Review Article

Molecular Detection of Bacterial and Viral Pathogens–Where Do We Go From Here?

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Abstract

Nucleic acid amplification tests (NATs) are rapidly becoming the cornerstone of clinical virology laboratories around the world. While polymerase chain reaction (PCR) amplification has served laboratories well since the 1990s, providing sensitive and specific tests to detect clinically important viruses, PCR tests have significant disadvantages as they are cumbersome, labor intensive, and relatively slow compared with newer isothermal amplification methods. Commercially available multiplex PCR tests have recently become popular and are being implemented in many laboratories. Following the introduction of the first isothermal amplification formats of the 1990s, newer isothermal methods have been developed including loop-mediated amplification (LAMP) or recombinase polymerase amplification (RPA), which can yield results in as little as 10 to 20 minutes. Specimen preparation has now fallen behind advances in amplification technology with specimen preparation now taking longer than these newer isothermal amplification methods. Coupled with advances in amplicon detection employing microfluidics, biosensors and nanotechnology, these new isothermal amplification methods provide unique opportunities for the development of new laboratory-based tests and inexpensive, one-time use, point-of-care (POC) diagnostics.

Keywords: Respiratory viruses; Diagnosis; Rapid detection; Molecular testing; PCR; Isothermal amplification; Point-of-care diagnostics

Introduction

Acute respiratory disease (ARD) accounts for a large proportion of all acute morbidities in developed countries, and the majority of these infections (approximately 80%) have a viral etiology. Acute viral respiratory tract infection is the leading cause of hospitalization for infants and young children in developed countries and is a major cause of death in developing countries. Upper respiratory tract infections (URTIs) causing rhinitis, pharyngitis, and laryngitis are among the most common infections in children, occurring several times per year in infants and young children with the incidence varying inversely with age. The Centers for Disease Control and Prevention's National Vital Statistics Report indicates that there are between 12 and 32 million episodes of URTI each year in children aged 1-2 years [1], and over 300,000 children are hospitalized each year in the United States at an annual cost of about $1 billion [2,3]. Respiratory tract infection can lead to acute asthma exacerbations, acute otitis media or other lower respiratory tract presentations including bronchitis, brochiolitis, and pneumonia. In 2002, LRTI accounted for approximately 4 million deaths world-wide, and 7% of all deaths [4]. In clinical practice, a specific virus is often not identified due to the lack of available sensitive tests, the presence of as-yet unidentified pathogens, or the failure to use appropriate tests.

The past 15 years has seen a major shift away from traditional virus testing methods such as culture, serology, and ELISA for antigen detection towards nucleic acid amplification tests (NATs). Molecular testing is rapidly becoming the cornerstone of clinical virology laboratories in many countries around the world. While polymerase chain reaction (PCR) amplification has provided diagnostic laboratories with sensitive and specific tools to detect clinically important viruses, PCR methods have significant disadvantages as they are cumbersome, labor intensive, slow, or have low test throughput or turn around times. Following the introduction of the first isothermal amplification formats in the 1990s, newer isothermal methods have been developed and some of these methods, including loop-mediated amplification (LAMP) or recombinase polymerase amplification (RPA), can yield positive results in under 20 minutes. Specimen preparation has become the rate limiting step and bottleneck for molecular testing with specimen preparation now taking longer than some isothermal amplification methods. Coupled with recent advances in amplicon detection employing microfluidics, biosensors and nanotechnology, these isothermal amplification methods provide new opportunities for the development of laboratory and point-of-care tests that are less expensive and provide faster results without a loss of sensitivity.

Traditional Testing Methods

Traditional diagnostic testing for respiratory virus infections involve virus isolation in cell culture, serology, ELISA, and direct fluorescent antigen (DFA) staining of NP specimens and Shell vial culture (SVC) using a panel of monoclonal antibodies [5]. In the early 1990s with the development of specific monoclonal antibodies, respiratory viruses could be detected in 3 hours by DFA staining viral antigens or 1-2 days by SVC for slowly growing viruses using commercially available R-Mix Shell vials (Diagnostic Hybrids, OH.) instead of the 8-10 days required for tube culture. DFA staining of cells collected using nasopharyngeal (NP) swabs or NP aspirates (NPA) became the mainstay for many laboratories in the 1990s, but today is

being phased out and replaced by molecular tests. Rapid EIA tests developed in the 1980s and 1990s for point-of-care testing have lacked sensitivity and have not been adopted in hospital settings. The clinical sensitivities of these tests are in the range of 20 to 90% and vary widely with the patient population being tested. Rapid EIA tests should not be used in critical care settings due to their low sensitivities. NATs, especially real time PCR, multiplex PCR, and more recently isothermal amplification methods have replaced the conventional methods for detecting respiratory viruses largely because these molecular tests detect 30 to 50% more positives compared with traditional tests [6,7].

Molecular Methods

Many nucleic acid based amplification approaches including polymerase chain reaction (PCR), nucleic acid sequence-based amplification (NASBA), transcription mediated amplification (TMA), strand displacement amplification (SDA), loop-mediated isothermal amplification (LAMP), rolling circle amplification (RCA), helicase dependent amplification (HDA) and multiplex ligation-dependent probe amplification (MLPA) have been applied for the detection of respiratory viruses. Some of these amplification methods viz. multiplex PCR, LAMP and HDA have been used to detect more than one virus by simultaneously amplifying multiple virus gene targets. Many of these highly sensitive NATs are now routinely used in clinical laboratories and are revolutionizing our approach to diagnostic virology. Several excellent reviews describing these isothermal amplification methods and how they have been used for detecting respiratory viruses are available [5,8,9].

Specimens and pre-analytical concerns

Many factors should be considered when selecting a molecular test for respiratory virus detection including the patient population being tested, age of the patient, immune status of the patient, and reason for testing (diagnosis, outbreak assessment, vaccine monitoring or treatment considerations including drug resistance). Diagnosis of a virus infection for which no treatment is available can be cost effective if the testing results reduce the use of other diagnostic tests or expensive and unnecessary treatments [10,11]. The time from symptom onset to testing may influence the sensitivity of testing. The choice of specimen is influenced by staff training and intrinsic sensitivity of the specimen type; nasopharyngeal aspirates or swabs are preferred in inpatient settings while nasal or oropharyngeal (throat) swabs are better for community surveillance. Newer flocked swabs may sample more cells than rayon swabs [12]. Flocked nasal mid-turbinate swabs enable self-collection and serial specimen collection, have sensitivity similar to nasopharyngeal swabs, and are being increasingly used in outpatient settings [13,14]. Finally, samples such as sputum, tracheal aspirates, bronchoalveolar lavage, and even blood or stool that may contain respiratory viruses may be relevant for certain patient populations or specific viruses, but the extraction methods for NATs used for these specimen types may require additional validation.

Another important pre-analytical aspect of specimen collection is the transport media. These include viral transport media, universal transport media, or alcohol-based transport media [15]. Many transport media were developed for viral culture or for antigen detection, but are poorly characterized with respect to stability of nucleic acids at various temperatures and for various time durations (although preservation of virus viability may imply stability of nucleic acids). With a changing emphasis on molecular diagnostics, transport

media that ensures specimen stability, minimizes biohazards, and enables timely processing needs to be developed and validated. The transition from traditional to molecular testing has precluded the ability to assess specimen quality using methods such as counting respiratory epithelial cells with DFA staining. While many NATs include an extraction and/or amplification control, most in-house and commercial molecular assays lack a means to assess specimen adequacy, and quality indicators are required to ensure adequacy of collection, transportation, extraction and nucleic acid detection.

Historically, molecular tests require extraction of nucleic acids from the specimen to remove amplification inhibitors. Most RT-PCR amplification assays used in clinical laboratories today take 90 to 120 minutes following nucleic acid extraction, which usually takes 45-60 minutes. The time required for amplification has been reduced with the introduction of isothermal amplification techniques that do not require thermal cycling. Considerable efforts are now being put into reducing the time for nucleic acid extraction. Ongoing improvements in specimen preparation coupled with faster isothermal amplification and detection methods should reduce the total time from specimen collection to results to under 30 minutes (see section on isothermal amplification).

Respiratory Virus Pathogens

The major causes of acute respiratory disease (ARD) in both children and adults are influenza virus types A, B and C, Parainfluenza virus (PIV) types 1,2, and 3, Respiratory syncytial virus (RSV), Adenovirus and Rhinovirus (Mahony et al 2008). In the past decade, several new respiratory viruses including avian influenza viruses (H5N1, H7N7, H7N3), human metapneumovirus (hMPV), SARS Coronavirus (CoV), human Coronaviruses (HCoV) NL63 and HKU1, Polyomaviurs WU/KI, Parvovirus 4 and 5, Mimivirus and the swine-origin pandemic A/H1N1 influenza have emerged and are now considered significant causes of upper or lower respiratory tract infection (RTI). A number of excellent reviews on the epidemiology and diagnosis of respiratory virus infections are available [5,16-19]. These viruses cause both upper and lower tract infections and have overlapping clinical presentations, and physicians cannot typically identify the causative agent without laboratory assistance.

NATs have played an increasingly important role in diagnosing respiratory virus infection since the first report in 1991 of influenza detection using RT-PCR by Zhang and Evans [20]. Over the past 20 years, NATs have been developed for all respiratory viruses (both conventional and emerging viruses), and single virus assays have rapidly been replaced by multiplex assays [5,9,18,21]. Molecular tests have played an important role in improving our understanding of the epidemiology of emerging virus infections such as SARS Coronavirus and the recent swine-origin pandemic H1N1 influenza of 2009. As genome sequences of these new emerging viruses have become available, several laboratories including our own developed molecular tests in a matter of days or weeks for SARS CoV [22,23] or pandemic 2009 H1N1 [24-26]. Several multiplex assays have now been developed for respiratory viruses with sensitivities exceeding 90-95% for several targets [21,27], and several studies have shown that the use of molecular tests has increased the yield of respiratory viruses by up to 50% [6,7]. The increased sensitivity of these NATs has been verified by confirming additional positives as true positives using a second gene target for amplification or by using a combined reference standard defining a positive specimen as being positive in two or more assays.

Multiplex Assays

Several commercially available multiplex assays have recently been approved (either CE marked or FDA approved) for use in clinical laboratories (Table 1). These multiplex assays have quickly evolved from the first cleared assay detecting 6 viruses (Hexaplex assay from Prodesse now owned by GenProbe) to assays now capable of detecting up to 19 virus types and subtypes [6,7,28]. These include the ResPlex II (Qiagen), MultiCode-PLx RVP Assay (EraGen Biosciences), SeeplexTM RV (Seegene Inc., Korea), NGENTM RVA ASR kit (Nanogen Inc., San Diego) and the xTAGTM RVP Assay (Luminex Molecular Diagnostics, Toronto, Ontario). The first two multiplex assays approved by the FDA were the ProFLu+ Assay (Prodesse Inc.), which is a modification of the ProFlu-1 assay that detects influenza A, influenza B and RSV [29] and the xTAGTM RVP Assay which was approved for the detection of 12 respiratory viruses. The xTAGTM RVP test was the first test approved for both identification and subtyping of H1 and H3 influenza-A virus. Despite their multiplex formats with multiple primers, these assays have excellent sensitivities and specificities for most viral targets [30-33]. The MultiCode-PLX and the ResPlex II tests are research use only (RUO) tests that also employ the Luminex xMAPTM system, and these tests have reported performance claims by the manufacturer similar to the xTAGTM RVP test [32,33]. Head to head comparisons of these commercial multiplex assays are beginning to appear, revealing performance differences between the assays. Thus, laboratories should choose tests that best meet the needs of their clients [34-37]. Larger comparisons of three and four tests will be required to determine the true performance characteristics of these assays.

Name	Manufacturer	Number Viruses detecteda	Strengths	Weaknessess	Status
Pro-Flu +	GenProbe(formerly Prodesse)	3		Results in 4 hr	IVD,CE
Pro-Flu ST		3			IVD, CE
Xpert Flu	Cepheid	Flu A/B	Minimal hands-on time	16 samples/hr	IVD
Infinity RVP Plus	AutoGenomics	15	48 tests in 5.5 hr	Limited performance data	RUO
Verigene RVNATsp	Nanosphere	3	Fully automated including extraction PCR, detection	Low throughput 8 samples/4 hr	RUO
Film Array	Idaho Technology Inc.	20	Minimal hands-on time, Excellent sensitivity	Low throughput 28 samples/8 hr	IVD, CE
Seeplex® RV-12	Seegene	12	Run time ≈ 7 hr	Moderate throughput	RUO ,CE
Seeplex® RV-15	Seegene	14	Run time ≈ 7 hr	Moderate throughput	RUO, CE
Seeplex® RV-5 Screening	Seegene	9c	Run time ≈ 7 hr	Moderate throughput	RUO, CE
CLART© Pneumovir	Genomica	13	Run time ≈ 7 hr	Moderate throughput	RUO, CE
xTAG™ RVP	Luminex Molecular Diagnostics	12	Run time ≈ 7 hr	Moderate throughput Only FDA approval test for Rhinovirus	IVD,CE
RVP Fast v 2.0	Luminex Molecular Diagnostics	18	Run time ≈ 3 hr	Moderate throughput	RUO, CE
MultiCode PLx	EraGen (now owned by Luminex)	17	Results in 4.5 hr	Detection uses Illumic VeraCode	RUO
ResPlex II	Qiagen	16	Run time ≈ 3 hr		RUO
NGEN Respi Finder	Nanogen Patho Finder	15		Limited performance data	CE
Simplexa FluA/B +RSV Flu A/H1N1	Focus Diagnostics	3	Uses 3M cycler, 96 results in 33 min		IVD, CE

aNumber of viruses detected does not include subtypes/genotypes for influenza A, RSV, hMPV, Enteroviruses or Rhinoviruses but does include Coronaviruses

bIVD, FDA approved for use as in vitro diagnostic; EUO, FDA approved for emergency use only (expired June 2010); RUO, Research use only test; CE, European Union marked.

cDiscriminates only Influenza A, Influenza B, RSV. Adapted from Mahony 201121.

Table 1: Commercially available multiplex assays for detecting respiratory viruses.

Isothermal Amplification Methods

The single reaction temperature required for isothermal amplification can translate into less expensive instrumentation, positioning these methods well for future POC tests. Transcription-mediated amplification (TMA, GenProbe), nucleic acid sequence-based amplification (NASBA, bioMerieux), and strand displacement amplification (SDA, Becton Dickinson) are well established isothermal amplification methods that have been implemented in many clinical laboratories for many years. Eleven newer isothermal amplification methods have been described, including include loop-mediated amplification (LAMP), cross-priming amplification (CPA), recombinase polymerase amplification (RPA), rolling circle amplification (RCA), helicase-dependent amplification (HDA), single-mediated amplification of RNA technology (SMART), nicking enzyme-mediated amplification (NEMA), isothermal chain amplification (ICA), smart amplification (Smart-AMP), exponential amplification reaction (EXPAR), and ramification amplification (RAM) (see reviews by Wu and Tang8; Niemz et al.9). These methods can be grouped based on amplification principle and are based on either RNA transcription (TMA, NASBA, SMART), enzymatic duplex melting and primer annealing (HDA, RPA, RCA), strand displacement using polymerase with multiple primers (LAMP, CPA, Smart-AMP) or strand displacement from a circularized target (RCA, RAM). Other isothermal amplification methods use polymerase extension in conjunction with single-strand cutting (SDA, NEMA, ICA, EXPAR). These methods all involve isothermal generation of ssDNA, exponential amplification of DNA and intermediate target generation. To achieve polymerase-based amplification isothermal, the target ssDNA must be obtained at the same temperature that is required for annealing and extension. To obtain efficient target amplification, amplicons must cycle back into the reaction. In most methods, the amplicon either re-primes or self-primes, and exponential amplification occurs asynchronously unlike in PCR where duplex melting, annealing, and extension occur synchronously through cycling temperatures. Various isothermal amplification methods require temperatures between 30 and 65°C, which is determined by the stability and activity of the polymerase used in the reaction.

development of highly sensitive tests for near-patient settings (Table 2) [9]. Real-time or end point fluorescence detection of isothermal amplification reactions can be mediated with intercalating dyes. Compared with PCR amplification, less expensive and less complex instrumentation is required for isothermal amplification detection. LAMP products can be detected using turbidity as pyrophosphate ions generated as a bi-product of DNA synthesis are precipitated in the presence of metal ions. Turbidity can be read by eye or using the Loopamp Realtime Turbidimeter (LA-200, Eiken). Small, portable, battery operated instruments for the detection of LAMP (Genie II, Optigene) or RPA (Twista, TwistDX) amplicons have been introduced and some assays have been developed where positives can be detected in <20 min [38-41]. Our lab has recently developed LAMP amplification assays using 6 primers for influenza or RSV that can detect positives in 8-10 minutes using the Genie II instrument [27,41]. Nucleic acid lateral flow (NALF) devices that use passive diffusion and perform endpoint detection are well established in POC diagnostics [42]. NALF devices are capable of detecting <10 copies of target nucleic acid per amplification reaction without a reader. NALF devices can be either antibody-dependent or antibody-independent and the latter usually involves antigenic tags (ex. Biotin, Digoxigenin or FITC) that are incorporated into the amplicon via primer labeling or using labeled dNTPs [43]. The BESt Cassette (BioHelix and U-Star Biotech, China) uses antibody-dependent NALF to detect labeled amplicon and has been designed as a single use device to minimize amplicon contamination. NALF is amenable to low level multiplexing and has been used with a variety of amplification methods including PCR, NASBA, HAD, LAMP, RPA, CPA and other isothermal amplification methods. For example, the IsoAmp HSV Assay from BioHelix uses HDA amplification and the BESt Cassette for detection. Other detection approaches have used optical including electronic biosensors for detection. For example, isothermal amplification products have been used together with an on-chip microarray for multiple pathogen detection [44] and electrochemical biosensors have been miniaturized and integrated into microfluidic devices [45]. Single use sample preparation devices have been interfaced with LAMP, RPA and HAD isothermal amplification [39,46,47]. In addition, LAMP has been combined with a lateral flow dipstick for rapid detection of Candidatus liberibacter asiaticus [48].

Novel Detection Methods for Point-of-Care Diagnostics

Recently, isothermal amplification methods have been coupled with novel detection methods, providing a significant advance in the

Platform	Manufacturer	Sample prep included	Amplification method	Detection methoda	Time to result (min)
GeneXpert	Cepheid	Y	PCR	RTF	90-120
Liat Analyzer	IQuum	Y	PCR	RTF	<60
Film Array	Idaho Technolgies	Y	PCR	EPF	60
LA-120	Eiken	N	LAMP	RTT	<60
Twista	TwistDx	N	RPA	RTF	<20
Genie II	Optigene	N	LAMP	RTF	<20
BESt Cassette	U-Star Biotech BioHelix	N	Not included, usually isothermal	NALF	N/A

Simplexa Direct Amplification Discb	Focus 3M Integrated Cycler	Y	PCR	RTF	~60
[a]RTF, real time fluorescence; NALF, nucleic acid lateral flow; EPF, end point fluorescence.					
[b]The Simplexa Direct Amplification Disc can be considered a POC platform since it includes a built in extraction and amplification reagents. Adapted from Niemz et al. 20119					

Table 2: Examples of commercially developed POC test platforms.

Conclusion and Critical Commentary

Clinical laboratories are diagnosing more infections following the adoption of molecular tests due in part to the increased sensitivity of NATs for individual viruses combined with the increased number of viruses that can be detected with multiplex assays. Clinicians will therefore need to become familiar with obtaining reports for uncommon viruses such as Bocavirus or CoV NL63. Molecular tests will also provide positive results on some patients with few or no symptoms since these patients may be at the end of an infection and be shedding low levels of virus. The benefit of a more accurate diagnosis is three-fold: first, it benefits the patient in terms of receiving the appropriate anti-viral drugs such as oseltamivir in the case of influenza; second, it assists infection control practicioners in providing appropriate infection control measures to lower the rate of nosocomial spread; and third, it provides more accurate information to public health authorites regarding what viruses are circulating in the community so they can set public health policy accordingly. In an era of emerging pandemics (ie. SARS Co-V and the 2009 A/H1N1 influenza), accurate and sensitive molecular tests have played a crucial role in indentifying the etiologic agent, tracking the outbreak and understanding the epidemiology of these new virus infections.

Despite the improved sensitivity and specificity profiles of NATs, no molecular test will ever be 100% sensitive or 100% specific for several reasons. Extraction of low levels of viral nucleic acid from specimens with abundant human or bacterial DNA (ie. fecal specimens) is challenging due to competition for solid phase reagents and the presence of mucous or other amplification inhibitors in the specimen, which is often difficult to remove. The percent sensitivity and specificity will also vary depending on the reference standard being used. Just as culture can in theory amplify and detect a single virus particle, the complexing of virus with neutralizing antibody renders culture less sensitive; similarly, molecular tests that are capable of detecting 1-3 viral genomes are rendered ineffective in the presence of amplification inhibitors. Althought the sensitivity of a molecular test may be >99% at the time of maximal viral shedding, the sensitivity may drop early or late in the course of infection when less virus is shed.

Although they have many advantages, molecular tests have some limitations that cannot be ignored. A point mutation in the primer binding site could result in a false negative result. For this reason, most primers are selected in a conserved region of the genome. Molecular tests can only be applied to a newly emerging virus once the viral genome has been sequenced. In the case of the SARS pandemic, the genome was sequenced in 2-3 weeks so molecular tests could be employed early in the pandemic. In addition, the detection of low levels of virus either late in the course of infection, in the case of a dual infection, or in people lacking any signs or symptoms makes it difficult to determine the clinical relevance of the identified virus. Molecular tests have been very useful in documenting horizontal transmission events during new pandemics and have helped elucidate the epidemiology of emerging virus infections. Limitations include the lack of available external reference standards for quality assurance purposes, a possible decrease in sensitivity of some multiplex assays compared with uniplex assays, and difficulties with virus quantitation when different types of clinical specimens (ie. NP, TS, BAL) are used and normalization is difficult to establish and determine their clinical relevance.

Evaluation of the performance of specific molecular tests using different specimen types is also critical since interference factors may vary from one specimen type to another and the ability of extraction kits to remove interference factors will vary. For example, sputum specimens present specific challenges for nucleic acid extraction due to the presence of excess mucous and the ability of a molecular test to detect a specific nucleic acid target will depend on the successful extraction of nucleic acid. Sputum lysis solution (SL-S from Copan, Italia) has recently been introduced to break down mucous [49]. We have used SLS for not only NP specimens, but also to increase the yield from stool specimens. Furthermore, natural history studies are required to determine the most appropriate clinical specimen for newly discovered viruses such as SARS CoV, which is readily detected in stool specimens [23].

The development and adoption of multiplex PCR tests for respiratory viruses will have a large benefit over the coming years since multiplex tests offer laboratories the ability to detect a large number of viral infections. Dual respiratory virus infections are poorly detected using DFA and SVC; however, multiplex assays have detected dual or triple infections in 8-11% of positive specimens [5,18]. The ability to easily detect dual infections provides both the means and impetus for studies aimed at determining the clinical importance of dual infections, who is at risk for obtaining dual infections and whether dual infections result in poorer outcomes for the patient. As mentioned earlier, the use of multiplex NATs has increased the diagnostic yield for respiratory viruses by 30-50% over conventional test methods. Quantitative assays for determining viral loads in respiratory specimens have recently appeared in the literature, and this development holds promise for establishing correlations between viral load and severity of clinical disease. Multiplex tests will contribute to our understanding of the epidemiology of viral respiratory infections as large numbers of specimens can be tested for multiple viruses providing a wealth of new information on seasonality, geographical distribution, and risk groups. Finally, genotyping assays to detect antiviral resistance have also appeared recently and these tests will provide clinicians with new information to improve patient management [50].

Where Do We Go From Here?

During the past decade, advances in isothermal amplification and detection methods have surpassed advances in specimen preparation and nucleic acid extraction. Nucleic acid extraction is now the bottleneck for molecular testing in both low and high volume laboratories and improvements in sample preparation for molecular testing is therefore warranted. Looking back to the first commercially available NAT for C. trachomatis, which involved detergent-based cell lysis and release of nucleic acid without purification, two commercial companies have recently developed tests for detecting C. difficile in stool specimens using either LAMP or PCR that use a 10 minute heat treatment (95°C) of stool suspensions without target purification. Other specimen processing approaches such as nanoparticle-based target capture and sequestration of nucleic acid away from amplification inhibitors using microfluidics should be exploited. Novel specimen preparation approaches will be required to take advantage of the tremendous potential of isothermal amplification in building the next generation of diagnostics. To date, a few single-use devices that couple sample preparation and isothermal amplification have been described [40,46,47,51]. Integration of new specimen preparation methods with isothermal amplification and miniaturization onto chip-based detection devices involving microfluidics and/or electrochemical biosensors will be paramount in the development of the next generation of diagnostics for resource poor and POC settings. These recent pioneering efforts will usher in a new dawn of diagnostics showcasing exciting and amazing landmark accomplishments that will far surpass those of real time and multiplex PCR.

Acknowledgment

Declaration of interest

The author has received speaking honoraria and consulting fees from Luminex Molecular Diagnostics and EraGen Biosciences. He has been a scientific advisor for Luminex, Chemicon Corporation (now Millipore), and EraGen Biosciences. He received a grant from TmBiosciences Corporation (now Luminex Molecular Diagnostics) to assist with the development of the xTAG™ RVP test and is co-holder of a patent on this product. No writing assistance was utilized in the preparation of this manuscript.

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