PRIORITIES AND FUTURE OF DIAGNOSIS OF EMERGING VIRAL DISEASES

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Abstract
This review discusses the most important emerging viral pathogens responsible for human infections in India. It also documents viral diseases recognized in India along with the conventional diagnostic techniques and their limitations. Additionally, the review examines the priorities in viral diagnostics and briefly discusses the new strategies of genomics and proteomics, such as next-generation sequencing, used for identifying known/unknown viral infections. The paper also explores the future of rapid viral diagnostic techniques and the challenges posed in point-of-care diagnosis of viral infections.

Introduction

The World Health Organization defines emerging infectious diseases (EID) as diseases of infectious origin whose incidence in humans has increased in the recent past or threatens to increase in the near future. EID take a heavy toll on mortality, morbidity, and economy, particularly in developing and underdeveloped regions. EID lead to 12 million deaths globally and account for 7 of the top 10 causes of death in children.1

Emerging and re-emerging pathogens pose several challenges to diagnosis, treatment, and public health surveillance. The identification
of an emerging pathogen by conventional methods is difficult and time-consuming because of the “novel” nature of the agent and requires a large array of techniques, including cell cultures, animal inoculation, cultivation in artificial media, histopathological evaluation of tissues (if available), and serological techniques using surrogate antigens. Looking back at past epidemics or outbreaks caused by previously unknown infectious agents, it is clear that the identification and characterization of a new infectious agent can take years to decades or even centuries. Such time frames have been decreased to weeks or months by the use of powerful molecular techniques, as illustrated by the identification of severe acute respiratory syndrome coronavirus (SARS-CoV) within weeks of the first cases being reported and the discovery of a new hantavirus in North America in 1993.2, 3 Molecular techniques offer several advantages over conventional methods, including high sensitivity and specificity, speed, ease of standardization, and automation. Other advantages include the identification of novel, non-cultivable, or very slowly growing organisms, strain typing in epidemiological studies, determination of antimicrobial susceptibility, and monitoring treatment by measuring bacterial or viral loads. Each diagnostic method has the potential to influence patient health outcomes and facilitate informed clinical decisions. The significance of diagnostics is anticipated to increase with advances in interventions and health information technology.

Emerging and future diagnostics will have wide-ranging impacts on all aspects of health care; further, they will help advance new fundamental concepts of care and improve the quality of health services. This review discusses in detail the important viral diseases in India, diagnostic assays
and their limitations, key priorities, new technologies in viral diagnostics, and the future of viral diagnostics.

Indian scenario —Viral diseases and their diagnosis

In India, the prevalence of a number of viruses, including dengue virus (DV), Japanese encephalitis virus (JEV), Chandipura virus (CHP), West Nile (WN) virus, Kyasanur forest disease virus (KFDV), measles virus, Chikungunya virus (CHIKV), enterovirus, influenza virus, and herpes viruses, has been identified. Major outbreaks of JEV, CHPV, DV, CHIKV, influenza virus, enterovirus, and measles virus have been documented in various parts of the country.4-9 Focal outbreaks of KFDV and Nipah virus are restricted to areas near the deep forests of Karnataka and those near the Bangladesh boarder of India, respectively.

The influenza pandemic caused by the new H1N1 virus has by now affected all parts of India. However, the extent of spread and possible impact are still unclear. Similar to seasonal flu, the illness is mild and self-limiting in the majority of cases, with only 1-2% of patients requiring hospitalization.5 In a few cases, the clinical course can deteriorate in a matter of hours, leading to severe complications and eventually death. The risk of complications is high among those who have preexisting diseases, such as asthma, heart disease, and kidney disease, and among pregnant women. Antiviral drugs are now available, and the most preferred one is oseltamivir, with zanamivir being an alternative.10 Antiviral treatment is not necessary for those who are otherwise healthy and have mild or uncomplicated illness. It is beneficial for patients with progressive lower respiratory tract disease or pneumonia, those with underlying medical conditions, and those pregnant. There is a limited
supply of the pandemic influenza vaccine in a few countries, and efforts to produce it in India are currently underway. The molecular diagnostic assays, such as qualitative real-time reverse transcription-polymerase chain reaction (RT-PCR), are considered most effective in the early detection of the virus. Another diagnostic test used is heamagglutinin inhibition assay (HIA) for the detection of the antibody against the HA gene of the influenza virus.

JEV is one of the most prevalent causes of encephalitis epidemics in Southeast Asian countries. JE is characterized by the encephalomyelitis syndrome associated with various movement disorders, anterior horn cell involvement, and typical radiological changes (thalamic, basal ganglia, and brainstem). In India, JE was first recognized in 1955 in southern India, and since then, it has been documented in several parts of the country. It has been found to be associated with a number of encephalitis outbreaks in various parts of the country, and currently, it is endemic in most of the southern and northern states. The pediatric age group is the most affected, with a fatality rate of 35-40%. However, JEV infection has been increasingly noted in adults during recent outbreaks. Serological (immunoglobulin (Ig)M enzyme-linked immunosorbent assay [ELISA]) and genome-based diagnostic techniques are routinely available for the detection of this disease.

Chandipura encephalitis is emerging as a major public health concern in India. Its association with several encephalitis outbreaks in the pediatric population has been documented in the states of Andhra Pradesh (2003) and Gujarat (2004), with minimum mortality rates of 50%. Since the window period between infection and acute clinical illness is short, the diagnosis of CHP encephalitis by IgM ELISA is not preferred. Molecular
diagnostic techniques are well established for CHPV and are preferred for human clinical specimens.

CHIKV, a mosquito-transmitted alphavirus, causes Chikungunya fever, which is characterized by fever, rash, and severe arthralgia. The association of CHIKV with human infection in India was first documented during human outbreaks in the 1960s.16 Until 2006, an estimated 1.3 million human cases of CHIKV infection has been documented in India.17-19 Neurological complications in pediatric CHIKV infections are increasingly being documented, and the virus has been detected in cerebrospinal fluid (CSF) samples collected from cases.20, 21 Direct PCR detection is considered the most appropriate technique for diagnosing CHIKV infection, rather than testing for IgM antibodies, which may persist for several months after infection and can reflect coincidental infection rather than an acute infection.22

Measles is the leading killer among vaccine-preventable diseases, responsible for an estimated 44% of the 1.7 million vaccine-preventable deaths among children annually.23 An increasing number of cases with neurological complications (fatal encephalopathy) are being documented in several measles outbreaks.24-26 Subacute sclerosing panencephalitis (SSPE), a rare progressive degenerative disease, is caused by persistent infection with a defective measles virus.27 Mutations in matrix genes have been frequently documented in strains associated with SSPE.28 SSPE is characterized by progressive mental deterioration, motor decline, and myoclonus, leading to death within 1-3 years of onset. SSPE is essentially a disease of childhood, and commonly occurs at the age of 5-15 years. Early diagnosis of measles may help in selecting the
appropriate medical interventions and administering necessary counseling.

Mumps is an acute infectious disease caused by the mumps virus, which is a member of the paramyxoviridae family. Mumps (epidemic parotitis) is an acute communicable disease characterized by the painful enlargement of the salivary glands, particularly the parotid glands. Neurologic and ocular manifestations of mumps are well documented but seldom appear concomitantly. Meningitis and mild meningoencephalitis are the most frequent complications of mumps in children. The epidemiology of mumps in India is not well understood. Despite the availability of an effective vaccine, it continues to occur in epidemic proportions, with significant morbidity in the pediatric age group. The clinical diagnosis of mumps is made on the basis of the presence of acute unilateral or bilateral parotitis. Laboratory confirmation of mumps infection is achieved through the isolation of IgM-specific antibodies to mumps virus in acute-phase serum samples, mumps virus in cell culture, or RNA of the mumps virus by RT-PCR.

WN virus has been reported to cause mild, febrile illness in southern India. Severe WN encephalitis has been documented in the pediatric and elderly populations of the Kolar region of Karnataka (during 1977, 1978, and 1981) and Dibrugarh region of Assam (during 2008). Serological tests confirmed the diagnosis in these cases as WN fever, and the WN virus was isolated from human clinical specimens. Additionally, febrile illness in epidemic form and clinically overt encephalitis cases have been documented in the Udaipur area of Rajasthan and Buldhana, Marathwada, and Khandesh districts of Maharashtra. Neutralizing antibodies specific to the WN virus have been detected in about 20-30%
of human serum samples collected from Tamil Nadu, Karnataka, Andhra Pradesh, Maharashtra, Gujarat, Madhya Pradesh, Orissa, and Rajasthan. All these studies indicate continuous WN virus activity in India. However, in most areas, JE and WN viruses coexist, and these cases remain undiagnosed because of the lack of virus-specific diagnostic tests.

DV is one of the most prevalent viral species in India. It causes dengue, which has a clinical presentation ranging from a self-limiting disease to more severe forms, such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Acute febrile encephalopathy (AFE) is a common cause of childhood hospital admission in many parts of India. The association of DV with neurological conditions, such as encephalitis, myelitis, mononeuropathies, acute disseminated encephalomyelitis, and Guillain-Barré syndrome has been documented. The frequency of dengue in patients with encephalitis, assessed on the basis of the presence of anti-dengue IgM in the serum, has been shown to range from 5% to 20%. The pathogenesis of the neurological manifestations is not clearly defined, but some reports have attributed it to the encephalopathy present in severe cases of DHF. Encephalopathy/encephalitis-like complications have also been recently documented in DV infections in northern and eastern India, other Asian countries, and South America. However, the involvement of the brain and spinal cord is uncommon in DV infection, and the prognosis is poor in patients presenting with encephalitis or myelitis. DV has been isolated from the blood of patients having severe neurological complications.
Paramyxoviruses have been implicated in both animal and human infections. Morbilliviruses are responsible for large-scale epidemics. Fatal infection in horses and humans was determined to be caused by a new paramyxovirus, Hendra virus (HeV) in Australia, and a closely related virus—Nipah virus (NiV)—was responsible for fatal infections in pigs and humans in Malaysia. Human cases of NiV and HeV virus (paramyxoviruses) infections with high mortality have been documented in Australia, Malaysia, Singapore, India, and Bangladesh. The first known NiV encephalitis outbreak occurred in Siliguri region (West Bengal), India, during 2001.6 Relapse encephalitis and long-term neurological defects have been documented in a significant portion (~10%) of the patients recovering from acute Nipah virus infection. In outbreak situations in Bangladesh and India, the mortality rate rose to approximately 70%.46, 47 The multiple outbreaks of NiV in Bangladesh and the 2001 outbreak in West Bengal show a continued risk for spillover infection between bats and humans in this region.48 Henipavirus infections are probably more widespread than current estimations and therefore warrant intense monitoring, especially in countries where large-scale deforestation occurs.49

KFD was discovered in 1957 in the Mysore forest region of south India, where 400-500 persons per year are infected with the virus.50 KFD virus has been found only in monkeys, humans, and Haemaphysalis spinigera ticks in the KFD-epidemic region of south India.51 KFD antibodies have been detected in residents of north and northeast India, and the KFD seropositive rate is especially high among residents of India’s Andaman and Nicobar Islands. KFD antibodies also were detected in both human and bird sera in the Chinese districts of Guangdong, Guangxi, Guizhou,
Hubei, Henan, Xinjiang, and Qinghai in 1983. Recently, KFD has also been isolated from humans in China.52 Considering the spread of KFD to newer areas, there is clearly a need for developing a rapid diagnostic system for KFD. In view of the changing ecology, the spread of KFD virus warrants attention since it can extend to localities never affected before. Among the major infections of viral origin are encephalitis due to herpes simplex (HSV), Epstein-Barr virus, cytomegalovirus, or herpes zoster virus, which can be treated with special antiviral drugs.53 HSV encephalitis is a rare but extremely serious brain disease caused by HSV-1 in the pediatric population, except newborns. In about 70% of infant HSV encephalitis, the disease occurs when a latent HSV-2 virus is activated. When left untreated, HSV encephalitis is fatal in over 70% of the cases in India. Each year, about 2,100 cases of encephalitis are reported from the USA, while the exact incidence of encephalitis in India is not known. Therefore, there is a need to develop sensitive and specific diagnostic tests for the early detection of the herpes group of viruses.

Currently, enteroviruses are posing a global health concern and cause a wide spectrum of diseases that includes myocarditis and pericarditis, exanthems and enanthems, conjunctivitis, and meningitis. The association of a few enteroviruses has been identified with human CNS infections leading to fatal neurological disease. Enteroviruses also cause seasonal aseptic meningitis and meningoencephalitis in young infants. Recently, the association of enterovirus 76 has been recognized in 30% of the encephalitis cases in northern India.54 In spite of the best efforts, more than 70% of the cases of viral encephalitis remain undiagnosed and pose a challenge to both clinicians and virologists. However, except the
JE virus, other viral agents associated with the remaining 70% of the encephalitic cases have not been characterized because of the lack of specific and sensitive diagnostic techniques. Rapid and specific diagnosis of the infectious agent in emergency situations assists the clinician in monitoring the patients, which would help decrease the morbidity and mortality.

**Different viral diagnostic assays and their limitations**

With the large number of tests available for the detection of infectious agents affecting humans, it is often not recognized that most of these tests are indirect. For infections caused by agents such as hepatitis C virus (HCV), hepatitis B virus (HBV), human immunodeficiency virus (HIV), or other agents of viral or bacterial origin, commonly used tests determine the presence of specific antibodies produced by the patient’s immune system in response to the infectious agent. These tests are useful for blood screening and, to a limited extent, as diagnostic tools, but because they offer only an indirect measure of infection, they do not clarify whether the infection is past or current or whether there is a response to the therapy. An antibody-based test can also miss a recent infection, because generally, it may take several days to weeks for the immune system to mount an antibody response to the infectious agent. This can be especially dangerous if the infectious agent rapidly spreads in the patient or if the blood from an apparently healthy but recently infected person (with HIV or HCV) is used for blood transfusion.

A new generation of tests has been developed to directly measure the concentration of the infectious agent within the patient’s sample. These tests detect the presence of nucleic acids (the genetic material) of the
infections agents in the blood or other samples from the patient. Two major methods have been developed and accepted for detection of infectious agents in patient samples. The most common is the polymerase chain reaction (PCR), which uses an enzymatic reaction to amplify specific nucleic acid sequences from the infectious agent if they are present in the sample. 55-60 PCR techniques allow for the in vitro synthesis of millions copies of the gene of interest, enabling the rapid detection of as few as 10 to 100 copies from the original sample. This technique has been shown to have a high degree of sensitivity and specificity when applied to the diagnosis of several viral infections. 61-64

There are several problems with this method because PCR uses specific nucleic acid sequences (primers) from an already known sequence of the infectious agent. Therefore, if the infectious agent has not been sequenced, PCR cannot be used. Similarly, if the infectious agent mutates very rapidly, the primers may not recognize the infectious agent and the result will be false negative. This is a major problem with the detection of HIV, which causes AIDS, because HIV undergoes mutations very rapidly, especially in response to drug treatment. The PCR uses an enzymatic reaction, and enzymes can be inhibited by impurities in the patient sample, thereby affording false-negative results. In addition, the primers, which have short sequences, are only specific for an infectious agent at certain temperatures, making these tests dependent on very strict conditions.

The diagnosis of viral infectious diseases has been revolutionized by the advent of new PCR-based technologies for identifying etiological agents of viral diseases; its applications in the quantitation of viral load to monitor the duration and adequacy of antiviral drug therapy are being...
evaluated. The conventional PCR methods have been revolutionized to increase its rapidity, specificity, sensitivity, and utility in the form of real-time PCR. These techniques can be performed rapidly and inexpensively, within a shorter time than that required for conventional PCR. It is also preferable for serologic testing, which requires 2 to 4 weeks after the acute infection for the development of a detectable rise in antibody titers and is of limited value for viruses with high basal seroprevalence rates. Unlike serologic testing, PCR yields positive results during acute infection, when the amount of the replicating virus is maximal.

Despite the use of newer techniques, including molecular techniques like PCR, 35-75% of the viral infections remain of unknown etiology worldwide. The failures in the diagnostic ascertainment have been attributed to the lack of a standard diagnostic algorithm for all the viral infectious diseases. It is not possible to screen the specimen for the entire range of viruses that are able of causing infections in humans. Hence, there is a need to develop the diagnostic approaches that will be rapid and help in the detection of the maximum number of viruses from clinical specimens.

**Priority: Rapid viral diagnostic testing**

Despite myriad publications on the rapid viral diagnostic testing (RVDT) methodologies for infectious diseases, such tests have become neither commonplace nor an integral component of the services offered by clinical microbiology laboratories in India. In the current era of managed care, the need for RVDT is underscored by the emergence of virulent strains of influenza virus and novel pathogens, such as the coronavirus
that causes the SARS, as well as the often grave consequences of healthcare-associated infections caused by the recently reported CCHF virus.66

Debate on the value of RVDT has broadened and the technique now encompasses infections caused by HSV, JEV, WN virus, HIV, and pandemic influenza virus. Exorbitant costs and questionable cost-effectiveness of certain rapid tests are usually implicated in the unavailability of RVDT.67 To be reliable and cost-effective, a test must have sufficient diagnostic value, and its use must be limited to the organisms most likely to be clinically relevant and to circumstances in which earlier diagnosis can have an impact on patient management.68, 69 For a diagnostic test to be “clinically relevant,” the following questions must be addressed:

- What is the reason for requesting the test?
- Will the result help in patient management?
- Would another cheaper test give the same information?
- Will the use of a specific RVDT enhance the understanding of the medical condition?
- Is it possible to avoid RVDT for the clinical situation under consideration?
- Is this test having public health or clinical importance? 67
- Is the test trustworthy?70
• Even if the RVDT is inexpensive, will it be cost-effective and sustainable in the long term?

This is particularly relevant for less-developed countries and even for countries such as India, where, in a recent trend, healthcare facilities (including academic centres) are purchasing microbiology services from private, rather than hospital-based, laboratories.

In the conventional view of RVDT, a clinical specimen (serum, plasma, saliva, urine, stool, tissue, or body fluids) is processed in a single step at the site where it is collected, and a qualitative or quantitative result is available within 20 minutes—the basis of point-of-care testing. However, RVDT now encompasses more than just a single-step testing procedure. A specimen is often sent to a laboratory for immediate workup, which might involve several steps, ending with the availability of results within 2 hours to 20 hours, although in certain cases, RVDT results are obtained within 18 hours, instead of 2 days (e.g., for influenza).

The properties of RVDT kits play an enormous role in determining their utility in the diagnosis of infectious diseases. The prerequisites of the ideal RVDT are as follows:

• High sensitivity, specificity, and reproducibility;

• Relatively high negative and positive predictive values;

• Rapid turnaround time;

• Availability and reporting of results to those who need them in a timely manner; and
• Affordable pricing.

How these requirements fit in with the standard clinical practice in an Indian hospital is currently undetermined. A low sensitivity will result in patients with true infection being falsely reassured by a negative test result, whereas a low specificity will lead to a relatively high number of false-positive test results.

Rapid and accurate diagnosis of an infection should enhance patient outcome by enabling early initiation of appropriate therapy and implementation of relevant infection-control measures and eliminating unnecessary diagnostic testing and treatment. Many of the current RVDT techniques involve genomic testing methodologies, such as nucleic acid hybridization with RNA or DNA probes, amplification, PCR technologies, or nucleic acid sequencing.71, 72 Tests used for the direct detection of organisms in clinical specimens must be highly sensitive; otherwise, processing will require an amplification step.

For countries with adequate resources, relatively older RVDT techniques, such as diagnostic electron microscopy, need not be expensive or difficult to perform if executed in a diagnostic network, by recruiting and using instruments and electron microscopists working in other departments or services. Because unusual and unexpected agents can be rapidly identified, electron microscopy is a major fixture in rapid diagnostic virology services, especially in the current era of vigilance for potential bioterrorist events, emerging pathogens, and new and unusual cases in which an infectious etiology is suspected.73, 74 Nonetheless, in the 21st century, genomic testing platforms are the principal
technologies upon which rapid diagnoses of infectious diseases are based.

New technologies in viral diagnostics

**Real time PCR:** The real-time PCR assay has many advantages over conventional PCR methods, including rapidity, quantitative measurement, lower contamination rate, higher sensitivity, higher specificity, and easy standardization. Thus, nucleic acid-based assays or real-time quantitative assay might eventually replace virus isolation and conventional RT-PCR as the new gold standard for the rapid diagnosis of virus infection in the acute-phase samples.75

Real-time PCR has promoted a wider acceptance of PCR-based tests because it improved the rapidity, sensitivity, and reproducibility of the tests and reduced the risk of carry-over contamination. Real-time PCR assays used for quantitative RT-PCR combine the best attributes of both relative and competitive (end-point) RT-PCR in that they are accurate, precise, capable of high throughput, and relatively easy to perform. The majority of diagnostic PCR assays reported to date have been used in a qualitative, or “yes/no” format. The development of real-time PCR has brought true quantitation of target nucleic acids out of the pure research laboratory and into the diagnostic laboratory.76

**Microarray or DNA chips:** Microarrays or DNA chips are one of the latest tools for rapid infectious disease diagnostics. Microarrays are a recent adaptation of the northern blot technology.77, 78 The ability of microarrays to label nucleotide sequences with fluorescent tags, much like the fluorescent antibody technology, has increased their use in
diagnostics. Microarrays are small, solid supports (typically glass slides) on which DNA sequences are attached or spotted at fixed, orderly, addressable locations. DNA is composed of short, single-stranded fragments, typically 5- to 50-nucleotides long. Microarrays can have up to tens of thousands of spots, allowing the collection of a large amount of data for each sample tested. Microarrays depend on the annealing of 2 nucleic acid strands to function. When sample DNA is prepared, usually through polymerase-based amplification, fluorescent dyes are incorporated into the amplicon so that hybridization can be detected. The kind of information required from microarrays drive how the arrays are developed and used. Microarrays can be spotted with known sequences of a variety of oligonucleotides for basic genomic investigation. Gaining wider acceptance is the use of microarrays to “resequence” organisms. Sequence differences can be determined by utilizing known sequences from already sequenced organisms and hybridizing genomic material from organisms not previously sequenced. With more than 10,000 sequences (and growing, as automated systems improve) to interrogate on a single chip, variation in genomic sequences can enable accurate determination of species and subspecies. Microarray-based diagnostic assays have also been used to characterize previously unknown viruses, such as SARS-CoVs, but require information on the genome of the virus or closely related viruses that are under investigation.79, 80

Virus discovery cDNA amplified fragment length polymorphism analysis

The human population is exposed to an increasing burden of infectious diseases caused by the emergence of new previously unrecognized
viruses. Climate changes, globalization, settlements near animal and livestock habitats, and the increased number of immunocompromised persons probably contribute to the emergence and spread of new infections.81 Thus, there is a need to improve methods for the identification of unsuspected viral pathogens or new viruses. Subtractive techniques, such as representational difference analysis or random sequencing of plasmid libraries of nuclease-resistant fragments of viral genomes, have led, in the past, to the discovery of several viruses, including human herpes virus type 8, human GB virus, Torque Teno Virus, bocavirus, human parvovirus 4, WU polyomavirus and Kl polyomavirus.82-88 One of these methods is termed virus discovery cDNA amplified fragment length polymorphism analysis (VIDISCA), which can be applied to sterile specimens, such as cell culture supernatants.89 In this method, samples are ultracentrifuged for viral particle enrichment and treated by DNase and RNase to digest away cellular nucleic acids. Capsid-protected viral nucleic acids are then purified, converted to double stranded DNA, digested with restriction enzymes, and ligated to oligonucleotide adaptors, which are used as primer-binding sites for comparative PCR. This method was described originally in the context of the discovery of severe acute respiratory syndrome SARS-CoV in 2004.90 These techniques are poorly sensitive and time-consuming and are thus unsuitable for large-scale analysis. For these purposes, next-generation sequencing (NGS)-based methods have been developed.

Next-generation sequencing

High-throughput NGS techniques represent a powerful tool that can be applied to metagenomics-based strategies for the detection of unknown disease-associated viruses and for the discovery of novel human
viruses. Compared to microarray-based assays, NGS methods offer the advantage of higher sensitivity and have the potential to detect the full spectrum of viruses, including those unknown and unexpected. One of the first applications of NGS for pathogen discovery was the investigation of 3 patients who died of a febrile illness a few weeks after the transplantation of solid organs from a single donor and for whom conventional microbiological and molecular tests, as well as microarray analysis for a wide range of infectious agents, were inconclusive.

The first high-throughput NGS technology, the 454 FLX pyrosequencing platform, which was developed by 454 Life Sciences and later bought by Roche, became available in 2005. In early 2007, Illumina released the Genome Analyzer, developed by Solexa GA, and more recently, SOLiD was released by Applied Biosystems. This field is rapidly expanding, and novel and improved platforms, such as Heliscope by Helicos, Ion Torrent PGM by Life Technologies, and a real-time sequencing platform by Pacific Biosciences, are continuously being developed and released. While the platform developed by Pacific Biosciences, as well as other novel sequencing platforms, are referred to as “third-generation” platforms because they progressively sequence single, large DNA molecules without the need to halt between read steps, the 454 pyrosequencer, Illumina Genome Analyzer, and SOLiD methods represent the “second generation” systems, which can sequence populations of amplified template-DNA molecules with a typical “wash-and-scan” technique. Given these criteria, Ion Torrent PGM and Heliscope occupy a position between “second-” and “third-generation” technologies since they do not completely fulfil the features assigned to either category.
Diagnostic virology is one of the most successful applications of NGS, and exciting results have been achieved in the discovery and characterization of new viruses; detection of unexpected viral pathogens in clinical specimens; ultrasensitive monitoring of antiviral drug resistance; investigation of viral diversity, evolution, and spread; and evaluation of the human virome. With the decrease of costs and improvement of turnaround time, these techniques will probably become essential diagnostic tools in clinical routines.

**Proteomics**

The proteome is the protein content of a cell, tissue, or entire organism in a defined state. Proteomics is the study of the full array of proteins produced by an organism. For infectious diseases, proteomics involves the profiling of proteins generated by human cells in response to stimuli from infectious agents and their products of metabolism. Characterization of a proteomic profile of a microorganism is complicated, largely because various unique proteins can be produced by the same gene product and because of the chemical diversity of the organism’s proteins. Technologies used in proteomics analysis are complex, labor intensive, and fraught with difficulty. The technologies most widely used to screen and analyze the proteome are 2-dimensional gel electrophoresis and mass spectrometry.

**Laser ablation electrospray ionization mass spectrometry**

This technology enables the analysis and imaging of cells and tissues and the identification of proteins, peptides, lipids, metabolites, and other biomolecules directly and rapidly in any sample that contains water.
Laser ablation electrospray ionization-mass spectrometry (LAESI-MS) allows the direct identification of biomolecules in tissue sections and cells, so that the destruction of the source sample is minimized. LAESI-MS provides both qualitative and quantitative data with 2-dimensional and 3-dimensional spatial analysis and is able to identify biomolecules and metabolites in cell structures, tissues, and fluids.

LAESI-MS is minimally invasive and does not destroy tissues and living cells; it is extremely sensitive and ideally suited for the direct analysis of biofluids and other aqueous samples that contain peptides, proteins, metabolites, and other biomarkers for clinical, diagnostic, and discovery workflows.94

Surface-enhanced laser desorption/ionization-time of flight

Surface-enhanced laser desorption/ionization-time-of-flight (SELDI-TOF) is an ionization method in MS that is used for the analysis of protein mixtures. SELDI-TOF is typically used to detect proteins in tissue samples (blood, urine, or other clinical specimens). The proteins are ionized with lasers and separated by size. Comparison of protein levels between patients with and without an infection can be used for biomarker discovery.

An advantage of this technology is that it characterizes the patterns of combinations of proteins or peptides in blood or other body tissues that uniquely define a specific infectious disease, rather than identifying only a single marker. The SELDI-TOF methodology is relatively insensitive, remains restricted to cultured specimens, and requires inoculums of at least 106 organisms. On the other hand, fingerprints to define disease
states rather than just pathogen detection require no assumptions about
the nature of proteins and protein identification and are, therefore, not
essential for diagnostic utility.

Current applications of SELDI-TOF include rapid diagnosis of sleeping
sickness, invasive aspergillosis, tuberculosis, and Chagas disease. In viral
diagnostics, this technology has been used in the detection of the
immune-dominant proteins of the viruses and serum biomarkers.95, 96
Additional work is necessary, however, before it can assume the role of a
routine technology in the clinical microbiology.

The future of RVDT

Despite the rapid progress of RVDT technologies, these diagnostic
modalities have not yet made many inroads toward replacing standard
identification tests in medical microbiology or virology laboratories.
More often than not, RVDTs based on molecular platforms have relatively
lower sensitivities or positive predictive values than traditional
methodologies for the investigation or diagnosis of infectious diseases.
Moreover, reliable molecular diagnostic tests for many infectious agents
are not readily available.

Obstacles to the sustainability of RVDTs include specimen transport
issues, low concentrations of infectious agent, genetic changes in
primer-binding sites, final assay volume, inhibition, contamination,
nonspecific amplification, and operator error. Further, viral genome
sequencing is subject to error because of sequence homology among
different viruses, database problems, and mutations. The consequences
of all these limitations include false-negative and false-positive
amplification results and misdiagnoses. Although the extent of viral nucleic acid in “normal” host tissues is unknown, the speed and sensitivity of methods such as real-time PCR have rendered this particular RVDT almost routine for the detection and ascertainment of viral etiology in both research and clinical specimens.

Although included in the diagnostic workup of influenza; SARS; and, more recently, CCHF, RVDTs have not been proven to actually improve the diagnostic capabilities of laboratories or enhance patient outcomes to any significant degree. That said, RVDTs will almost certainly continue to play an important role in the effort to enable the rapid diagnosis in patients with HIV, influenza virus, HSV, and new unknown virus infections. It will become a tool in public health endeavours and in the screening of asymptomatic patients for infection, where the possibility of a lack of follow-up is real. RVDT methods may be implemented as adjuncts to the epidemiologic investigation of infectious disease outbreaks. As the sensitivity, specificity, positive predictive value, and negative predictive value of RVDTs continue to improve and as RVDTs become more widely appreciated through the production of less expensive and more user-friendly platforms, it will become necessary to formulate responsible guidelines for their appropriate and optimal use in clinical practice, for clarifying infectious disease diagnoses and improving patient outcomes.

References


