foundation for Innovative New Diagnostics (FIND) and the US Centers for Disease Control and Prevention (among others), selected manufacturers were invited to submit their tests for evaluation against panels of parasitized patients (parasite-negative panels were also used as controls). In particular, thermal stability at high temperature and humidity (similar to conditions found in tropical environments) was tested for some months. Results for several tested kits showed easy detection of low parasitemia of both *Plasmodium falciparum* and *Plasmodium vivax*, with few false-positive results and good stability at tropical temperatures. However, varying performances between different lots were observed and even higher variation between different (although similar) products. The outcomes of this study suggested the need for standardized testing of different lots soon after purchase and prior to use in the field, as well as for manufacturers to compare their products with reference materials for product development.

Selection of the best products must take into account parameters other than those concerning the product itself, namely training for test use, supply chain and availability of proper infrastructure. BinaxNOW® (Inverness Medical Professional Diagnostic, Princeton, NJ), a test kit for malaria approved by the US Food and Drug Administration (FDA), is based on the histidine-rich protein-2 (HRP-2) which, like pLDH, is a *Plasmodium falciparum*-specific antigen.[79] It reaches an overall sensitivity of 82% and specificity for *Plasmodium falciparum*.[80] This test is approved by FDA to evaluate symptomatic patients with negative results requiring confirmation. However, the FDA has argued that this kit should only be used in laboratories able to acquire *Plasmodium falciparum*-containing blood samples for use as a positive control. In addition, this and many other diagnostic kits does not differentiate viable from non-viable parasites, which not only prevents monitoring the parasitemia level (on which management decisions are based), but also the therapeutic response as antigens persist even after elimination of the parasite.[28] Furthermore, positive rheumatoid factor has been associated with false-positive results.[30]

Recently, a magneto-optic technology was developed for rapid and quantitative diagnosis of malaria in an *in vivo*, non-invasive format. (18) It detects hemozoin, a by-product of malaria parasite action on hemoglobin that, under an applied magnetic field, gives rise to an induced optical dichroism characteristic of its concentration. Such dichroism correlates with hemozoin concentration in infected blood and tissues, and superior performance over other diagnostic techniques has been claimed after a preliminary clinical trial. A major advantage of magnetic detection assays is that few biological compounds are magnetically active.[2] Microfluidic devices have also been developed for inexpensive and point-of-care malaria diagnosis. Since the microfluidic channels can be tailored to mimic capillary blood vessels, host-parasite interactions and infected erythrocytes can be accurately assessed.[81] It is known that the sensitivity and thus the performance of rapid diagnostic tests for malaria strongly depend on parasite density in target populations; therefore, detection of low parasite densities

is becoming increasingly important in countries that are in the process of reducing or even eliminating malaria.[78]

Not only high sensitivity but also high specificity is required to avoid misleading diagnosis of the etiologic agent in order to prevent inappropriate treatment, and thus unnecessary drug pressure (strongly related with the genetic basis of antimicrobial resistance), especially in regions of low incidence of non-*falciparum* species of *Plasmodium* parasites. In addition, specificity is also required in order to yield more precise estimates about the real local burden of malaria. Local epidemiological patterns may be crucial in order to define strategies concerning the utilization of rapid diagnostic tests. In fact, HRP-2-based kits may be preferred for regions where *Plasmodium falciparum* circulates alone or with non-*falciparum* species as co-infectants. On the other hand, regions where *falciparum* and non-*falciparum* species co-circulate require tests for differential diagnosis between them, whereas *falciparum*-free regions with endemic malaria may need a generic test for *Plasmodium* species, or often specific tests for *Plasmodium vivax*.[28]

The adoption of such simple guidelines is not always straightforward. In fact, recent observations have shown that HRP-2 can exhibit high polymorphism among isolates from the same region.[82] In addition, monoclonal antibodies raised against HRP-2 can cross-react with other histidine-rich proteins,[83] and deletions in the parasite HRP-2 gene may test negative for HRP-2 rapid tests. Therefore, epidemiological studies related to the geographical distribution of endemic *Plasmodium falciparum* variants lacking the HRP-2 gene prior to large-scale implementation of HRP-2-targeted rapid tests are crucial for successful combatting of malaria in those regions.[84]

Dengue

Dengue is currently the most important arthropod-born disease. It is caused by females of *Aede*s spp. mosquitoes infected with RNA-containing dengue virus. Dengue may occur in the form of four antigenically-related viral serotypes and affects mainly urban populations in the tropical world, but can be a serious threat to more temperate countries. Very often, hemorrhagic fever or even shock syndrome (both conditions requiring critical health care) may arise. In the absence of approved vaccines or therapeutics which should be effective against all the four serotypes, accurate and rapid diagnoses are urgently needed.

Direct detection of the virus and RT-PCR is often required for early diagnosis due to the short time period (less than one week) in which viremia can be detected. Thus, serological diagnosis through the measurement of nonstructural dengue proteins, mainly the nonstructural 1 (NS1) protein, and of specific immunoglobulins G or M (IgG or IgM, respectively) dengue antibodies, has been frequently employed for routine dengue diagnosis. In this context, colorimetric IgM monoclonal antibody capture EIA test (MAC-EIA) is the most widely used technique for dengue serological diagnosis. However, serology frequently renders misleading diagnosis of dengue (a) with other febrile illnesses, especially other flaviviruses (*e.g.* yellow fever and viral encephalitis); (b) in secondary infections, due to cross-reactivity with dengue antibodies of serotypes from previous infections; and (c) in individuals previously vaccinated against yellow fever or Japanese encephalitis, also due to cross-reactivity.[31] Moreover, the MAC-EIA test is not always available in resource-limited health care settings.

Infection with a given dengue serotype usually confers life-long immunity against that serotype, but enhances susceptibility to the other serotypes. Therefore, accurate identification of prevalent circulating serotypes may be crucial for predicting epidemiological implications and patterns during epidemic outbreaks. Thus, biosensors not only for dengue detection, but also for unambiguous serotype identification are currently envisaged. Despite the availability of commercial MAC-EIA-based kits for dengue diagnosis,[85,86] their cost remains a barrier to purchase by developing countries where dengue exists.

Among the reported dengue biosensors is an immunochip employing two specific monoclonal antibodies, immobilized into a piezoelectric transducer, for detection of glycoprotein-E and NS1 protein.[87] Multi-antibody coating allows the capture of several different antigens, thus enhancing the detection signal. This layout exhibited 100-fold higher sensitivity than conventional EIA and a short response time (30–60 min). In another approach, a genosensor employed ferrocene, an electroactive hybridization indicator that binds single-chain DNA with less affinity than that for double-chain DNA, thus resulting in unequal concentrations near the electrode surface and therefore a variation of the electrochemical response. This was used for simple electrochemical detection of a dengue-related oligonucleotide sequence with a chitosan-coated glassy carbon electrode through cyclic voltammetry.[88] Instead of the common methods that use an oligonucleotide probe with a covalently linked label (*e.g.* a fluorophore), a cheap and simple dengue biosensor employing a liposome labeled universal reporter-probe (to bind any of each dengue serotype-related targets) and four sequence-specific probes (one for each serotype) was reported,[89] with the liposomes allowing signal amplification. Rapid

electrochemiluminescent detection (within 15 min) and serotype-discrimination were carried out by nucleic acid sequence-based amplification (NASBA), a PCR-based technique for thermal cycling amplification of RNA.[90] This sandwich system integrates a liposome-coupled reporter-probe and a membrane-immobilized biotinylated capture-probe, being the amount of tagged liposomes proportional to that of sample RNA.

When employing NASBA, care must be taken in handling and storing their molecular targets, since RNA is more labile than DNA. Compared to fluorescence, chemiluminescence offers the advantages of not requiring an excitation optical source and of not being prone to quenching phenomena. Moreover, it is also highly sensitive and relatively low in cost.[91] A pioneering chemiluminescent optical fiber immunosensor for detection of IgM dengue antibodies in serum samples was developed and compared with three available MAC-EIA schemes (chemiluminescent, colorimetric and commercial Panbio® (Brisbane, Australia)).[92] For this optical fiber sensor, a lower detection limit than the three MAC-EIA formats and sensitivity at least 10–100 times higher than that of the respective colorimetric test were claimed, and this may be particularly useful in detecting typical asymptomatic forms of dengue disease. In addition, the time of analysis with the chemiluminescent optical fiber sensor was shorter than with the chemiluminescent MAC-EIA assay. Nevertheless, MAC-EIA tests showed better reproducibility than this optical fiber sensor, probably due to difficulties in surface binding.

A slight variation of the above described liposome-based electrochemiluminescent layout reports immobilization of a magnetic sphere-attached capture-probe onto a permanent magnet in a detection region, built in a microfluidic format.[93] Liposomes were filled with a dye marker, thus yielding high signal amplification and sensitivity by fluorescence microscopy. Based upon the two layouts described above, an optical biosensor with a polyethersulfone membrane-immobilized universal reporter-probe and dye-filled liposomes was applied to dengue detection.[22] The reporter and capture probes can be easily and quickly changed for a specific target sequence. The same detection scheme was applied to the specific identification of four dengue virus serotypes in a single multianalyte assay instead of using four independent assays.[94] The reporter and capture probes may be easily and quickly changed to be specific for the target sequence.

More recently, an electrochemical dengue biosensor was developed using gold electrode- immobilized concanavalin-A by means of gold nanoparticles and polyvinyl butyral.[95] As with other lectins, the specificity of concanavalin-A glycoprotein for carbohydrate moieties can be used for developing specific biosensors for sugars. Confirmed dengue fever, dengue hemorrhagic fever and non-diseased sera samples were compared by electrochemical impedance spectroscopy (EIS), a sensitive and effective technique to probe the interfacial properties of modified electrodes. The distinctive patterns of impedimetric responses observed were ascribed to different glycoprotein patterns in the different sera. This type of biosensor showed potential applicability for the recognition of different patterns of serum glycoproteins. Since impedance spectroscopy requires the recording of a full spectrum within a broad frequency region, it is a time-consuming technique. Therefore, its use for bioanalytical purposes has been limited basically to characterization features and as an auxiliary technique.[96] Nevertheless, impedimetry-based biosensors may find a market where low cost, portability and speed of analysis are required and moderate sensitivity is sufficient.[97]

Specificity is of utmost importance for analysis of clinical samples, and in a recent work reporting the development of an immunoassay for detection of specific IgM dengue antibodies in serum through indirect competitive inhibition,[98] this was achieved by self-assembled monolayer (SAM)-based covalent immobilization onto a gold chip surface of a bioreceptor conjugate of a dengue antigen and bovine serum albumin (BSA). This protein is commonly employed as a blocking reagent in biosensing methodologies in order to minimize nonspecific binding of interferents that typically occurs in clinical samples. In this study a SPR transducer was used with an increase in the resonance angle in the presence of dengue virus. Compared to traditional immunoassay formats SPR allows real-time and label-free analysis, although miniaturization of optical and electronic components of SPR-based devices is still a difficult issue with regard to point-of-care applications.[10]

Recognizing the enormous potential threat of the introduction of dengue disease to Europe, in 2006 the European Union started the DENFRAME project, aiming to develop, implement and standardize novel rapid diagnostic kits for dengue, on the basis of ligand binding molecules and chemiluminescence. [http://www.denframe.org/objectives.html.].

Tuberculosis

TB is an old and often deadly respiratory disease that affects about one third of the world's population. It is caused by *Mycobacterium tuberculosis,* and after its disappearance in many parts of the world following decades of intensive efforts, TB rose again in the form of multi-resistant strains due to the intensive use of

antibiotics (a serious problem in intensive care health units), as well as in immunocompromised individuals, especially after the advent of AIDS. Estimates point to around two million human deaths each year caused by TB. If appropriate control measures are not undertaken in a short time, one billion people may become infected by 2020.[14] The rise of TB in the world, even in developed countries, is also strongly associated with the decrease of its surveillance (the disease was considered eradicated in many regions worldwide until recently) and is highly correlated with impoverishment and decline of lifestyle and quality of life.

The smear sputum test usually employed for its diagnosis identifies only active pulmonary TB, precluding extra-pulmonary symptoms common among human HIV patients. In addition, the test shows decreased sensitivity in resource-poor settings.[99] Clinical diagnosis of the disease is complicated because opportunistic infections in immunocompromised patients produce symptoms similar to those of TB.[14] As both the time and cost of TB screening are relatively high, rapid and inexpensive detection methods are required.

An electrochemical biosensor for determination of short sequences of *Mycobacterium tuberculosis* DNA with carbon paste and microfabricated carbon-strip electrodes has been described.[100] Chronopotentiometry was employed for label-based detection of the hybridization event, and after a short 5–15 min hybridization period, ng/mL levels of target-DNA were detected. $Co(phenantroline)_3^{3+}$, a very common electroactive hybridization indicator, was used in this work. The use of cheap carbon paste electrodes allows low background currents to be obtained and broad potential windows to be employed.[101]

Recently an optical genosensor for detection of the TB bacterium by SPR was used. It used a cysteine-modified NH_2-end peptide nucleic acid (PNA)- and 5′-thiol end-labeled DNA-probes, immobilized on gold-coated glass plates.[102] The shift in the resonance angle when the target DNA binds the surface-immobilized probes was used to signal the presence of the pathogen.

A piezoelectric immunosensor has been described for the rapid diagnosis of *Mycobacterium tuberculosis*. It is based on the frequency shift caused by antigen binding to rabbit IgG against the TB bacterium, immobilized in an ordered orientation onto the crystal surface.[103] Detection was performed within the range of 10^5–10^8 cells/mL. Other methods such as the bulk acoustic-wave impedance biosensor[104] and the multichannel series piezoelectric quartz crystal sensor system,[105] have been developed for rapid detection of *Mycobacterium tuberculosis*.

A microfluidic scheme for PCR-based amplification of DNA from *Mycobacterium tuberculosis* was also developed.[106] The device uses a low-cost and low-power halogen lamp heat source that, together with the need for smaller volumes of required sample and reagents, will allow the reduction of the thermocycling step from hours to minutes and therefore enhance usefulness for the development of a portable field device.[10]

Given the extreme ease of contamination with the TB pathogen, especially within health care settings, there is a strong demand for a rapid TB test in suspected decedents, as the most common test requires one month (at least) for results. This would certainly benefit those who had close contact with infected individuals, enabling timely diagnosis and treatment. Thus, a portable and automated fiber-optic, evanescent-wave biosensor (RAPTOR platform) was recently developed to detect *Mycobacterium tuberculosis* from lung tissue homogenates,[107] with results obtained in 3 h. An alternative application for this assay is on biopsy tissue from living individuals with heavy pulmonary or disseminated infections by the TB pathogen.

Visceral leishmaniasis

Considered one of most neglected of tropical diseases,[108] leishmaniasis may occur in the cutaneous or visceral forms, with the visceral leishmaniasis (VL) variant being potentially fatal and responsible for thousands of deaths, mainly among children. More than 90% of cases of VL (also known as kala-azar or dumdum fever) occur in Brazil, India, Sudan and Bangladesh. Even in Europe, several countries have notified cases.[14] The disease is caused by protozoa parasites of the genus *Leishmania* and transmitted to humans by female *Phlebotomus* sandflies. VL is also considered a zoonosis, since other mammals, including dogs, may be affected.

Presumptive treatment of VL is inadequate because of the potentially serious side effects of current chemotherapy. Direct microscopic examination and/or culture of spleen tissue aspirates have been considered the reference tests for diagnostic confirmation, although not recommended as first-line health care measures in endemic areas. Bone marrow and lymph node aspirates can also be used, although this method is much less sensitive.[109] On the other hand, PCR has been shown to provide a marker for infection rather than for clinical disease, at least in VL endemic regions.[110] Although parasitology is quite reliable for VL diagnosis, unless spleen aspirates can be taken sensitivity is no higher than 90%.[111] In the absence of a true "gold-standard" method for diagnosis of VL, the validity estimates of new tests under scrutiny can be affected due to their dependence on the adopted reference test.[112] Consequently, cheap and reliable

serological tests have been proposed as desirable alternatives to parasitology.

A few decades ago a direct agglutination test (DAT) for VL was developed and proposed for applications in the field,[113] although low specificity for this test has been claimed.[114] In some remote regions, treating patients for VL upon clinical suspicion and/or diagnosis with a classical formol gel (aldehyde) test (FGT)[115] is still practiced. Meanwhile a dipstick test (rK39) was developed for VL,[116] although the high validity claimed has been contested. Moreover, commercial production of the device has been interrupted several times.[117] In short, both the low specificity of serological tests for VL and their irregular supply have hampered a wider use in health care settings.[109]

For validation purposes a comparative study using the latent class analysis (LCA) technique to estimate disease parameters (prevalence, sensitivity and specificity), of five diagnostic tests for VL was reported. These tests were: FGT, DAT, an indirect fluorescence antibody test, the rK39 dipstick test and a hematological sign (pancytopenia).[109] This study corroborated the high sensitivity and good specificity of the DAT test. It also concluded that the DAT and the rK39 tests can replace parasitology for treatment prescription with regard to validity criteria, while simplicity of use favors the rK39 dipstick test. Nevertheless, final decisions regarding diagnosis must take other criteria into account, namely disease prevalence, reproducibility, cost, sustainability and particular medical contexts.

Chagas disease

Chagas disease occurs in the Americas and is caused by the hemoflagellate *Trypanosoma cruzi*, with millions of people infected from southern Chile and Argentina to southern USA. The disease is usually asymptomatic in the early phase, when parasites are invading vital organs. Fatal damage of these organs may occur in the chronic phase.[14] Insecticide-based disease control measures against hematophagous insect vectors have been successfully undertaken, but contamination by infected blood transfusion in countries that do not screen blood donor banks for this particular pathogen remains a major burden.

For Chagas disease diagnosis, direct visualization of the parasite and serology (detection of specific antibodies) are the most common choices. Serology is the preferred option for etiological diagnosis in the chronic phase in routine clinical settings. The usual serological tests are indirect hemagglutination, indirect immunofluorescence and EIA, while western blot may sometimes be used for confirmatory results. Lack of sensitivity and slow turnaround are, however, significant drawbacks.

Among the few works published about biosensors for Chagas disease is the report of a label-based electrochemical scheme that uses EIS for transduction and adsorption onto gold and platinum electrodes, of a polypeptide chain composed of recombinant antigens and *Trypanosoma cruzi*-specific antigens.[118] In general, results showed good antigen stability during interaction with serum from chagasic patients and therefore viability of the technique.

In another scheme, a chronoamperometric immunosensor for detection of *Trypanosoma cruzi* proteins from epimastigote membranes was built, and its performance was compared with that of conventional EIA.[13] In the modified gold electrode the detection reaction used peroxidase-labeled IgG conjugate, H_2O_2 and iodide substrate. While EIA required an incubation time around 1 h, the immunosensor required no more than 20 min. Some cross-reactivity with VL and schistosomiasis was observed, which should be eliminated by purification of reactive antigens.[119] The method may be important not only for detecting the presence of chagasic antibodies in blood donors, but also to follow antibody decay during treatment of infected patients with available drugs.

Schistosomiasis

Schistosomiasis is typically caused by parasitic worms of the *Trematoda* class, usually from *Schistosoma* spp., of which *Schistosoma mansoni* and *Schistosoma japonicum* are among the most well known. It is usually endemic in sub-Saharan Africa and is considered one of the major helminthic diseases of the world. Despite the availability of an effective drug and of successful control programs, the number of infected cases has remained almost constant in the last few decades, at around 200 million people.[120] Schistosomiasis is propagated through contaminated drinking water and, although many infected cases are initially asymptomatic, the disease may evolve over time with severe infestation.[121] Repeated infections may lead to damage of liver, intestines, lungs and bladder.[14]

Classical schistosomiasis diagnosis has been based on detection of parasite eggs in urine and feces, but the amount of excreted eggs is often low and variable. Antibody detection is highly sensitive and specific, but interpretation of results is not straightforward; while travelers returning from non-endemic areas usually exhibit high antibody responses, travelers with a lifelong history of exposure have low or even negative responses. Serum levels of adult worm-associated

gut antigens correlate with worm burden, but unlike antibodies, rapidly decrease after drug treatment.[122]

Circulating anodic antigen (CAA), is commonly detected by monoclonal antibody-based EIA with around 100% sensitivity for all *Schistosoma* spp.[123] in heavy infections, but lower performance in lighter infections, which occur mainly in international travelers.[124] Furthermore, the CAA-EIA test is not sufficiently robust when used for single-case identification. For such situations, a reliable biosensor for detection of infection by *Schistosoma* in urine or serum would be valuable. A rapid lateral-flow test strip for detection of CAA in single serum samples of *Schistosoma*-infected patients was developed.[121] It uses the same specific monoclonal antibodies of standard CAA-EIA and fluorescent submicron-sized up-converting phosphor technology (UPT) reporter particles. Unlike conventional labels (*e.g.* fluorescent), UPT reporters are claimed to be highly sensitive, long-lived and low-cost.[125] In this study, the UPT lateral flow format exhibited higher sensitivity and accuracy than the CAA-EIA test and showed suitability for laboratory diagnosis of *Schistosoma* infections, mainly in immigrants and international travelers.

Another biosensor for *Schistosoma* detection relies on amperometric transduction with a functionalized epoxy-graphite electrode for targeting the anti-apyrase antibody of *Schistosoma mansoni*.[126] The function of epoxy resin is to harden the carbon powder of the manufactured electrode. This strategy of surface modification renders a suitable platform for protein immobilization on protein arrays and immunosensors. The EIA-based test is thus well suited for laboratory diagnosis of individual *Schistosoma* infections.

Leprosy

Leprosy is a chronic disease caused by *Mycobacterium leprae*, ranging from asymptomatic infections to a severe disfiguring condition. Almost 200 million people are exposed to this infection, which affects mainly the tropical regions. Despite being an old and well-known disease, its eradication is difficult, due to its complex epidemiology.[127] Given the steady number of newly infected patients, instituted control measures have not been sufficiently effective. In addition to the lack of a specific vaccine, diagnostic and prognostic tests are not feasible or not well established in clinical routine settings.[128] Bacterial culture of *Mycobacterium leprae* is not possible, which greatly limits the possibilities of diagnosis. Moreover, EIA and PCR have a very limited approach for leprosy, which must be considered a neglected disease. IgM antibodies against the specific *Mycobacterium leprae* phenolic glycolipid-1 (PGL-1) antigen may reflect the total bacterial load in the body,[129] although blood levels may be absent in cases of low bacteremia. Of note, the relative risk of developing leprosy is about six-fold greater in AIDS-infected individuals.[130] Hence, there is a need for new sensitive and specific diagnostic tests for leprosy.

Protein microarrays[131] and phage display[132] have been used for such purpose, with the potential for discovery of new disease biomarkers. As many different peptide antigens are produced by the leprosy bacterium, leading to different antigenic responses in different individuals,[133] it is likely that a multiple-target assay would improve leprosy diagnosis. Nevertheless, anti-PGL-1 assays are sufficiently simple, cheap and easy for routine use in endemic countries.[130]

An interesting electrochemical biosensor for detection of *Mycobacterium leprae* DNA was obtained after immobilization of PCR amplicons from genomic DNA of the bacterium onto a functionalized electrode.[134] Target DNA was detected by using ferrocenecarboxyaldehyde as the hybridization indicator. The rapid 3-min detection of non-amplified *Mycobacterium leprae* DNA clearly suggests the possibility of producing a portable device for detection in the field.

Human African trypanosomiasis

Human African trypanosomiasis (HAT), or sleeping sickness, may be caused by *Trypanosoma brucei gambiense* (in chronic infection) or *Trypanosoma brucei rhodesiense* (in acute infection), and transmitted by tsetse flies (genus *Glossina*), mainly in poor and rural sub-Saharan regions. If untreated, this disease can cause severe symptoms (*e.g.* mental deterioration) and death. HAT presents essentially two disease stages: an early hemolymphatic, and a late, more serious, encephalitic one. Early diagnosis is crucial to prevent both the onset of irreversible neurological disorders and transmission.[14] Efficient disease control is dependent on accurate infection diagnosis and prompt treatment.

The major obstacle for molecular diagnosis of this infection is the so-called antigenic variation, which relies on the high frequency of change in the structure of immunologically unrelated variant surface glycoproteins (VSGs) that cover a huge portion of trypanosome cell surfaces. Classical molecular diagnosis of HAT usually relies on the detection of specific antibodies against the parasite with a card agglutination test, a method that may fail in terms of some lack of specificity,[135] and on detection of parasites in blood or lymph by microscopy, which is often hindered by extremely low parasitemia, especially in the case of *Trypanosoma brucei gambiense*. As a consequence,

20–30% of infected patients remain undiagnosed by standard parasitological techniques.[136]

The mini anion exchange centrifugation technique (mAECT), probably the most sensitive method to detect trypanosomes in blood, is able to detect around 100 parasites per mL of blood.[137] It involves a separating anion exchange chromatography followed by concentration by low-speed centrifugation and can be coupled with microscopic examination before the concentration step in order to increase the sensitivity of parasite detection.[135] Provided a centrifuge and electricity are available, mAECT can be deployed in the absence of an efficient cold storage. For field applicability, however, the need for electricity is a serious hurdle in many poor and under-developed regions.

PCR, real-time PCR and other PCR-based techniques have been used for detection and discrimination between *Trypanosoma* spp.[138,139] Their suitability, however, encompasses research and disease surveillance purposes, precluding routine clinical diagnostics,[140] partly due to labor intensity and to hazardous characteristics of common chemical reagents. In particular, sub-species or even strain identification may be useful for epidemiological studies of HAT.[141] Interestingly, there is evidence of integration of *Trypanosoma* spp. within the human genome,[142] which may explain contradictory results between PCR and serology for HAT diagnosis.[143]

Simple and rapid dipstick layouts for HAT can be developed using oligochromatography (OC), where PCR products are detected through hybridization with gold-conjugated probes. Detection is carried out in only 5 min, without the need for heavy equipment. To date, one of the few reported rapid tests for HAT diagnosis concerns the use of a *Trypanosoma brucei*-specific PCR-OC test (known as HAT-PCR-OC) for blood analysis.[144] The 18S ribosomal RNA (rRNA) was chosen as the target for being a multi-copy gene containing sequences conserved among trypanosomatids, as well as species-specific sequences.[145] This test combines the sensitivity and specificity of PCR with the rapidity and simplicity of membrane chromatography. Unlike other conventional amplicon detection techniques, OC does not require post-amplification preparation or sophisticated equipment.

The HAT-PCR-OC test is tailored for moderately equipped clinical facilities, but further improvements should take into account the need for eliminating the PCR thermocycling steps. In this regard, loop-mediated isothermal amplification (LAMP) has been applied as a promising PCR-based technique for clinical sample analysis due to its ability to amplify genomes without the demanding thermal cycling conditions of conventional PCR and real-time PCR. This technique has been successfully applied to the amplification and detection of HAT-related DNA[146,147] and, coupled to NASBA, to HAT-related rRNA[148] sequences. By coupling the high sensitivity of NASBA with membrane oligochromatography, a scheme was developed for simpler and less demanding formats.[149] In this context a user-friendly layout based on fluorescence *in situ* hybridization with PNA probes was developed to target the parasite rRNA.[150] It is limited as a point-of-care resource as a result of some lack of sensitivity and requirement for electricity, although fluorescence microscopes for parasite detection based on battery-powered light-emitting diodes (LEDs) may constitute a valuable solution.[151] In the future, applications in the field may benefit from simple fluorescence detection devices with a previous step for parasite concentration.[135]

Improvements in HAT diagnosis are expected from nanotechnology, especially from VSG-specific RNA aptamers, which are able to recognize and bind VSG variants with sub-nanomolar affinity. In this way, RNA for aptamers can bind the surface of living trypanosomes, thus acting as carrier-type delivery molecules for antibodies, with obvious advantages for therapeutics and diagnosis.[152] Given the very low volume of liquid allowed for microscopic examination and the relatively low parasite load in body fluids, prior concentration steps are still needed to reach the same analytical sensitivity of mAECT.[135]

Lymphatic filariasis

Filariasis is propagated by mosquitoes and caused by lymphatic-dwelling filarial nematodes, namely *Wuchereria bancrofti* (mainly) and *Brugia malayi*. About 120 million people are infected, mainly in Asia and Africa. Apart from psychiatric illness, filariasis is the main cause of long-term disability in the world. Filariasis may be asymptomatic, or result in acute or chronic infections in endemic areas.[153] Even in the early stages, permanent abnormalities of lymphatic vessels (*e.g.* dilatation) may occur.[154] The most common chronic manifestation is lymphedema, which may progress to highly disfiguring elephantiasis. A major pitfall of classical laboratory diagnosis of filariasis is the failure to diagnose the clinical and sub-clinical stages of the infection. Although the detection of specific antifilarial antibodies in serum is useful for diagnosing clinical filariasis, it cannot differentiate microfilaria carriers from clinical filarial subjects, and hence is not appropriate to differentiate between the microfilaremic and the chronic stages. Such inability to distinguish between the two stages of the disease also applies to PCR-based assays. In short, better

diagnostic biomarkers for diagnosing acute and chronic infections are needed.

Immune complexes formed after antibody onset in the body (in response to antigen releasing, from living and dying parasites, into the circulation) have been shown to be suitable biomarkers for serodiagnosis of chronic lymphatic filariasis.[155] Highly accurate and specific filarial antigen detection assays are already available for detection of *Wuchereria bancrofti*, both as immunochromatographic and EIA-like assays.[156,157] Taking advantage of the enormous potential of optical fibers for remote sensing, a fiber-optic immunosensor for filarial detection was developed,[39] with outputs given in 15 min with this scheme. An advantage of this method over other conventional methods is that extensive washing steps are not required to separate bound and unbound tagged antibodies. This technique can be tailored to become a portable device for epidemiological studies in the field.

Cholera

Cholera is caused by the toxin-producing *Vibrio cholera* bacterium, a natural inhabitant of aquatic environments (both free and in association with various marine animals and plants), and hence is mainly transmitted by contaminated water. It is a frequent cause of rapid dehydration, diarrhea and death in poor countries, and the reason why the monitoring of such liquid losses is so important for surveillance and epidemiological studies. Thus, concomitant monitoring of the bacterium and its enterotoxin in water sources and supplies is of utmost importance for effective public health protection from cholera. Conventional methods for diagnosing and classifying *Vibrio cholerae* usually require prolonged incubation and growth on selective media to reduce the amount of nonspecific organisms.[158] Besides being time-consuming and laborious, such methods do not allow screening of a large number of bacteria, nor its detection within heterogeneous populations. Other molecular and immunological techniques have enabled specific detection of this bacterium.[159,160]

In this regard, an amperometric immunosensor with disposable screen-printed electrodes was developed for environmental detection of *Vibrio cholerae* O1.[161] For electrode construction, graphite powder was mixed with polystyrene polymer. This polymer, which is used for making EIA plates, was chosen for easily adsorbing proteins. This sensor is able to detect as few as 8 CFU/mL in ground water and seawater in less than 1 h.

Other immunosensors have been reported for detection of *Vibrio cholera* O1 through SPR[162] and of the *Vibrio cholera* O139 strain through piezoelectricity.[163] Aiming to detect the cholera antitoxin immunoglobulin A (IgA), a chemiluminescence-based immunosensor was developed by immobilizing the pentameric cholera toxin B subunit (the immunogenic, though safe, portion of the cholera toxin) onto fiber-optic tips.[164] The antitoxin analyte was tagged with a horseradish peroxidase (HRP)-labeled secondary antibody. The system exhibited good sensitivity, specificity and rapidity, while not requiring a purification step.

A different version of this chemiluminescent detection scheme consisted of immobilizing the cholera toxin B subunit onto optical fibers coated with a conductive indium–tin oxide (ITO) thin film, onto which a polypyrrole-biotin coating was formed by electropolymerization.[165] Biotin-labeled cholera toxin B subunit was attached to this film through highly specific and strong avidin-biotin linkages. By providing a hydrophobic environment, the poly(pyrrole-biotin) coating seems to help to decrease nonspecific adsorption of avidin and the biotinylated cholera toxin B subunit to the sensor surface. Strong reduction of nonspecific binding induced by cholera toxin B peptides may be important to preclude unwanted binding of similar enterotoxins from other pathogens, *e.g. Escherichia coli*. The biosensor was claimed to be sensitive, to have a wide linear range and to be reusable after breaking avidin-biotin linkages apart with a surfactant.[166]

The performance of this HRP-based sandwich immobilization procedure was compared in terms of sensitivity and detection limit by amperometric transducing with those of two other enzyme markers for cholera antitoxin antibody tagging: biotinylated polyphenol-oxidase (here used for the first time as an enzyme marker for immunosensors) and biotinylated glucose-oxidase.[167] In this system the cholera toxin B subunit was immobilized through the avidin-biotin system onto an electropolymerized copolymer poly(pyrrole-biotin)/poly(pyrrole lactobionamide), used as the initial anchoring layer.

Compared to the HRP-based configuration, the polyphenol-oxidase and the glucose-oxidase layouts require two additional incubation steps, and unexpectedly display lesser sensitivities and poorer detection limits. This lower performance of the immunosensor is probably due to steric constraints imposed by the probe immobilization procedure, which may decrease the accessibility of generated electroactive species to the electrode surface. Nevertheless, all the three layouts performed well in detecting the anti-cholera antibody.

A chemiluminescent biosensor based on a supported lipid bilayer incorporated with ganglioside GM1 was recently developed for detection of the cholera toxin.[168] The biosensing planar-supported lipid

membrane was prepared through spontaneous spread of ganglioside-incorporated phospholipid vesicles on the gold surface which was coated with octadecanethiol. This biosensor uses a GM1-functionalized liposome probe and HRP in a sandwiched format. An HRP-catalyzed chemiluminescent reaction provides the signal for detection of the target cholera toxin. The main advantages over more standardized biosensing schemes include easy renewal of the sensing surface, small background signal due to low nonspecific adsorption of serum compounds on the lipid membrane, and effective immobilization of multiple biocatalytic amplifiers and recognition components through common phospholipid reagents. Correlation with cholera toxin concentration within the range of 1 pg/mL and 1 ng/mL was achieved.

HIV/AIDS

HIV is a lentivirus that causes AIDS, a human condition in which the immune system begins to fail, leading to life-threatening opportunistic infections. HIV, which basically includes the HIV-1 and HIV-2 sub-categories, affects millions of people worldwide, as with many other sexually transmitted diseases, mostly in developing countries (mainly in sub-Saharan Africa and India), where the burden of the disease is enormous. Propagation occurs basically through sexual transmission or via contaminated body fluids. Since the incubation period may be several years, wide propagation among asymptomatic and unaware individuals is a major concern. Estimates suggest that only up to 10% of people are aware of their HIV sero-status.[169] The resultant silent progression of the disease is a main cause of the disease burden even in developed countries.

Over the past two decades, standard HIV diagnosis has been based mainly on an EIA assay for $CD4^+$ T-lymphocytes (host cells). Apart from the need for expensive optical microscopes for cell counting, the occurrence of many false-positive results, especially when screening low-prevalence populations, has been a considerable limitation. Retroviral RNA quantification (indicative of viral load) has also been used for HIV monitoring. Nowadays, standardized HIV diagnosis is rather complex and time-consuming, as it includes EIA, and for positive cases confirmation by western blot. On the whole, conventional methods for HIV diagnosis (flow cytometry and quantitative PCR) are time-consuming and require appropriately skilled manpower.

In parallel, many HIV-infected patients do not get tested until later (and often irreversible) symptoms arise. Furthermore, the growing availability for antiretroviral treatment creates an increased demand for simple and affordable tests in developing countries.[2] Currently available HIV rapid tests provide sensitivities and specificities comparable to those of standard laboratory tests. In they offer a suitable solution to avoid positive-tested individuals failing to return to the health care facility in order to get the test results. It is estimated that this may occur with 30–40% of tested patients and represents a missed opportunity for those individuals to immediately commence follow-up treatment.[170] Various reports have shown that the proportion of patients who receive post-test counseling increased substantially after the introduction of rapid testing.[171] By offering an opportunity for routine testing for AIDS in hospitals, rapid tests avoid huge numbers of HIV-infected patients remaining undiagnosed for long periods of time.[172]

Comprehensive studies including test methods, statistical methods, test results and estimates and participant data are crucial information for program management and policy making for appropriate disease monitoring and management.[173] By using a specific RNA aptamer, a piezoelectric biosensor for the protein trans-activator of transcription (Tat) of HIV-1 was built, through binding between the biotinylated aptamer and gold surface-deposited streptavidin.[56] This model system exhibited comparable performance to that of the homologous anti-Tat immunosensor. However, both sensors failed to reach the sensitivity for Tat detection in real samples. A thiolated CNT/gold nanoparticle modified gold electrode surface was used for self-assembled deposition of ferrocene-pepstatin and subsequent detection of HIV-1 protease by cyclic voltammetry and EIS.[174] Remarkable detection sensitivity and a detection limit around 0.8 pmol/L were claimed. Aiming to simultaneously detect HIV-1 and HIV-2 genome-related oligonucleotides, thiolated hairpin DNA probes were self-assembled onto gold electrodes in a simple and inexpensive multi-electrode array layout. The hybridization events were monitored by square-wave voltammetry, using methylene blue (which binds single-stranded DNA with higher affinity than double-stranded DNA) as the hybridization redox indicator.[175] This sensor array showed good specificity for both HIV-1 and HIV-2, including discrimination between single-base mismatches.

Recently, DNA sequences coding the immune system modulator interleukin-2, commonly used for HIV diagnosis, were probed with an ultrahigh-density nanoarray based on fiber-optics.[62] The nanowell arrays created by the etching process were filled with specific oligonucleotide probe-modified nanospheres and used for DNA-target detection. Unlike previous reports of similar systems, this nanoarray reached densities above a million array elements per mm^2,

while retaining the features of previously designed fiber-optic microarray technology.[176]

Most rapid tests already approved for field use rely on immunochromatographic or finger stick layouts for serum or plasma analysis. Among them for HIV-1 screening are the Uni-Gold Recombigen (Trinity Biotech, Bray, Ireland), the OraQuick® Rapid HIV-1 Antibody Test (OraSure Technologies, Bethlehem, PA) - both for storage at room temperature - and the Reveal G2 (MedMira, Halifax, Nova Scotia), for storage under refrigeration. For both HIV-1 and HIV-2 screening there is the Multispot HIV-1/HIV-2 (Bio-Rad Laboratories, Redmond, WA) test, which requires refrigerated reagents. In general, these tests are moderately complex for operation by medium-skilled laboratory personnel, with test completion times ranging from 5 to 20 min. Some of these tests are already being used with oral rather than blood fluids, allowing less invasive testing.[170]

In a convenient merging between micro- and nanotechnology, several portable and cost-effective lab-on-a-chip devices have been reported for HIV capture and imaging from whole blood, *e.g.* a scheme for quantification of serum anti-HIV-1 antibodies[177] limited by questionable correlation between HIV positive diagnosis and actual disease states, a disposable RT-PCR layout for detection of HIV viral capsid p24 and gp120 proteins,[178] although still relatively complex for point-of-care in poor regions, a label-free $CD4^+$ blood cell counter[179] which still requires an expensive optical microscope for detection, a relatively cheap lensless CCD[44] which needs further optimization in field conditions, and a nanosized QD-based scheme for HIV imaging of unprocessed infected whole blood.[180] In the future, more accurate diagnosis of HIV is expected from the development of nanofluidic platforms for viral load determination by proper retention of viral particles and concomitant removal of macro-sized cells from infected whole blood.[10]

Management and surveillance of tropical diseases

As portable and easy-to-use devices, biosensors may be useful not only for point-of-care and intra-clinical testing of biological samples, but also as part of an inter-clinical (multi-center) approach for disease

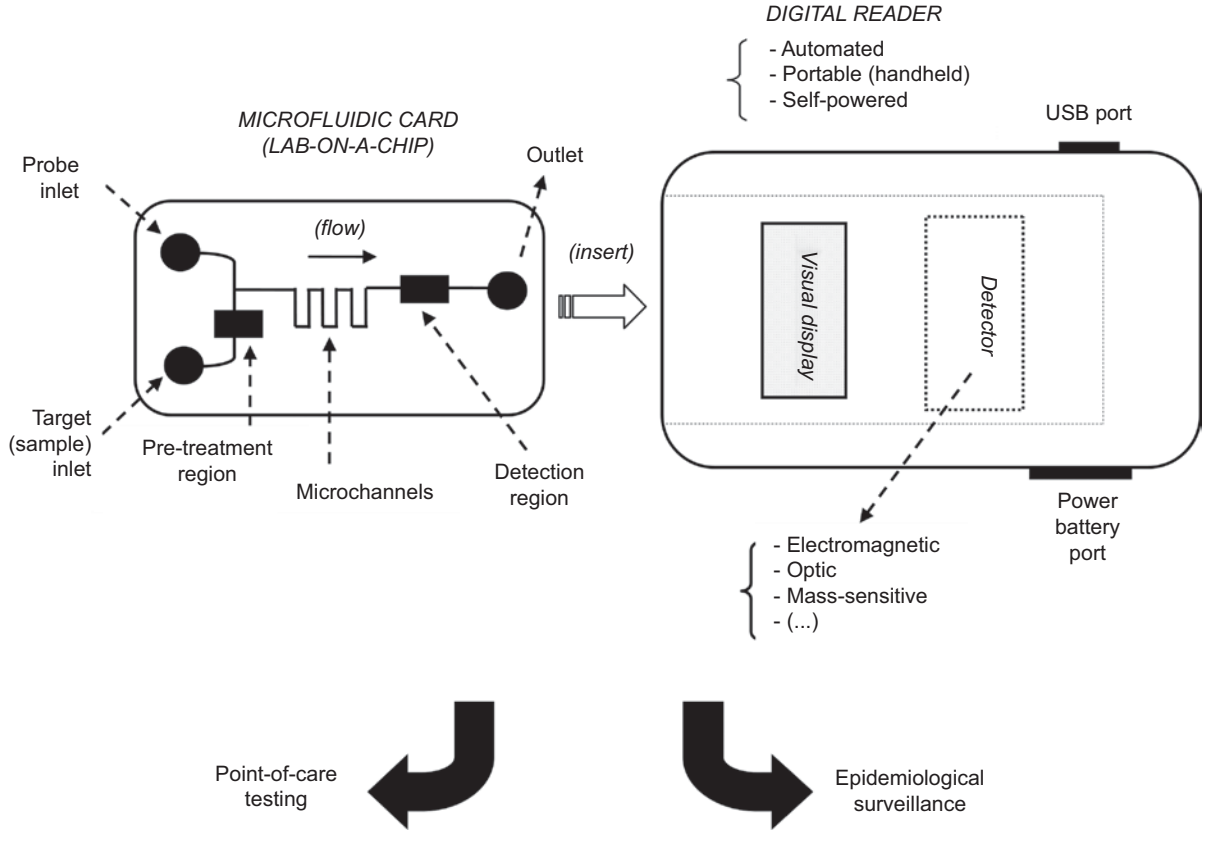

Figure 2. Scheme of a card-like microfluidic device (lab-on-a-chip) for decentralized analysis of clinical samples. Stored data can be used either for point-of-care testing (at the individual and intraclinical level) or epidemiological surveillance (at the population and interclinical level).

epidemiological surveillance (Figure 2). Since many available diagnostic tests have low sensitivity, many undiagnosed people suffer and eventually die. As these people may act as infection reservoirs, it becomes obvious that sensitive diagnostics are required not only for case detection and management, but also for disease surveillance and epidemiological studies. In this regard, pathogen identification at the sub-species level may be especially useful.[135]

Most of the infectious diseases in the tropics are treatable, but accurate identification of patients requiring treatment remains a hard task for disease control. Improved diagnostic tests will likely have little or no impact for diseases which are easily recognized by their clinical symptoms and for which syndromic treatment is recommended. These include deadly acute respiratory infections, diarrheas and pneumonia, for which immunization and case-management are crucial, as well as bacterial infections which can be treated syndromically with broad-spectrum antibiotics (except for TB or leprosy). For some of these diseases the WHO recommends supportive treatment for children, including targeting of common etiological agents and appropriate fluid replacement measures. In these situations there is no clinical need to identify the causal agent, and therefore diagnostic tests are not required for disease management.

By contrast, such tests are necessary for asymptomatic diseases, diseases with misleading symptoms, diseases requiring different treatments or diseases that by their complex or costly treatment require previous case confirmation (some of them demand, for example, intravenous, prolonged and expensive toxic treatments). Such groups include several parasitic infections, such as HAT. However, even for the former diseases periodic surveillance is needed to identify changes in disease spectrum and antimicrobial susceptibility. Surveillance and ongoing control measures must be maintained in developing countries in order to deal with the risk of major epidemics. It must be taken into account that surveillance for elimination-targeted diseases should be improved as a way to detect sporadic although persistent cases of infection.[2] As shown by the examples of re-emergent treponemal infections[181] and TB, surveillance must be maintained even in cases of decreased disease burden, which usually diminishes the perceived importance of diagnosis and of its cost-effectiveness.

Depending on the control strategy, better diagnostic tests may be needed for case finding/case management and for improvement of disease surveillance. In the first case they can be used to screen asymptomatic individuals at risk of disease, or to confirm/rule out a clinical diagnosis in symptomatic patients. Investment in high-quality diagnostic tests may be expensive, but the essential issue is the overall cost of patient health care, especially in cases of long and costly treatment, not to mention the predictable pitfalls of lack of treatment or even wrong or delayed treatment. It is estimated that about 25% of the current burden of infectious diseases in developing countries could be averted if 80% of populations from low-income countries could receive a minimum clinical package (including prenatal/delivery care, family planning, management of sick children, treatment of TB and case-management of sexually transmitted diseases) at a cost below US$10 per person per year.[182]

Difficulties and challenges

Many of the difficulties and challenges in biosensor technology relate to technical aspects of their development and production. In particular, the behavior of active biomolecules when adsorbed on a surface is highly unpredictable, together with troublesome interaction of matrix compounds which may complicate determination of selectivity, sensitivity, limit of detection and response time of the biosensor.[183] Furthermore, mass production of biosensors with a reproducible output often requires large amounts of the purified biomolecule from a biological source with a reasonable degree of purity, except in the case of the highly specific monoclonal antibodies. These are more specific than polyclonal antibodies, while being less sensitive. Furthermore, the source of antigen used (purified native proteins, recombinant proteins or peptides) may significantly influence the performance of rapid diagnostic tests.[28]

Certain biological components of the biosensor may also be released over time, thus contaminating the biological sample. Today's biosensors also have poor stability and are difficult to sterilize, thus explaining the increasing interest and investment in single-use (disposable) devices and integrated low-cost chip technology.[33] Regardless of the undeniable advantages of disposability (single testing) compared to reusability when dealing with infected samples, this should comply with affordability conditions, a link that sometimes fails in resource-depleted regions and settings. It is likely that the use of disposable diagnostics will eventually dominate as production costs per unit will further decrease as a consequence of mass-production through cheaper and simpler manufacturing processes and materials.

The lack of robustness in real samples may be attributed to the usually low operational and/or long-term stability of the biological receptor and/or the physical transducer.[184] The modest stability of biomolecules for biosensing under working conditions *e.g.* room

temperature, may limit the performance of the biosensor after some months. Nevertheless, storage under refrigeration and immobilization of the biological sensing element in membranes or gels may be employed to overcome these obstacles.

Another setback in biosensor research and development is low selectivity due to nonspecific interfering signals at the level of the transducer element. Research about the effects of interfering substances has been performed in pure synthetic model samples rather than in complex real samples, while prior knowledge of the selective molecular recognition processes is needed.[185] In general, current biosensors still need sensitivity improvement in order to achieve the extremely small detection limits in the attomolar range[186] required for diagnostic assays with real, nonamplified biological samples, whereas this is often beyond the fundamental limits of common sensing devices.

Genome amplification-based diagnostics, despite being typically more sensitive than amplification-independent ones, are still too complex and costly for first line point-of-care diagnosis. An appropriate diagnostic may not only require detection, but also quantification of the disease-causing substance, since pathological states are usually associated with high serological levels of these compounds. Many read-out devices, however, are only able to yield qualitative outputs on a "yes/no" basis. Multiplexed simultaneous analysis of many targets must be improved in several current layouts under research and development in order to accomplish the needs for high-throughput analysis. Very often, this may be more dependent on electronic read-out improvements than on the mechanical assembly of the testing device itself. Rapid multiplexing diagnostics will be particularly useful, for example, in cases of co-infections, where unambiguous identification of the disease-causing pathogen(s) is of utmost importance for prompt and adequate case management and treatment. The accuracy of many biosensing devices and methods is still questionable because most still lack proper evaluation, validation and standardization. Ultimately, this will be important for integration and successful use of rapid molecular diagnostics in disease control programs.

Upon development, these diagnostics need to be tested regarding their usage and effect in a sequence of steps.[187] Usually only a few go beyond the first evaluation stage, as testing is mostly carried out indoors rather than in the health care settings where these diagnostics are ultimately supposed to be employed. The UNICEF/UN Development Program/World Bank/WHO TDR Program was created in 1975 for research aimed at the development of new tools for tropical disease control and for training researchers from disease-endemic countries, through public–private partnerships. Such tools include drugs, vaccines and diagnostics, as well as socio-economic issues. In particular, the development of new, accurate, user-friendly and affordable point-of-care tests for health care settings in developing countries is envisaged, but also timely test evaluation and standardization in developing countries by establishing specimen and strain banks in several regions. Wider access to high-quality, low-cost tests is ensured when they are included in the WHO bulk-procurement scheme.[14] Moreover, the TDR Program collaborates with *in vitro* diagnostic manufacturers and other institutions to award grants for the application of new technologies for disease diagnosis. TDR now publishes guidelines about the process of diagnostics evaluation for several diseases; these guidelines have been the basis on which to formulate regulatory approval standards in developing countries.[188] Leading journals now use a TDR checklist to improve standards for publication of diagnostic trials.[189]

Some years ago the TDR Program acquired an additional expertise in care improvement for patients with sexually transmitted infections in resource-limited settings through better diagnostic options. Public sector partnerships such as this are beginning to address the traditional failure in the developed-country market concerning the development through private sector investments, of diagnostic tests for diseases of the developing world. In order to specifically assign the development of new diagnostic tools for neglected infectious diseases to the advent of new biotechnological outcomes, the Bill and Melinda Gates Foundation created the FIND in 2003, the only not-for-profit organization totally devoted to the development of diagnostic tests for infectious diseases. FIND aims to create a model for public action that addresses the current failure of market stakeholders to provide diagnostics for neglected diseases.

The unprecedented opportunities for innovations in new diagnostic tools have benefited from the increased potential for the discovery of novel diagnostic biomarkers through genomics and proteomics, better understanding of pathogen virulence factors and host gene expression (*e.g.* through microarrays) and developments in cutting-edge materials science and nanotechnology. Improved materials have been investigated for use either as matrices for surface biomolecule immobilization (namely new polymers) and as substrates for manufacturing the device body itself (especially from planar fabrication technologies). Predictably, such uniform fabrication will produce featured devices as similar as possible with reproducible characteristics.[23]

In addition to these highlights, future automated and portable diagnostic devices will likely be connected to wireless communication and information systems,

taking advantage of camera-equipped mobile phone networks coupled to web databases, with expected applications to diagnostic imaging and telemedicine in resource-limited settings.[190] By constituting a viable alternative to bulky and expensive camera-equipped microscopy systems, new possibilities for automatic, remote, interactive and nearly real-time data transmission and processing are opened, and hence enormous improvements in disease and patient management,[70] especially in peripheral clinical settings and in rural areas.

Point-of-care devices must comply with standard evaluation criteria that include performance (sensitivity and specificity), simplicity of usage, conditions of use/storage and shelf-life.[191] The application of this wide technical capacity to the enormous medical needs of developing countries is a major challenge for the public sector, given the lack of obvious attractive commercial markets for private entrepreneurs. However, many manufacturers worldwide, particularly in middle-income countries, already show strong capacity and interest in the development and commercialization of these technologies.[2] For commercialization purposes in particular, a major challenge in the development of point-of-care devices is minimization of coupled peripheral components.[192] The Clinical Laboratory Improvement Amendments (CLIA), a regulatory guidance of the FDA, categorizes (through a certification process) commercially marketed *in vitro* diagnostic tests into three categories: waived tests, tests of moderate complexity, and tests of high complexity.

The first category concerns tests that are excluded from certain performance requisites and thus can be used outside traditional laboratory settings. These waived tests, which do not have to comply with heavy regulatory restrictions, can be used, in general, by physician offices that do not have a laboratory for moderately complex testing. In order to achieve the desired coverage and impact, however, new diagnostics must reach and benefit those who really need it. Economical evaluations have shown the advantages of using rapid diagnostic tests in peripheral rural areas with limited access to standard laboratory diagnosis.[193] This ultimately depends on the infrastructure and manpower labor contexts in which diagnostic testing takes place in developing, low-income countries. Not surprisingly, those determinants are usually disrupted and collapsed in such countries, as limited laboratory facilities, few skilled laboratory personnel, unreliable basic health information, competing vertical initiatives, scarce drugs, and weak policy capacity often occur.[188]

Expected economic revenues from capital investment by commercial suppliers in the development, production and marketing of novel rapid diagnostic kits for use in tropical developing countries are unattractive.[12] Since local non-governmental organizations have supported mainly fundamental research rather than industrial and commercial endeavors, additional efforts for wider promotion of rapid diagnostic tests are expected from international public health organizations. Of course, this will largely depend on the establishment of evaluation regulations and standards for clinical use of point-of-care devices.[191]

Conclusion

Biosensors represent a novel technology very different from that currently used in clinical facilities worldwide. In addition, biosensors often face difficulties in obtaining regulatory approval for testing and commercialization. *In vitro* diagnostic devices will likely be expensive to purchase over a one-test basis, although the overall cost may be lower due to the minimal requirement for laboratory manpower. The need for point-of-care testing, especially in resource-limited settings, requires simpler and cheaper instrumentation. In addition, the production and commercialization of high-throughput devices may require investments in the order of several hundred million dollars, a serious obstacle for small start-up companies. The need and market appeal for mass-production of rapid diagnostic tests has pointed not only towards automated fabrication (leading, for instance, to preferable solid-state biosensor designs), but also for entirely new fabrication technologies; in this regard, large industries with expertise in semiconduction, electronics and advanced printing, rather than in biosensing itself, have been in the forefront of knowledge and development.

Constraints about the interpretation of results by non-clinical laboratory staff and maintenance of historical patient records must also be considered in biosensor-based diagnosis, also bearing in mind the need for evaluation of diagnostic procedures among different laboratories. Wider acceptance of these new diagnostic tools by the clinical community may be impaired by controversial usefulness of monitoring one single analyte as a disease biomarker. In this respect, multiplexed and multi-disease tests, for concomitant detection of various infectious targets within a syndrome, can be more clinically useful and cost-effective. Biosensor platforms for clinical diagnosis must fill the need for lower cost in more flexible and diversified end-user environments, while providing a higher level of diagnostic information. Improved micro and especially nanotechnological platforms may thus perform a special role in this regard. On the other hand, the still limited availability of biosensors in the market may be due mainly to a lack

of appropriate technology for their manufacture at a competitive cost rather than a lack of fundamental knowledge. Future advances in biosensor technology will require integration of analytical chemistry, surface chemistry, materials engineering, biological sciences, microelectronics, microfabrication and automation before these devices can become more widely accepted by the clinical community as valuable diagnostic tools for diagnosis of tropical and other infectious diseases.

Many available rapid tests for the diagnosis of tropical diseases lack well-established performance features, are relatively expensive, and have not received regulatory approval either in developing countries or in the countries of origin. Given the world distribution of tropical infectious diseases, the validation of relevant diagnostic kits is usually carried out in geographically diverse settings within the affected developed countries, and thus researchers and manufacturers must bring the requisites of decentralized, point-of-care testing, including standard evaluation criteria, to the implementation of biosensing platforms in developing countries. A more systematic implementation of these novel diagnostic tests as a first-line health care approach will require carrying out extensive cost/benefit studies to forecast the potential impact and advantages of such technologies. This will largely depend on the specific social, economic and technical contexts in which they will be used. It is anticipated that in the future the advanced level of medical diagnosis will be largely dependent on the successful development and implementation of biosensors in such contexts and conditions.

Acknowledgements

We acknowledge Jorge Atouguia and Aida Esteves from the Instituto de Higiene e Medicina Tropical for some useful discussions.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

1. World Health Organization. (2010). The world health report 2004 [Online] Available at: http://www.who.int/whr/2004/en/report04_en.pdf. Accessed on 6 March 2010.
2. Mabey D, Peeling RW, Ustianowski A, Perkins MD. Diagnostics for the developing world. *Nat Rev Microbiol* 2004;2:231–240.
3. Lupi O, Tyring SK. Tropical dermatology: viral tropical diseases. *J Am Acad Dermatol* 2003;49:979–1000.
4. Bell D, Wongsrichanalai C, Barnwell JW. Ensuring quality and access for malaria diagnosis: how can it be achieved? *Nat Rev Microbiol* 2006;4(Suppl):682–695.
5. Strömberg M, Göransson J, Gunnarsson K, Nilsson M, Svedlindh P, Strømme M. Sensitive molecular diagnostics using volume-amplified magnetic nanobeads. *Nano Lett* 2008;8:816–821.
6. Bier FF, Kleinjung F. Feature-size limitations of microarray technology - a critical review. *Fresen J Anal Chem* 2001;371:151–156
7. Kuno G, Cropp CB, Wong-Lee J, Gubler DJ. Evaluation of an IgM immunoblot kit for dengue diagnosis. *Am J Trop Med Hyg* 1998;59:757–762.
8. Miller BL, Henke RR. (2009). Using DNA in multiplexed detection schemes. *IVDT* 2009;Nov:35. [Online] Available at: http://www.devicelink.com/ivdt/archive/09/11/003.html. Accessed on 6 March 2010.
9. Patel PD. (Bio) sensors for measurement of analytes implicated in food safety: a review. *Trends Anal Chem* 2002;21:96–115.
10. Lee WG, Kim Y-G, Chung BG, Demirci U, Khademhosseini A. Nano/microfluidics for diagnosis of infectious diseases in developing countries. *Adv Drug Deliv Rev* 2010;62:449–457.
11. Dineva MA, Mahilum-Tapay L, Lee H. Sample preparation: a challenge in the development of point-of-care nucleic acid-based assays for resource-limited settings. *Analyst* 2007;132:1193–1199.
12. Wilde H, Suankratay C. There is need for antigen-based rapid diagnostic tests to identify common acute tropical illnesses. *J Travel Med* 2007;14:254–258.
13. Ferreira AAP, Colli W, da Costa PI, Yamanaka H. Immunosensor for the diagnosis of Chagas' disease. *Biosens Bioelectron* 2005;21:175–181.
14. Mondesire RR. (2005). Global efforts to develop IVDs for tropical diseases. *IVDT* [Online] 2005;Jul: 24. Available at: http://www.devicelink.com/ivdt/archive/05/07/005.html. Accessed on 6 March 2010.
15. D'Acremont V, Lengeler C, Mshinda H, Mtasiwa D, Tanner M, Genton B. Time to move from presumptive malaria treatment to laboratory-confirmed diagnosis and treatment in African children with fever. *PLoS Med* 2009;6(art.e252):1–3.
16. Toovey S, Jamieson A. Rolling back malaria: how well is Europe doing? *Travel Med Infect Dis* 2003;1:167–175.
17. Daar AS, Thorsteindottir H, Martin DK, Smith AC, Nast S, Singer PA. Top ten biotechnologies for improving health in developing countries. *Nat Genet* 2002;32:229–232.
18. Newman DM, Heptinstall J, Matelon RJ, Savage L, Wears ML, Beddow J, Cox M, Schallig HDFH, Mens PF. A magneto-optic route toward the *in vivo* diagnosis of malaria: preliminary results and preclinical trial data. *Biophys J* 2008;95:994–1000.
19. Zelada-Guillén GA, Riu J, Düzgün A, Rius FX. Immediate detection of living bacteria at ultralow concentrations using a carbon nanotube based potentiometric aptasensor. *Angew Chem Int Ed* 2009;48:7334–7337.
20. Pividori MI, Merkoçi A, Alegret S. Electrochemical genosensor design: immobilisation of oligonucleotides onto transducer surfaces and detection methods. *Biosens Bioelectron* 2000;15:291– 03.
21. Ferreira AW. *Testes sorológicos*. In: Coura JR, ed. Dinâmica das doenças infecciosas e parasitárias (vol. I). [Dynamics of

infectious and parasitic diseases]. Rio de Janeiro: Guanabara Koogan, 2005:196.
22. Baeumner AJ, Pretz J, Fang S. A universal nucleic acid sequence with nanomolar detection limits. *Anal Chem* 2004;76:888–894.
23. Diamond D. *Overview*. In: Diamond D, ed. Principles of chemical and biological sensors. Toronto: John Wiley, 1998:7–12.
24. Teles FRR, Fonseca LP. Trends in DNA biosensors. *Talanta* 2008;77:606–623.
25. Wang T-H, Peng Y, Zhang C, Wong PK, Ho C-M. Single-molecule tracing on a fluidic microchip for quantitative detection of low-abundance nucleic acids. *J Am Chem Soc* 2005;127:5354–5359.
26. Baird CL, Myszka DG. Current and emerging commercial optical biosensors. *J Mol Recognit* 2001;14:261–268.
27. Petti CA, Polage CR, Quinn TC, Ronald AR, Sande MA. Laboratory medicine in Africa: a barrier to effective health care. *Clin Infect Dis* 2006;42:377–382.
28. Murray CK, Bennett W. Rapid diagnosis of malaria. *Interdiscip Perspect Infect Dis* 2009; Article ID 415953 (7 pages).
29. Nayak M, Kotian A, Marathe S, Chakravortty D. Detection of microorganisms using biosensors – a smarter way towards detection techniques. *Biosens Bioelectron* 2009;25:661–667.
30. Murray CK, Gasser RA Jr, Magill AJ, Miller RS. Update on rapid diagnostic testing for malaria. *Clin Microbiol Rev* 2008;21:97–110.
31. Teles FRR, Prazeres DMF, Lima-Filho JL. Trends in dengue diagnosis. *Rev Med Virol* 2008;15:287–302.
32. Peters RHP, Agtamael MA van, Danner SA, Savelkoul PHM, Vandenbroucke-Grauls CMJE. New developments in the diagnosis of bloodstream infections. *Lancet Infect Dis* 2004;4:751–760.
33. Griffiths D, Hall G. Biosensors – what real progress is being made? *Trends Biotechnol* 1993;11:122–130.
34. McCormack T, Keating G, Killard A, Manning BM, O'Kennedy R. *Biomaterials for biosensors*. In: Diamond D, ed. Principles of chemical and biological sensors. Toronto, Canada: John Wiley 1998:186–187.
35. Mikkelsen SR. Electrochecmical biosensors for DNA sequence detection. *Electroanalysis* 1996;8:15–19.
36. Campbell NF, Evans JA, Fawcett NC. Detection of poly (U) hybridization using azido modified poly (A) coated piezoelectric crystals. *Biochem Biophys Res Commun* 1993;196:858–863.
37. Turner APF. Biosensors – sense and sensitivity. *Science* 2000;290:1315–1317.
38. Wang H-S, Ju H-X, Chen H-Y. Simultaneous determination of guanine and adenine in DNA using an electrochemically pretreated glassy carbon electrode. *Anal Chim Acta* 2002;461:243–250.
39. Mohan TM, Nath N, Anand S. Detection of filarial antibody using a fiber optics immunosensor (FOI). *Indian J Clin Biochem* 1997;12(Suppl):17–21.
40. Dittrich PS, Tachikawa K, Manz A. Micro total analysis systems. Latest advancements and trends. *Anal Chem* 2006;78:3887–3908.
41. Bang H, Yun H, Lee WG, Park J, Lee J, Chung S, Cho K, Chung C, Han D-C, Chang JK. Expansion channel for microchip flow cytometers. *Lab Chip* 2006;6:1381–1383.
42. Kerman K, Kobayashi M, Tamiya E. Recent trends in electrochemical DNA biosensor technology. *Meas Sci Technol* 2004;15:R1–R11.
43. Lee WG, Bang H, Yun H, Lee J, Park J, Kim JK, Chung S, Cho K, Chung C, Han D-C, Chang JK. On-chip erythrocyte deformability test under optical pressure. *Lab Chip* 2007;7:516–519.
44. Moon SJ, Keles HO, Ozcan A, Khademhosseini A, Haeggstrom E, Kuritzkes D, Demirci U. Integrating microfluidics and lensless imaging for point-of-care testing. *Biosens Bioelectron* 2009;24:3208–3214.
45. Goddard GR, Sanders CK, Martin JC, Kaduchak G, Graves SW. Analytical performance of an ultrasonic particle focusing flow cytometer. *Anal Chem* 2007;79:8740–8746.
46. Sato H, Sasamoto Y, Yagyu D, Sekiguchi T, Shoji S. 3D sheath flow using hydrodynamic position control of the sample flow. *J Micromech Microeng* 2007;17:2211–2216.
47. Berg A van den, Wessling M. Nanofluidics: silicon for the perfect membrane. *Nature* 2007;445:726.
48. Jain KK. Applications of nanobiotechnology in clinical diagnostics. *Clin Chem* 2007;53:2002–2009.
49. Yager P, Domingo Gj, Gerdes J. Point-of-care diagnostics for global health. *Ann Rev Biomed Eng* 2008;10:107–144.
50. Vaddiraju S, Tomazos I, Burgess DJ, Jain FC, Papadimitrakopoulos F. Emerging synergy between nanotechnology and implantable biosensors: a review. *Biosens Bioelectron* 2010;25:1553–1565.
51. Yonzon CR, Stuart DA, Zhang X, McFarland AD, Haynes CL, van Duyne RP. Towards advanced chemical and biological nanosensors – an overview. *Talanta* 2005;67:438–448.
52. Chung S, Yun H, Kamm RD. Nanointerstice-driven microflow. *Small* 2009;5:609–613.
53. Martinez AW, Phillips ST, Wiley BJ, Gupta M, Whitesides GM. FLASH: a rapid method for prototyping paper-based microfluidic devices. *Lab Chip* 2008;8:2146–2150.
54. Niemeyer CM. Semi-synthetic nucleic acid–protein conjugates: applications in life sciences and nanobiotechnology. *Rev Mol Biotechnol* 2001;82:47–66.
55. Gruner G. Carbon nanonets – spark new electronics. *Sci Am* 2007;17:48–55.
56. Minunni M, Tombelli S, Gullotto A, Luzi E, Mascini M. Development of biosensor with aptamers as bio-recognition element: the case of HIV-1 Tat protein. *Biosens Bioelectron* 2004;20:1149–1156.
57. De-los-Santos-Álvarez N, Lobo-Castañón MJ, Miranda-Ordieres AJ, Tuñón-Blanco P. Aptamers as recognition elements for label-free analytical devices. *Trends Anal Chem* 2008;27:437–446.
58. Shamah SM, Healy JM, Cload ST. Complex target SELEX. *Acc Chem Res* 2008;41:130–138.
59. Hohng S, Ha T. Single-molecule quantum-dot fluorescence resonance energy transfer. *Chem Phys Chem* 2005;6:956–960.
60. Alivisatos AP. Less is more in medicine. *Sci Am* 2003; 17:73–79.
61. Whitesides GM, Love JC. The art of building small. *Sci Am* 2007;17:13–21.
62. Tam JM, Song L, Walt DR. DNA detection on ultrahigh-density optical fiber-based nanoarrays. *Biosens Bioelectron* 2009;24:2488–2493.
63. Bhardwaj U, Papadimitrakopoulos, Burgess DJ. A review of the development of a vehicle for localized and controlled drug delivery for implantable biosensors. *J Diabetes Sci Technol* 2008;2:1016–1029.
64. Wu Y, Rojas AP, Griffith GW, Skrzypchak AM, Lafayette N, Bartlett RH, Meyerhoff ME. Improving blood compatibility of intravascular oxygen sensors via catalytic decomposition of *S*-nitrosothiols to generate nitric oxide in situ. *Sensors Actuat B* 2007;121:36–46.
65. Onuki Y, Bhardwaj U, Papadimitrakopoulos, Burgess DJ. A review of the biocompatibility of implantable devices: current challenges to overcome foreign body response. *J Diabetes Sci Technol* 2008;2:1003–1015.

66. Kvist PH, Iburg T, Aalbaek B, Gerstenberg M, Schoier C, Kaastrup P, Buch-Rasmussen T, Hasselager E, Jensen HE. Biocompatibility of an enzyme-based, electrochemical glucose sensor for short-term implantation in the subcutis. *Diabetes Technol Ther* 2006;8:546–559.
67. Adiga SP, Curtiss LA, Elam JW, Pellin MJ, Shih C-C, Shih C-M, Lin S-J, Su Y-Y, Gittard SD, Zhang J, Narayan RJ. Nanoporous materials for biomedical devices. *J Miner Met Mater Soc* 2008;60:26–32.
68. Asuri P, Karajanagi SS, Kane RS, Dordick JS. Polymer-nanotube-enzyme composites as active antifouling films. *Small* 2007;3:50–53.
69. Luckarift HR, Dickerson MB, Sandhage KH, Spain JC. Rapid, room-temperature synthesis of antibacterial bionanocomposites of lyzozyme with amorphous silica or titania. *Small* 2006;2:640–643.
70. Industrial Science and Technology Working Group (ISTWG). Roadmapping converging technologies to combat emerging infectious diseases. Bangkok, Thailand: Asia-Pacific Economic Cooperation (APEC) 2008.
71. Sampath R, Hofstadler SA, Blyn L, Eshoo M, Hall T, Massire C, Levene HM, Hannis JC, Harrell PM, Neuman B, Buchmeier MJ, Jiang Y, Ranken R, Drader JJ, Samant V, Griffey RH, McNeil JA, Crooke ST, Ecker DJ. Rapid identification of emerging pathogens: coronavirus. *Emerg Infect Dis* 2005;11:373-379.
72. Sampath R, Russell KL, Massire C, Eshoo MW, Harpin V, Blyn LB, Melton R, Ivy C, Pennella T, Li F, Levene H, Hall TA, Libby B, Fan N, Walcott DJ, Ranken R, Pear M, Schink A, Gutierrez J, Drader J, Moore D, Metzgar D, Addington L, Rothman R, Gaydos CA, Yang S, George KS, Fuschino ME, Dean AB, Stallknecht DE, Goekjian G, Yingst S, Monteville M, Saad MD, Whitehouse CA, Baldwin C, Rudnick KH, Hofstadler SA, Lemon SM, Ecker DJ. Global surveillance of emerging Influenza virus genotypes by mass spectrometry. *PLoS ONE* 2007;2:e489 (9 pages).
73. Moody A. Rapid diagnostic tests for malaria parasites. *Clin Microbiol Rev* 2002;15:66–78.
74. Aguirre AMR, Zavalaga LFL, Belaunde MT. Relación costo-efectividad del uso de pruebas rápidas para el diagnóstico de la malária en la Amazonia peruana [Cost-effectiveness relation for the use of rapid tests for diagnosis of malaria in the Amazon region of Peru]. *Rev Panam Salud Publica* 2009;25:377–388.
75. World Health Organization. *Guidelines for the treatment of malaria*, 2nd ed. Geneva: World Health Organization, 2010.
76. Forney JR, Wongsrichanalai C, Magill AJ, Craig LG, Sirishaisinthop J, Bautista CT, Miller RS, Ockenhouse CF, Kester KE, Aronson NE, Andersen EM, Quino-Ascurra HA, Vidal C, Moran KA, Murray CK, DeWitt CC, Heppner DG, Kain KC, Ballou WR, Gasser Jr RA. Devices for rapid diagnosis of malaria: evaluation of prototype assays that detect Plasmodium falciparum histidine-rich protein 2 and a Plasmodium vivax-specific antigen. *J Clin Microbiol* 2003;41:2358–2366.
77. World Health Organization. (2003). Malaria rapid diagnosis – making it work. WHO Meeting Report (20–23 Jan) [Online] Available at: http://www.who.int/malaria/publications/atoz/rdt2.pdf. Accessed on 6 March 2010.
78. World Health Organization. Malaria rapid diagnostic test performance. Summary results of WHO product testing of malaria RDTs: rounds 1 and 2 (2008–2009). Geneva: World Health Organization, 2010.
79. Banoo S, Bell D, Bossuyt P, Herring A, Mabey D, Poole F, Smith PG, Sriram N, Wongsrichanalai C, Linke R, O'Brien R, Perkins M, Cunningham J, Matsoso P, Nathanson CM, Olliaro P, Peeling RW, Ramsay A. Evaluation of diagnostic tests for infectious diseases: general principles. *Nat Rev Microbiol* 2006;4(Suppl):S20–S32.
80. Gasser Jr RA, Magill AJ, Ruebush II TK, Miller RS, Sirichaisinthop J, Forney JR, Bautista CT, Arevalo I, Rhorer J, Wittes J, Wongsrichanalai C. Malaria diagnosis: performance of NOW® ICT malaria in a large scale field trial. *Proceedings of the 54th Annual Meeting of the American Society of Tropical Medicine and Hygiene,* December 2005; Washington, USA.
81. Antia M, Herricks T, Rathod PK. Microfluidic modeling of cell-cell interactions in malaria pathogenesis. *PLoS Pathog* 2007;3:e99(939–948).
82. Baker J, McCarthy J, Gatton M, Kyle DE, Belizario V, Luchavez J, Bell D, Cheng Q. Genetic diversity of *Plasmodium falciparum* histidine-rich protein 2 (PfHRP2) and its effect on the performance of PfHRP2-based rapid diagnostic tests. *J Infect Dis* 2005;192:870–877.
83. Lee N, Baker J, Andrews KT, Gatton ML, Bell D, Cheng Q, McCarthy J. Effect of sequence variation in *Plasmodim falciparum* histidine-rich protein 2 on binding of specific monoclonal antibodies: implications for rapid diagnostic tests for malaria. *J Clin Microbiol* 2006;44:2773–2778.
84. Gamboa D, Mei-Fong H, Bendezu J, Torres K, Chiodini PL, Barnwell JW, Incardona S, Perkins M, Bell D, McCarthy J, Cheng Q. A large proportion of *P. falciparum* isolates in the Amazon region of *Peru lack* pfhrp2 and pfhrp3: implications for malaria rapid diagnostic tests. *PLoS ONE* 2010;5:e8091(1–7).
85. Groen J, Koraka P, Velzing J, Copra C, Osterhaus AD. Evaluation of six immunoassays for detection of dengue virus-specific immunoglobulin M and G antibodies. *Clin Diagn Lab Immunol* 2000;7:867–871.
86. Vasquez S, Hafner G, Ruiz D, Calzada N, Guzman MG. Evaluation of immunoglobulin M and G capture enzyme-linked immunosorbent assay Panbio kits for diagnostic dengue infections. *J Clin Virol* 2007;39:194–198.
87. Su C-C, Wu T-Z, Chen L-K, Yang H-H, Tai D-F. Development of immunochips for the detection of dengue viral antigens. *Anal Chim Acta* 2003;479:117–123.
88. Teles FRR, Prazeres DMF, Lima-Filho JL. Electrochemical detection of a dengue-related oligonucleotide sequence using ferrocenium as a hybridization indicator. *Sensors* 2007;7:2510–2518.
89. Tam-Chang S-W, Carson TD, Huang L, Publicover NG, Hunter Jr KW. Stem–loop probe with universal reporter for sensing unlabeled nucleic acids. *Anal Biochem* 2007;366:126–130.
90. Baeumner AJ, Schlesinger NA, Slutzki NS, Romano J, Lee EM, Montagna RA. Biosensor for dengue virus detection: sensitive, rapid, and serotype specific. *Anal Chem* 2002;74:1442–1448.
91. Dodeigne C, Thunus L, Lejeune R. Chemiluminescence as a diagnostic tool. A review. *Talanta* 2000;51:415–4439.
92. Atias D, Liebes Y, Chalifa-Caspi V, Bremand L, Lobel L, Marks RS, Dussart P. Chemiluminescent optical fiber immunosensor for the detection of IgM antibody to dengue virus in humans. *Sensors Actuat B* 2009;140:206–215.
93. Kwakye S, Baeumner A. A microfluidic biosensor based on nucleic acid sequence recognition. *Anal Bioanal Chem* 2003;376:1062–1068.
94. Zaytseva NV, Montagna RA, Lee EM, Baeumner AJ. Multi-analyte single-membrane biosensor for the serotype-specific detection of dengue virus. *Anal Bioanal Chem* 2004;380:46–53.
95. Oliveira MDL, Correia MTS, Diniz FB. Concanavalin A and polyvinyl butyral use as a potential dengue electrochemical biosensor. *Biosens Bioelectron* 2009;25:728–732.
96. Guan J-G, Miao Y-Q, Zhang Q-J. Impedimetric biosensors. *J Biosci Bioeng* 2004;97:219–226.

97. Daniels JS, Pourmand N. Label-free impedance biosensors: opportunities and challenges. *Electroanalysis* 2007;19:1239–1257.
98. Kumbhat S, Sharma K, Gehlot R, Solanki A, Joshi V. Surface plasmon resonance based immunosensor for serological diagnosis of dengue virus infection. *J Pharm Biomed Anal* 2010;52:255–259.
99. Ridderhof JC, Deun A van, Kam KM, Narayanan PR, Aziz MA. Roles of laboratories and laboratory systems in effective tuberculosis programmes. *Bull World Health Organ* 2007;85:354–359.
100. Wang J, Rivas G, Cai X, Dontha N, Shiraishi H, Luo D, Valera FS. Sequence-specific electrochemical biosensing of *M. tuberculosis* DNA. *Anal Chim Acta* 1997;337:41–48.
101. Liu GD, Wu ZY, Wang SP, Shen GL, Yu RQ. Renewable amperometric immunosensor for Schistosoma japonicum antibody assay. *Anal Chem* 2001;73:3219–3226.
102. Prabhakar N, Arora K, Arya SK, Solanki PR, Iwamoto M, Singh H, Malhotra BD. Nucleic acid sensor for *M. tuberculosis* detection based on surface plasmon resonance. *Analyst* 2008;133:1587–1592.
103. He F, Zhang L. Rapid diagnosis of *M. tuberculosis* using a piezoelectric immunosensor. *Anal Sci* 2002;18:397–401.
104. He F, Zhao J, Zhang L, Su X. A rapid method for determining *Mycobacterium tuberculosis* based on a bulk acoustic wave impedance biosensor. *Talanta* 2003;59:935–941.
105. Ren J, He F, Yi S, Cui X. A new MSPQC for rapid growth and detection of *Mycobacterium tuberculosis*. *Biosens Bioelectron* 2008;24:403–409.
106. Ke C, Berney H, Mathewson A, Sheehan MM. Rapid amplification for the detection of *Mycobacterium tuberculosis* using a non-contact heating method in a silicon microreactor based thermal cycler. *Sens Actuat B* 2004;102:308–314.
107. Denton KA, Kramer MF, Lim DV. Rapid detection of *Mycobacterium tuberculosis* in lung tissue using a fiber optic biosensor. *J Rapid Methods Autom Microbiol* 2009;17:17–31.
108. Trouiller P, Olliaro P, Torreele E, Orbinski J, Laing R, Ford N. Drug development for neglected diseases: a deficient market and a public-health policy failure. *Lancet* 2002;359:2188–2194.
109. Boelaert M, Rijal S, Regmi S, Singh R, Karki B, Jacquet D, Chappuis F, Campino L, Desjeux P, Le Ray D, Koirala S, Van der Stuyft P. A comparative study of the effectiveness of diagnostic tests for visceral leishmaniasis. *Am J Trop Med Hyg* 2004;70:72–77.
110. Deborggraeve S, Boelaert M, Rijal S, Doncker S, Dujardin J-C, Herdewijn P, Büscher P. Diagnostic accuracy of a new *Leishmania* PCR for clinical visceral leishmaniasis in Nepal and its role in diagnosis of disease. *Trop Med Int Health* 2008;13:1378–1383.
111. Zijlstra EE, Ali MS, Hassan AM el, Toum IA el, Satti M, Ghalib HW, Kager PA. Kala-azar: a comparative study of parasitological methods and the direct agglutination test in diagnosis. *Trans R Soc Trop Med Hyg* 1992;86:505–507.
112. Staquet M, Rozencweig M, Lee YJ, Muggia FM. Methodology for the assessment of new dichotomous diagnostic tests. *J Chronic Dis* 1981;34:599–610.
113. Harith A el, Kolk AH, Kager PA, Leeuwenburg J, Muigai R, Kiugu S, Bovier PA, Desjeux P, Le Ray D, Koirala S, Loutan L. A simple and economical direct agglutination test for serodiagnosis and sero-epidemiological studies of visceral leishmaniasis. *Trans R Soc Trop Med Hyg* 1986;80:583–587.
114. Zijlstra EE, Ali MS, Hassan AM el, Toum IA el, Satti M, Ghalib HW, Kager PA. Direct agglutination test for diagnosis and sero-epidemiological survey of kala-azar in the Sudan. *Trans R Soc Trop Med Hyg* 1991;85:474–476.
115. Chowdhury MA, Rafiqueuddin AK, Hussain A. Aldehyde test (formol-gel test) in the diagnosis of kala-azar (visceral leishmaniasis). *Trop Doct* 1992;22:185–186.
116. Sundar S, Reed SG, Singh VP, Kumar PC, Murray HW. Rapid accurate field diagnosis of Indian visceral leishmaniasis. *Lancet* 1998;351:563–565.
117. Chappuis F, Rijal S, Singh R, Acharya P, Karki BM, Das ML, Bovier PA, Desjeux P, Le Ray D, Koirala S, Loutan L. Prospective evaluation and comparison of the direct agglutination test and an rK39-antigen-based dipstick test for the diagnosis of suspected kala-azar in Nepal. *Trop Med Int Health* 2003;8:277–285.
118. Diniz FB, Ueta RR, Pedrosa AM da C, Areias M da C, Pereira VRA, Silva ED, Silva Jr JG, Ferreira AGP, Gome YM. Impedimetric evaluation for diagnosis of Chagas' disease: antigen-antibody interactions on metallic electrodes. *Biosens Bioelectron* 2003;19:79–84.
119. Umezawa ES, Nascimento MS, Stolf AMS. Enzyme-linked immunosorbent assay with *Trypanosoma* cruzi excreted-secreted antigens (TESA-ELISA) for serodiagnosis of acute and chronic Chagas' disease. *Diagn Microbiol Infect Dis* 2001;39:169–176.
120. Engels D, Chitsulo L, Montresor A, Savioli L. The global epidemiological situation of schistosomiasis and new approaches to control and research. *Acta Trop* 2002;82:139–146.
121. Corstjens PLAM, Lieshout L van, Zuiderwijk M, Kornelis D, Tanke HJ, Deelder AM, Dam GJ van. Up-converting phosphor technology-based lateral flow assay for detection of *Schistosoma* circulating anodic antigen in serum. *J Clin Microbiol* 2008;46:171–176.
122. Lieshout L van, Polderman AM, Deelder AM. Immunodiagnosis of schistosomiasis by determination of the circulating antigens CAA and CCA, in particular in individuals with recent or light infections. *Acta Trop* 2000;77:69–80.
123. Deelder AM, De JN, Boerman OC, Fillie YE, Hilberath GW, Rotmans JP, Gerritse MJ, Schut DWO. Sensitive determination of circulating anodic antigen in *Schistosoma* mansoni infected individuals by an enzyme-linked immunosorbent assay using monoclonal antibodies. *Am J Trop Med Hyg* 1989;40:268–272.
124. Lieshout L van, Polderman AM, Visser LG, Verwey JJ, Deelder AM. Detection of the circulating antigens CAA and CCA in a group of Dutch travelers with acute schistosomiasis. *Trop Med Int Health* 1997;2:551–557.
125. Zijlmans HJ, Bonnet J, Burton J, Kardos K, Vail T, Niedbala RS, Tanke HJ. Detection of cell and tissue surface antigens using u-converting phosphors: a new reporter technology. *Anal Biochem* 1999;267:30–36.
126. Bojorge-Ramírez NI, Salgado AM, Valdman B. Amperometric immunosensor for detecting *Schistosoma* mansoni antibody. *Assay Drug Dev Technol* 2007;5:673–682.
127. Goulart IMB, Goulart LR. Leprosy: diagnostic and control challenges for a worldwide disease. *Arch Dermatol Res* 2008;300:269–290.
128. Scollard MD, Adams LB, Gillis TP, Krahembuhl JL, Truman RW, Williams DL. The continuing challenges of leprosy. *Clin Microbiol Rev* 2006;19:166–168.
129. Bührer-Sékula S, Smits HL, Gussenhoven GC, Leeuwen J van, Amador S, Fujiwara T, Klatser PR, Oskam L. Simple and fast lateral flow test for classification of leprosy patients and identification of contacts with high risk of developing leprosy. *J Clin Microbiol* 2003;41:1991–1995.
130. Goulart IMB, Souza DOB, Marques CR, Pimenta VL, Gonçalves MA, Goulart LR. Risk and protective factors for leprosy development in an epidemiological surveillance of household contacts. *Clin Vaccine Immunol* 2008;15:101–105.

131. Groathouse NA, Amin A, Marques MAM, Spencer JS, Gelber R, Knudsen DL, Belisle JT, Brennan PJ, Slayden RA. Use of protein microarrays to define the humoral immune response in leprosy patients and identification of disease-state specific antigen profiles. *Infect Immun* 2006;74:6458–6466.
132. Capparelli FE, Oliveira JDD, Marangoni K, Goulart IMB, Goulart LR. Desenvolvimento de mimetopos protéicos de PGL-1 imunorreativos contra soro de pacientes com hanseníase [Development of protein mimetopes from PGL-1 immunoreactive against serum of patients with hanseniasis]. *Hansenol Int* 2005;30:120.
133. Spencer JS, Dockrell HM, Kim HJ, Marques MAM, Williams DL, Martins MVSB, Martins MLF, Lima MCBS, Sarno EN, Pereira GMB, Matos H, Fonseca LS, Sampaio EP, Ottenhoff THM, Geluk A, Cho S-N, Stoker NG, Cole ST, Brennan PJ, Pessolani MCV. Identification of specific proteins and peptides in *Mycobacterium* leprae suitable for the selective diagnosis of leprosy. *J Immunol* 2005;175:7930–7938.
134. Afonso AS, Goulart LR, Moura M, Goulart IMB, Madurro JM, Brito-Madurro AG. (2007). Detection of *Mycobacterium leprae* DNA onto graphite for leprosy diagnostics. San Francisco: Fourth International Congress of Nanotechnology, November 2007:1–2.
135. Deborggraeve S, Büscher P. Molecular diagnostics for sleeping sickness: what is the benefit for the patient? *Lancet Infect Dis* 2010;10:433–439.
136. Robays J, Bilengue MMC, Stuyft P Van der, Boelaert M. The effectiveness of active population screening and treatment from sleeping sickness control in the Democratic Republic of Congo. *Trop Med Int Health* 2004;9:542–550.
137. Büscher P, Ngoyi DM, Kaboré J, Lejon V, Robays J, Jamonneau V. Improved models of mini anion exchange centrifugation technique (mAECT) and modified single centrifugation (MSC) for sleeping sickness diagnosis and staging. *PLoS Negl Trop Dis* 2009;3:e471(1–3).
138. Becker S, Franco JR, Simarro PP, Stich A, Abel PM, Steverding D. Real-time PCR for detection of *Trypanosoma* brucei in human blood samples. *Diagn Microbiol Infect Dis* 2004;50:193–199.
139. Radwanska M, Chamekh M, Vanhamme L, Claes F, Magez S, Magnus E, Baetselier P, Büscher P, Pays E. The serum resistance-associated gene as a diagnostic tool for the detection of *Trypanosoma* brucei rhodesiense. *Am J Trop Med Hyg* 2002;67:684–690.
140. Chappuis F, Loutan L, Simarro P, Lejon V, Büscher P. Options for the field diagnosis of human African trypanosomiasis. *Clin Microbiol Rev* 2005;18:133–146.
141. Simo G, Njiokou F, Tume C, Lueong S, De Meeûs T, Cuny G, Asonganyi T. Population genetic structure of Central African *Trypanosoma* brucei gambiense isolates using microsatellite DNA markers. *Infect Genet Evol* 2010;10:68–76.
142. Hecht MM, Nitz N, Araujo PF, Sousa AO, Rosa AC, Gomes DA, Leonardecz E, Teixeira ARL. Inheritance of DNA transferred from American trypanosomes to human hosts. *PLoS One* 2010;12:e9181.
143. Jamonneau V, Solano P, Garcia A, Lejon V, Djé N, Miezan TW, N'Guessan P, Cuny G, Büscher P. Stage determination and therapeutic decision in human African trypanosomiasis: value of polymerase chain reaction and immunoglobulin M quantification on the cerebrospinal fluid of sleeping sickness patients in Côte d'Ivoire. *Trop Med Int Health* 2003;8:589–594.
144. Deborggraeve S, Claes F, Laurent T, Mertens P, Leclipteux T, Jujardin JC, Herdewijn P, Büscher P. Molecular dipstick test for diagnosis of sleeping sickness. *J Clin Microbiol* 2006;44:2884–2889.
145. Maslov DA, Lukes J, Jirku M, Simpson L. Phylogeny of trypanosomes as inferred from the small and large subunit rRNAs: implications for the evolution of parasitism in the trypanosomatid protozoa. *Mol Biochem Parasitol* 1996;75:197–205.
146. Kuboki N, Inoue N, Sakurai T, Cello F, Grab DJ, Suzuki H, Sugimoto C, Igarashi I. Loop-mediated isothermal amplification for detection of African trypanosomes. *J Clin Microbiol* 2003;41:5517–5524.
147. Njiru ZK, Mikosza ASJ, Matovu E, Enyaru JCK, Ouma JO, Kibona SN, Thompson RC, Ndung'u JM. African trypanosomiasis: sensitive and rapid detection of the sub-genus *Trypanozoon by loop-mediated isothermal amplification (LAMP) of parasite DNA*. *Int J Parasitol* 2008;38:589–599.
148. Mugasa CM, Schoone GJ, Ekangu RA, Lubega GW, Kager PA, Schallig HD. Detection of *Trypanosoma* brucei parasites in blood samples using real-time nucleic acid sequence-based amplification. *Diagn Microbiol Infect Dis* 2009;61:440–445.
149. Mugasa CM, Laurent T, Schoone GJ, Kager PA, Lubega GW, Schallig HD. Nucleic acid sequence-based amplification with oligochromatography for detection of *Trypanosoma* brucei in clinical samples. *J Clin Microbiol* 2009;47:630–635.
150. Radwanska M, Magez S, Perry-O'Keefe H, Stender H, Coull J, Sternberg JM, Büscher P, Hyldig-Nielsen JJ. Direct detection and identification of African trypanosomes by fluorescence in situ hybridization with peptide nucleic acid probes. *J Clin Microbiol* 2002;40:4295–4297.
151. Jones D, Nyalwidhe J, Tetley L, Barret MP. McArthur revisited: fluorescence microscopes for field diagnostics. *Trends Parasitol* 2007;23:468–469.
152. Lorger M, Engstler M, Homann M, Göringer U. Targeting the variable surface of African trypanosomes with variant surface glycoprotein-specific, serum-stable RNA aptamers. *Eukar Cell* 2003;2:84–94.
153. Palumbo E. Filariasis: diagnosis, treatment and prevention. *Acta Biomed* 2008;79:106–109.
154. Freedman DO, Filho PJ de A, Besh S, Silva MCM, Braga C, Maciel A. Lymphoscintigraphic analysis of lymphatic abnormalities in symptomatic and asymptomatic human filariasis. *J Infect Dis* 1994;170:927–933.
155. Dixit V, Gupta AK, Bisen PS, Prasad GBKS, Harinath BC. Serum immune complexes as diagnostic and therapeutic markers in lymphatic filariasis. *J Clin Lab Anal* 2007;21:114–118.
156. Nguyen NL, Plichart C, Esterre P. Assessment of immunochromatographic test for rapid lymphatic filariasis diagnosis. *Parasitology* 1999;6:355–358.
157. Weil G, Lammie PJ, Weiss N. The ICT filariasis test: a rapid format antigen test for diagnosis of bancrofitian filariasis. *Parasitol Today* 1997;13:401–404.
158. Tamrakar AK, Goel AK, Kamboj DV, Singh L. Surveillance methodology for *V. cholerae* in environmental samples. *Int J Environ Health Res* 2006;16:305–312.
159. Goel AK, Tamrakar AK, Kamboj DV, Singh L. Direct immunofluorescence assay for rapid environmental detection of *Vibrio cholerae* O1. *Folia Microbiol* 2005a;50:443–446.
160. Goel AK, Tamrakar AK, Nema V, Kamboj DV, Singh L. Detection of viable toxigenic *Vibrio cholerae* from environmental water sources by direct cell duplex PCR assay. *World J Microbiol Biotech* 2005;21:973–976.
161. Sharma MK, Goel AK, Singh L, Rao VK. Immunological biosensor for detection of *Vibrio cholerae* O1 in environmental water samples. *World J Microbiol Biotechnol* 2006;22:1155–1159.
162. Jyoung JY, Hong S, Lee W, Choi JW. Immunosensor for the detection of *Vibrio cholerae* O1 using surface plasmon resonance. *Biosens Bioelectron* 2006;21:2315–2319.

163. Carter RM, Mekalanos JJ, Jacobs MB, Lubrano GJ, Guilbault GG. Quartz crystal microbalance detection of *Vibrio cholerae* O139 serotype. *J Immunol Methods* 1995;187:121–125.
164. Marks RS, Bassis E, Bychenko A, Levine MM. Chemiluminescent optical fiber immunosensor for detecting cholera antitoxin. *Opt Eng* 1997;36:3258–3264.
165. Konry T, Novoa A, Cosnier S, Marks RS. Development of an "electroptode" immunosensor: indium tin oxide-coated optical fiber tips conjugated with an electropolymerized thin film with conjugated cholera toxin B subunit. *Anal Chem* 2003;75:2633–2639.
166. Dupont-Filliard A, Roget A, Livache T, Billon M. Reverse oligonucleotide immobilisation based on biotinylated polypyrrole film. *Anal Chim Acta* 2001;449:45–50.
167. Ionescu RE, Gondran C, Cosnier S, Gheber LA, Marks RS. Comparison between the performances of amperometric immunosensors for cholera antitoxin based on three enzyme markers. *Talanta* 2005;66:15–20.
168. Chen H, Zheng Y, Jian-Hui J, Hai-Long W, Guo-Li S, Ru-Qin Y. An ultrasensitive chemiluminescence biosensor for cholera toxin based on ganglioside-functionalized supported lipid membrane and liposome. *Biosens Bioelectron* 2009;24:684–689.
169. Pai NP. Oral fluid-based rapid HIV testing: issues, challenges and research directions. *Expert Rev Mol Diagn* 2007;7:325–328.
170. Franco-Paredes C, Tellez I, del Rio C. Rapid HIV testing: a review of the literature and implications for the clinician. *Curr HIV/AIDS Rep* 2006;3:169–175.
171. Kendrick SR, Kroc KA, Withum D, Rydman RJ, Branson BM, Weinstein RA. Outcomes of offering rapid point-of-care HIV testing in a sexually transmitted disease clinic. *J Acquir Immune Defic Syndr* 2005;38:142–146.
172. Liddicoat RV, Horton NJ, Urban R, Maier E, Christiansen D, Samet JH. Assessing missed opportunities for HIV testing in medical settings. *J Gen Intern Med* 2004;19:349–356.
173. Kimmel AD, Losina E, Freedberg KA, Goldie SJ. Diagnostic tests in HIV management: a review of clinical and laboratory strategies to monitor HIV-infected individuals in developing countries. *Bull World Health Organ* 2006;84:581–588.
174. Mahmoud KA, Hrapovic S, Luong JHT. Picomolar detection of protease using peptide/single walled carbon nanotube/gold nanoparticle-modified electrode. *ACS Nano* 2008;2:1051–1057.
175. Zhang D, Peng Y, Qi H, Zhang C. Label-free electrochemical DNA biosensor array for simultaneous detection of the HIV-1 and HIV-2 oligonucleotides incorporating different hairpin-DNA probes and redox indicator. *Biosens Bioelectron* 2010;25:1088–1094.
176. Epstein JR, Leung APK, Lee KH, Walt DR. High-density, microsphere-based fiber optic DNA microarrays. *Biosens Bioelectron* 2003;18:541–546.
177. Sia SK, Linder V, Parviz BA, Siegel A, Whitesides GM. An integrated approach to a portable and low-cost immunoassay for resource-poor settings. *Angew Chem Int Ed Engl* 2004;43:498–502.
178. Lee SH, Kim SW, Kang JY, Ahn CH. A polymer lab-on-a-chip for reverse transcription (RT)-PCR based point-of-care clinical diagnostics. *Lab Chip* 2008;8:2121–2127.
179. Cheng X, Irimia D, Dixon M, Sekine K, Demirci U, Zamir L, Tompkins RG, Rodriguez W, Toner M. A microfluidic device for practical label-free CD4(+) T cell counting of HIV-infected subjects. *Lab Chip* 2007;7:170–178.
180. Kim YG, Moon S, Kuritzkes DR, Demirci U. Quantum dot-based HIV capture and imaging in a microfluidic channel. *Biosens Bioelectron* 2009;25:253–258.
181. Meheus A, Antal GM. The endemic treponematoses: not yet eradicated. *World Health Stat Q* 1992;45:228–237.
182. World Bank. World Bank world development report 1993: investing in health. New York: Oxford University Press, 1993.
183. Mehrvar M, Abdi M. Recent developments, characteristics, and potential applications of electrochemical biosensors. *Anal Sci* 2004;20:1113–1126.
184. Andreescu S, Sadik OA. Trends and challenges in biochemical sensors for clinical and environmental monitoring. *Pure Appl Chem* 2004;76:861–878.
185. Cammann K, Lemke U, Rohen A, Sander J, Wilken H, Winter B. Chemical sensors and biosensors – principles and applications. *Angew Chem Int Ed Engl* 1991;30:516–539.
186. Caruso F, Rodda E, Furlong DN. Quartz crystal microbalance study of DNA immobilization and hybridization for nucleic acid sensor development. *Anal Chem* 1997;69:2043–2049.
187. The TDR Diagnostics Evaluation Expert Panel. Evaluation of diagnostic tests for infectious diseases: general principles. *Nat Rev Microbiol* 2006;4(Suppl):S20–S32.
188. Pang T, Peeling RW. Diagnostic tests for infectious diseases in the developing world: two sides of the coin. *Trans R Soc Trop Med Hyg* 2007;101:856–857.
189. Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig LM, Lijmer JG, Moher D, Rennie D, Vet HCW de, for the STARD Group. Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. *Vet Clin Pathol* 2007;36:8–12.
190. Breslauer DN, Maamari RN, Switz NA, Lam WA, Fletcher DA. Mobile phone based clinical microscopy for global health applications. *PLoS ONE* 2009;4:e6320(1–7).
191. Peeling RW, Smith PG, Bossuyt PM. A guide for diagnostic evaluations. *Nat Rev Microbiol* 2006;4:S2–S6.
192. Weigl B, Domingo G, Labarre P, Gerlach J. Towards non- and minimally instrumented, microfluidics-based diagnostic devices. *Lab Chip* 2008;8:1999–2014.
193. Bualombai P, Prajakwong S, Aussawatheerakul N, Congpoung K, Sudathip S, Thimasarn K, Sirichaisinthop J, Indaratna K, Kidson C, Srisuphanand M. Determining cost-effectiveness and cost component of three malaria diagnostic models being used in remote non-microscope areas. *Southeast Asian J Trop Med Public Health* 2003;34:322–333.