17 Advanced Immunotechnological Methods for Detection and Diagnosis of Viral Infections: Current Applications and Future Challenges

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Abstract

Diagnosis and identification of viruses is an important component of diagnostic virology laboratory. Although various modes of diagnostic methods are now available at disposal, a vast majority of the diseases across the globe remain undiagnosed. This is largely due to the overlapping undifferentiated set of symptoms across myriad set of RNA and DNA viral diseases. As such, it becomes critical to take into consideration several factors for viral diagnosis ranging from the type and quality of specimen collected, time of specimen collection, mode of transport, accuracy, specificity, sensitivity, and the type of diagnostic method used. This chapter broadly emphasizes various methods on diagnostic virology ranging from the classical methods of diagnosis to the most recently developed molecular methods of detection of virus.

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17.1 Introduction

Viruses are obligate intracellular parasites and their detection and identification is an imperative component clinically. Viral infectious signify a major portion in public health perspectives with thousands of deaths annually. Notwithstanding from highly contagious infections and serious pandemics to common influenza episodes, clinical diagnosis of viral infection often relies on early detection. Therefore, effectual detection of viruses is indispensable aid to avert transmission, initiate befitting therapy, and scrutinize response to treatment which leads to effective disease control and management (Souf 2016).

Viral diagnosis is a dynamic process. Although prophylaxis is better than cure, an accurate diagnosis of any virus fundamental to the infection is equally vital. Generally, diagnostic tests are categorized into three groups: direct detection, isolation of virus, and serology tests. Direct examination methods are merely faster and examined directly for presence of virus particles, virus antigen, or viral nucleic acids. Immunofluorescence assay is extensively used for rapid identification of virus infections through detection of virus antigen or virus-specific antibodies in clinical specimens. Viral diagnosis is a crucial revolution and renders more accessible and makes it potential to standardize the recently developed diagnostic methods. Serology essentially comprises bulk of work of any virology laboratory. A serological diagnosis can be identified by increasing titers of antibody among acute and convalescent stages of infection, or by the detection of Immunoglobulin M (IgM).

The diagnosis of viral infections were enhanced noticeably all through 1990s, with the onset of highly sensitive methods, viz. enzyme-linked immunosorbent assay (ELISA) and PCR are superseded for this reason. Nevertheless, during the last 20 years this technique is being utilized, due to its unique nature, making diagnosis probable through visualization of virus. Then after, introduction of ELISA then revolutionized viral diagnosis by simplifying detection and shortening the time (Torrance and Jones 1981). Molecular diagnostic tools for viral diagnosis has trialed speedy advancements in last few years (Hayden and Persing 2001), and revolutionized diagnosis of infectious diseases, particularly viral diseases. The use of amplification techniques, viz. PCR, RT-PCR, or NASBA (nucleic acid sequence-based amplification) (Van Belkum and Niesters 1995) for detection of virus, genotyping, and quantification have several advantages such as high reproducibility and sensitivity, including broad dynamic range (Ebner et al. 2005, 2006). Several molecular diagnostic techniques were recently swapped by fully automated devices that use less time, maneuver smaller volumes of liquids, and provide quantified results with improved accuracy. The current review emphasizes the advanced immunotechniques, how the specific characteristics of diagnostic methods revolutionized field of viral diagnosis clinically from a decade.

17.2 Recent Advances in Viral Disease Diagnosis

17.2.1 Advanced Methods in Diagnostic Virology

17.2.1.1 Microscopy

Transmission electron microscopy (TEM) is a merely imaging technique that permits direct image of viruses attributable to its nanoscale resolution. This is a classical technique owing to its advantage of direct visualization of virus. Amid the 1960s and 1990s, TEM contributed a breakthrough and doled out as a diagnostic tool for recognizing numerous virus unswervingly in any biological samples. For that reason, in modern years, role of TEM in diagnosis of anonymous infectious agents in specific viral outbreaks or transmission clusters shifted from regular use to an initial screening test. TEM is also considered an important method for assessment of viral safety in biopharmaceutical products. Application of TEM understands the unknown viruses for which there is an imminent risk of contamination, and TEM will undeniably be functional for documenting the subsistence of viruses or virus-like particles in these cells and the products derivative from them (Roingeard et al. 2019).

17.2.2 Advanced Serological Immunoassays

The immunoassays are primarily antibody/antigen dependent assays. This principle works on immunization with the antigens and activating, or infection. Therefore, measuring of IgG immunoglobulin and quantifying the antibodies are preferentially used as diagnostic markers. The antigens or antibodies are coated with conjugated labels like metals, radioactive isotopes, fluorescent tags, respectively. As a part of modern research on immunotechniques, a diagnostic approach for chronic hepatitis C infection (CHC), detects specific antibody to HCV (anti-HCV) (indirect tests) and assays that can detect, quantify, or characterize components of HCV viral particles, viz. HCV RNA and core antigen (direct tests). Quantification of HCV core antigen (cAg) as a one-step procedure has been indispensable. Hence, in order to evaluate the performance of cAg quantification in diagnosing CHC and how it is predisposed by concomitant HIV or HBV infections, cross-sectional validation assays, i.e., HCV Ag quantification as a one-step procedure in diagnosing CHC in Cameroon was designed to abridge the diagnostic process (Duchesne et al. 2017). It is pertinent to note that a multiplex microsphere immunoassay (MIA) recently developed possess diagnostic power to incarcerate viral envelope protein, which evokes to be strong cross-reactive antibodies to other *flaviviruses* and differential power of viral nonstructural proteins NS1 and NS5 was. Interestingly this serologic assay needs to be employed in rapid clinical diagnosis of ZIKV and/or dengue virus infections for screening immune responses in vaccine trials (Wong et al. 2017). It is noteworthy that oral fluid is a noninvasive biospecimen that can harbor pathogen-specific antibodies and reach potential to replace blood-based testing protocols. Therefore, a saliva-based oral fluid immunoassay was developed to assess past and recent hepatitis E virus (HEV) infections from noninvasive

sampling methods. The sensitivity and specificity of this assay was comparable to serum-based ELISAs. This salivary assay could improve our understanding of the ecology and natural history of HEV (Pisanic et al. 2017).

Diagnosing ZIKV remains a great challenge, as detection of viral RNA is only possible merely few days after onset of symptoms. Conversely, novel high-throughput image-based fluorescent neutralization method for identification of ZIKV was thoroughly evaluated and developed which reported higher sensitivity than Plaque reduction neutralization test (PRNT) and MAC-ELISA, respectively. This test might employ for clinical diagnosis, clinical trials, and confirmation and seroprevalence studies of ZIKV infection (Koishi et al. 2018). In one of the recent studies, detection of serum HEV antigen (Ag) is deemed to be sensitive and promising biomarker for HEV antigen diagnosis with HEV RNA in both acute and chronic genotypes. Strikingly an antigen assay was recently evaluated for diagnosing HEV genotypes with higher sensitivity than commercial anti-HEV IgM and HEV RNA ELISA tests (Zhang et al. 2019). Nonetheless, recent studies on respiratory syncytial virus (RSV) developed Luciferase Immunoprecipitation Systems (LIPS) assay to detect IgG Antibodies against Human RSV G-Glycoprotein. Moreover, Human RSV G-Glycoprotein also acts as biomarker for natural exposure or immunization. RSV genes encoding native and mutated G (mG) proteins from subgroups A and B strains were cloned, expressed as luciferase-tagged proteins, and experimented separately to spot anti-RSV-G specific IgG antibodies employing a high-throughput luciferase immunoprecipitation system (LIPS-G). It was pertinent to note that RSV monoclonal antibodies and polyclonal antisera explicitly bound in LIPS-G_A and/or -G_B assays (Crim et al. 2019).

The diagnosis of (ZIKV) and dengue virus (DENV) infections against viral envelope protein and nonstructural proteins (NS) was developed using flavivirus multiplex microsphere immunoassay (MIA). However MIA could not differentiate more recent from past infections, which represents a key diagnostic challenge; therefore, in a most recent report an immunoglobulin G (IgG)-based avidity assay was developed for its diagnostic performance to accurately differentiate between recent ZIKA and past dengue virus infections. This assay was found useful in patients with high risk of ZIKA complications, viz. pregnant women and monitoring immune responses in vaccine trials (Furuya et al. 2019). Consecutively to develop serological diagnosis of ZIKV-IgA and ZIKV-IgG, avidity assays were evaluated to characterize ZIKA infections in want of viremia. These assay facilitated construed low avidity of IgG and IgA results, enhanced the serological diagnosis of ZIKV (Amaro et al. 2019). In another study, homologous proteins of diverse flaviviruses exhibited high degrees of sequence uniqueness, mainly within subgroups. This led to prevalent immunological cross-reactivity. Therefore, a proportional deconvolution of complex B cell responses against ZIKV and other flavivirus were deliberated by screening with a microarray chip-based high-resolution serological analysis primed from overlapping peptides covering the whole amino acid sequence of ZIKV genomic polyprotein was developed. Additionally with advent of this assay several infections, viz. dengue, yellow fever, tick-borne encephalitis, and West Nile viruses shall be diagnosed (Hansen et al. 2019).

17.2.2.1 ELISA-Based Immunodetection

Enzymes are extensive tool for diagnosing virus which have various applications like enzyme immune assay, ELISA. Enzyme immune assay has different applications like fluorescence polarization immune assay (FPIA), micro-particle immune assay (MEIA), chemiluminescent (CLIA). Enzyme immune assays work with antigen–antibody interaction with the conjugated tags like fluorescent tags, chemiluminescent tags which are complemented with substrates like polarized light and fluorescent substrates. As a part of most advanced immunotechniques, an ultrasensitive colorimetric assay called magnetic nano(e)zyme-linked immunosorbent assay (MagLISA) was developed, wherein silica-shelled magnetic nanobeads (MagNBs) and gold nanoparticles were pooled to monitor influenza A virus up to femtogram per milliliter concentration (Oh et al. 2018). Sensitive and specific detection of Crimean–Congo hemorrhagic fever virus (CCHFV) was developed employing specific IgM and IgG antibodies in human sera using recombinant CCHFV nucleoprotein as antigen in μ-capture and IgG immune complex (IC) ELISA tests (Emmerich et al. 2018). Recently, truncated forms of HeV and NiV (Hendra and Nipah virus) G proteins as well as full-length NiV nucleocapsid (N) protein were used for detection of Hendra and Nipah virus specific antibodies in pigs. These recombinant proteins were expressed through diverse expression systems and an indirect ELISA was developed for detection (Fischer et al. 2018). A rapid diagnostic platform for colorimetric differential detection of DENV and CHIKV viral infections was recently developed with a possibility to alter clinical diagnosis of acute febrile illnesses in resource-limited settings. This platform principally facilitates consistent and accurate multiplexed detection of chikungunya and dengue IgM/IgG antibodies in human clinical samples within short stint (Wang et al. 2019).

17.2.2.2 Immunofluorescence-Based Immunodetection

Immunofluorescence (IF) is extensively used for speedy detection of viral infections clinically started in early 1970s. IF is used for diagnosis of virus antigen and virus-specific IgG/IgA/IgM antibody in clinical specimens. In this technique, fluorescein-labeled antibody to stain specimens containing specific virus antigens, were used for UV illumination. As a part of modern research on immunotechniques, new recombinant rabies virus expressing green fluorescent protein (rRV-GFP) is more rapid, simpler, and less expensive detection and for quantification of virus neutralizing antibodies in blood sera. This technique simplified multistep Rapid Fluorescent Focus Inhibition Test (RFFIT) procedure by purging immunostaining step (Qin et al. 2019a, b).

17.2.2.3 PCR/RT-PCR-Based Immunodetection

Nucleic acid (DNA and RNA) amplification assays are conventionally known as polymerase chain reactions (PCRs). Distinct PCRs exists based on type of nucleic acid and information known a propos the sequences of genomes for immunodetection. Rous Sarcoma Virus (RSV) is one of the most significant causative agents of respiratory tract infection in children and related with high morbidity and mortality. However, very little is known with reference to effects of respiratory viral

infections. A reverse transcription recombinase polymerase amplification assay (RT-RPA) is nucleic acid probe based on novel isothermal amplification technique which has been widely employed to detect human RSV. The results exemplified that concurrence rates between RT-RPA assay and qRT-PCR assay for clinical samples was 96%, demonstrating that RT-RPA assay holds better diagnostic presentation on clinical samples in remote rural areas in developing countries (Xi et al. 2019). Using quantitative PCR (qPCR) for common respiratory viruses and for two genes (*CCL8*/*CXCL11*) is recognized to be extremely upregulated in viral infections. Notably, RNA-seq virus detection achieved 86% sensitivity compared to qPCR-based screening in asthmatic children which consequently drives immune cell airway infiltration, cellular remodeling, and alteration of asthmogenic gene expression (Wesolowska-Andersen et al. 2017). Nevertheless as a part of latest advancements on immunotechniques, isothermal reverse transcription and recombinase polymerase amplification (RT-RPA) of synthetic RNA (Ebola virus) employing paper microfluidics devices was developed initially. Later on based on RNA detection and multiplexed analysis for Ebola virus diagnostics were optimized and demonstrated with high sensitivity. Additionally, nine-spot multilayered device achieving parallel detection of three distinct RNA sequences opens a route in the direction for detection of multiple viral pathogens (Magro et al. 2017).

Rabies virus (RABV) is one of the most significant global zoonotic pathogens. Two sensitive real-time quantitative RT-PCR assays were developed and validated for large-spectrum detection of RABV, with a focus on African isolates. The primer and probe sets were targeted for highly conserved regions of nucleoprotein (N) and polymerase (L) genes. Effective detection and high sensitivity of these assays can be effectively functional in general research and used in diagnostic process and epizootic surveillance (Faye et al. 2017). However, in recent times a fluorescent reverse transcription loop-mediated isothermal amplification (RT-LAMP) employing quenching probes for detection of Middle East respiratory syndrome coronavirus was developed. Additionally, detection efficacy of QProbe RT-LAMP was comparable to that of RT-PCR assay (Azhar 2018). Furthermore this assay can as well be used as authoritative diagnostic tool for rapid detection and surveillance of MERS-CoV infections (Shirato et al. 2018). Consecutively to assist detection of ZIKV infections, and distinguish these infections from DENV and CHIKV, Trioplex real-time RT-PCR assay was recently developed. However the performance of Trioplex real-time RT-PCR assay was particularly employed for the detection of ZIKV, DENV and CHIKV viruses. Simultaneous testing of more than one specimen type from each patient affords a superior diagnostic sensitivity of this technique (Santiago et al. 2018).

An in situ hybridization (RNA-ISH) assay was recently developed to detect viral hemorrhagic septicemia virus (VHSV), an OIE listed piscine rhabdovirus, in infected fish cells with fathead minnow (FHM) as model cell line. Two antisense RNA probes targeting fragments of N and G genes were amplified by RT-PCR employing VHSV-specific primers trailed by transcription reaction in presence of digoxigenin dUTP has competently localized VHSV mRNAs in infected cells. The diagnostic sensitivity of RNA-ISH assay was better than immunocytochemistry,

qRT-PCR and $TCID_{50}$ (Qadiri et al. 2019). Development and evaluation of a new one-step, real-time RT-PCR assay was developed for detecting latest H9N2 influenza viruses competent of causing human infection. The sensitivity of one-step, real-time RT-PCR assay was generally determined to be used in vitro transcribed RNA, devoid of any cross-reactivity against RNA from H1–15 subtypes of influenza viruses and other viral respiratory pathogens with no nonspecific reactions (Saito et al. 2019).

17.2.2.4 Spectroscopy-Based Immunodetection

In the current trends mass spectrometry (MS) is a benchmark for qualitative and quantitative diagnosis of viruses clinically. In clinical laboratories, MALDI (matrix-assisted laser desorption ionization) and ES (electrospray) ionization methods are most frequently used as they allow ionization of analyte in considerable amounts. The combination (RT-PCR/ESI-MS) was able to detect viral pathogens (acute viral respiratory infections and influenza A viruses) usually for those viruses which are undetected by regular testing methods as well as provides rapid and detailed data (about types and subtypes of virus) in short period (Deyde et al. 2010; Chen et al. 2011). Yet another study reported that near-infrared spectroscopy (NIRS) is a rapid, reagent-free, and cost-effective tool used to detect ZIKV noninvasively in heads and thoraces of intact *Aedes aegypti* mosquitoes with high prediction accuracies relative to quantitative RT-qPCR reaction. Perhaps this technique could be extended upon for identifying probable arbovirus hotspots to guide spatial prioritization of vector control (Fernandes et al. 2018). Recently developed surface plasma resonance (SPR) spectroscopy was developed to be a valuable optical biosensor and potential method for diagnosis of dengue virus E-protein and also for identification of antibodies to DENV antigen. The diagnosis limit, sensitivity, and selectivity of SPR sensing in DENV antigen was amazingly high. This technique was introduced as a novel 3D-PAMAM-SAM-Au multilayer thin film for future research on SPR sensing applications (Omar and Fen 2018).

17.2.2.5 miRNA-Based Immunodetection

miRNA are conserved small noncoding RNA with 19–24 nucleotides which regulates post-transcriptional modification. miRNAs are transcribed by RNA polymerase II into pre-miRNA which is processed by Dorsa/DGCR-8 and Dicer and transported to cytoplasm. RISC in cytoplasm processes pre-miRNA into mature miRNA and regulates post-transcriptional activities. As a part of the most advanced immunotechniques, exosomal microRNAs were recently studied as potential diagnostic markers for various malignancies, including hepatocellular carcinoma (HCC). Serum exosomal microRNAs combined with alpha-fetoprotein as diagnostic markers of hepatocellular carcinoma (Wang et al. 2018). In another study, synthetic miRNA-based approach was developed to express neutralizing antibodies directly in lung via aerosol, to prevent from human RSV and influenza infections. Engineered mRNA-expressed antibodies prevented RSV infection. It is noteworthy that an expressing membrane-anchored broadly neutralizing antibody in lungs could potentially be promising pulmonary prophylaxis approach (Tiwari et al. 2018). In a most recent

Table 17.1 Advanced immunotechnological techniques for detection and diagnosis of viral infections

S. No.	Techniques	Application to diagnosis of emerging viral infections
1.	Serological tests	Diagnostic approach for chronic hepatitis C infection, ZIKV, RSV, DENV, tick-borne encephalitis, and West Nile viruses
2.	Enzyme assays (fluorescence polarization immune assay (FPIA), microparticle immune assay (MEIA), chemiluminescent (CLIA)	Identification of structural proteins of Hendra and Nipah virus, Crimean–Congo hemorrhagic fever virus (CCHFV), chikungunya
3.	PCR-based immunodetection (RT-RPA, RT-LAMP, RNA-ISH, RT-PCR, Trioplex real-time PCR)	Detection of Ebola virus, Rabies virus, Middle East respiratory syndrome coronavirus, hemorrhagic septicemia virus, influenza viruses
4.	Mass spectrometry (MS/MS)	Prognosis of DENV antigen, ZIKV
5.	miRNA-based immunodetection	Circulating microRNA biomarkers in detection of arboviruses; hepatitis infection
6.	Next-generation sequencing (NGS)	Provides more exact and precise time estimates of infection, crucial for HIV-1 surveillance
7.	Metagenomics (panpathogen metagenomic sequencing assay)	Diagnostic standard techniques used in diagnosis and genetic analysis of influenza and other clinical respiratory viruses
8.	Immunosensors (sandwich-type electrochemiluminescence (ECL) immunosensor)	Diagnostic test for HBV, swine flu (H1N1) infection, H7N9 virus with high sensitivity
9.	Microfluidic technology	Used to test the convenience of neutralizing antibodies to explore impact on virus–cell interactions
10.	CRISPR/Cas system	Prognosis of coxsackievirus, hepatitis B virus, Zika virus
11.	Nanoparticles-based immunodetection	Nanoparticle-based lateral flow immunoassay as point-of-care diagnostic tool for infectious agents and diseases

study, endogenous microRNAs (miRNA) are evolutionarily conserved and their presence in biological fluids signifies regulatory role of circulating miRNAs in pathogenesis, immune responses, and viral infections. On the other hand, noninvasive diagnostic approach, using biomarkers, currently plays a central role in early diagnosis of viral infections. Given the fact, a recent report depicted numerous circulating microRNA biomarkers viz. miR155 and miR1260 in influenza; miR12, miRVP3p, and miR184 in arboviruses; and miR29b and miR125 in hepatitis infection for diagnostic function, respectively (Ojha et al. 2019) (Table 17.1).

17.2.2.6 Next-Generation Sequencing (NGS)-Based Immunodetection

Next-generation sequencing (NGS) is one of the noteworthy achievements recorded in the current era. Ahead from genome sequencing of known organisms, permitted breakthrough of new viruses dependable for unknown human diseases, for tracking viral outbreaks and pandemics as influenza to comprehend their emergence and transmission profiles. Viral diversity from next-generation sequencing of HIV-1 samples provides more exact and precise estimates of time since infection, consequently, the infection regencies are also crucial for HIV-1 surveillance and understanding of viral pathogenesis. NGS-derived average pair-wise diversity exhibited higher sensitivity and specificity compared to fraction of ambiguous nucleotides (Carlisle et al. 2019).

17.2.2.7 Metagenomics-Based Immunodetection

It is noteworthy that metagenomic next-generation sequencing assay (mNGS) for pan-pathogen detection has been effectively tested in patients with acute illness of several viral etiologies. In this connection a customized bioinformatics pipeline, SURPI+, was recently designed to quickly analyze mNGS data, generate an automated summary of detected pathogens, and provide a graphical user interface for evaluating and interpreting results (Miller et al. 2019). Panpathogen Metagenomic sequencing assay for SLEV (St. Louis encephalitis virus) infection in CSF (cerebrospinal fluid) is an unbiased approach to infectious disease testing, although several challenges still remain relating to test availability, interpretation, and validation that were reported. However, recently metagenomic next-generation sequencing was employed to diagnose fatal case of meningoencephalitis caused by SLEV (Chiu et al. 2017). In another study, metagenomic viral sequencing is the potential diagnostic test for influenza which also provides insights on transmission, drug resistance, evolution, and simultaneously detects other viruses. It is pertinent that Oxford Nanopore Technology was employed to metagenomic sequencing of respiratory samples. This technology operated with very high sensitivity compared to current diagnostic standard techniques and certainly this approach may show a great promise for nanopore platform to be used in diagnosis and genetic analysis of influenza and other clinical respiratory viruses (Lewandowski et al. 2019) (Table 17.1).

17.2.2.8 Monoclonal Antibodies-Based Immunodetection

In the recent past designing diagnostic and therapeutic platforms based on aptamer technology is undoubtedly a potential approach in viral infections. Nevertheless the oligonucleotide aptamers which are potential alternatives for monoclonal antibodies based detection could be aimed against any protein in infected cells and any components of viral particles are deemed as probable novel diagnostic molecules

against viral hepatitis. It is noteworthy that these aptamer molecules could be a favorable substitute for monoclonal antibody in near future (Mirian et al. 2017).

17.2.2.9 Immunosensors-Based Immunodetection

A sensitive and selective electrochemical immunosensor for label-free ZIKV protein detection was recently developed, employing functionalized interdigitated microelectrode of gold (IDE-Au) array. This ZIKV immune-sensing chip can be integrated with miniaturized potentiostat (MP)-interfaced with smart phone for rapid ZIKV-infection detection is obligatory for early stage diagnostics at point-of-care application (Kaushik et al. 2018). However, a cost-effective and portable graphene-enabled biosensor to detect ZIKV with a highly specific immobilized monoclonal antibody was recently developed. Field effect biosensing (FEB) with monoclonal antibodies covalently linked to graphene enables the real-time, quantitative detection of native ZIKV antigens. This assay is first-of-its-kind graphene-enabled ZIKA biosensor which makes it an ideal candidate for the development as a medical diagnostic test (Afsahi et al. 2018).

An accurate and timely diagnosis of any new reassortment of avian influenza is very crucial for controlling disease outbreaks. In view of this, a simple strategy for rapid and sensitive detection of H7N9 virus was achieved by employing an intensity-modulated surface plasmon resonance (IM-SPR) biosensor technique integrated with newly generated monoclonal antibody. This novel antibody demonstrates noteworthy specificity to identify H7N9 virus compared to homemade target-captured ELISA, qRT-PCR, and rapid influenza diagnostic test (RIDT) with high sensitivity (Chang et al. 2018).

In a recent study, sandwich-type electrochemiluminescence (ECL) immunosensor was developed for ultrasensitive determination of HBV surface antigen. The primary antibody of HBs (Ab_1) was immobilized on surface of the carboxyl-modified magnetic nanoparticles (MNPs). Then, the PAMAM dendrimer with many amine functional groups was employed as carrier for immobilizing CdTe@CdS quantum dots (QDs) and the secondary antibody (Ab_2) amplified ECL signal of QDs considerably improved sensitivity. Strikingly, this ECL sensor was designed based on signal amplification with dendrimer–quantum dots structures (Babamiri et al. 2018). In another study, hemagglutinin (HA), a glycoprotein present on the surface of influenza A subtype virus H1N1, virus binds to human cells with sialic acid on membrane of upper respiratory tract. For early detection of swine flu (H1N1) infection in human, an impedimetric hemagglutinin gene-based biosensor was developed by immobilizing amino-labeled single-stranded DNA probe onto cysteine modified gold surface of the screen printed electrode for early and rapid detection of H1N1 (swine flu) in human (Mohan et al. 2019).

17.2.2.10 Microfluidic Technology-Based Immunodetection

Microfluidic technique permitted research of viral measurement of fusion kinetics, viral infectivity, and screening of viral responses to neutralizing molecules. Besides that, microfluidic platforms also signify promising and innovative clinical tools with applications in clinical diagnostics including drug screening. Microfluidics

specifically emphasizes an array of techniques concerned with the precise control and manipulation of fluids, within microscopic channels (Whitesides 2006). This moderately clear-cut perception underpins a range of biological research techniques, from flow cytometric and DNA analyses to enzyme and immunoassays (Duncombe et al. 2015). A recent study investigating the use of an integrated microfluidic system for diagnostics utilized aptamers against IAV (H1N1) for viral detection (Tsang et al. 2016) was studied. Remarkably, two recent studies into the infectivity of murine norovirus (MNV) also utilized droplet-based microfluidic platforms to test the convenience of neutralizing antibodies and explored their impact on virus–cell interactions (Fischer et al. 2018). Since viral infections are complex and highly dynamic process, appreciably affected by the physical and chemical environment, studies into infection biology should preferably occur in microfluidics system

17.2.2.11 CRISPR/Cas System-Based Immunodetection

The use of CRISPR/Cas system for RNA-based gene therapy is currently raising numerous potential therapeutic applications. CRISPR/cas9 is the latest and unique RNA targeted gene therapy successfully applied in 2007 and observed in *Staphylococcus pneumonia*. The CRISPR is RNA sequence repeats which targets the foreign DNA cleavage by binding to the PAM flanking sequences which mediates the endonuclease called Cas through guide RNA (g-RNA) and responsible for the double strand breaks in the host or foreign DNA and silences the gene expression by nonhomologous end joining (NHEJ). There are different kinds which are mediated by different cas proteins. Notably like class 2, type II has cas9 where g-RNA complements cas protein and other system, eventually class II and type V complements with cas12a and the CRISPR codes crRNA acts as guide RNA by complementing the type V cas 12 protein complex. There are other 29 CRISPER/cas systems that were identified (Makarova et al. 2015). The application of CRISPR/CAS in viral diagnostics makes a breakthrough and it increased specificity. The type V CRISPR-CAS12a is used for the detection of the viral DNA. The CAS12a is designed specific to the viral DNA. The ssDNA is tagged with a Reporter which has fluorophore and quencher on both ends. The cleavage activity of viral DNA by cas12a confers the cleavage of the ssDNA. As a result flourophore reporter, the cleavage of ssDNA emits the fluorescence which is detected by quenchers and further amplified by recombinase polymerase PCR. The technique was characterized by DETECTR. There are other methods like SHERLOCK (Gootenberg et al. 2018) which works consiently based on the Cas13 system. This tool is specially applied in the ZIKA virus diagnosis (Table 17.1).

A novel arrayed CRISPR screen is aptly based on the plasmid library expressing single-guide RNA (sgRNA) and disrupted 1514 genes, encoding kinases, proteins related to endocytosis, and Golgi-localized proteins, individually using 4542 sgRNAs. This CRISPR screen uncovered host factors indispensable for infection by coxsackievirus B3 (CVB3) which comprises human pathogens causing diverse diseases. This technique is more sensitive as compared to arrayed screens based on

siRNA-mediated knockdown (Kim et al. 2018).The lenti viral-based CRISPR screen-based sg-RNA plasmid pool can be employed as potential diagnostic tool for HPV and can target other viral diseases. An automated POC system for EBV detection with RNA-guided RNA endonuclease Cas13a, employing its collateral RNA degradation subsequent to its activation was recently developed by Qin and colleagues. Followed by an automated microfluidic mixing and hybridization, nonspecific cleavage products of Cas13a were allowed to quantify by a custom-integrated fluorometer. This CRISPR-Cas13a based diagnostic method is rapid, amplification-free, simple, and sensitive, thus establishing a key technology toward a useful POC diagnostic platform (Qin et al. 2019a, b).

17.2.2.12 Nanoparticles-Based Immunodetection

The prologue of a new class of nanoscale materials with manifold exceptional properties and functions has sparked series of breakthrough applications in biomedical and diagnostic applications. It is pertinent to note that some recent advances in nanoparticle-based lateral flow immunoassay as point-of-care diagnostic tool for infectious agents and diseases has been recently developed for the detection of infectious viral agents. Lateral flow immunoassay (LFIA) technology is a paper-based, point-of-care strip biosensor designed to detect a specific analyte in virus-infected samples (Banerjee and Jaiswal 2018). AuNP-based detection techniques were reported by various groups of clinically relevant viruses with a unique focus on applied types of bio-AuNP hybrid structures, virus detection targets, and assay modalities and formats were recently developed (Draz and Shafiee 2018).

17.3 Future Perspectives and Conclusions

The most recently developed diagnostic viral techniques are redesigning the field of clinical virology, which could contribute to reduction in incidence of serious infectious viral diseases. The foremost advantages of molecular techniques are its higher sensitivity and specificity matched up with other diagnostic methods, viz. serological assays and culture methods, as well as its rapidity and possibility of automation. Nevertheless, the technological capabilities alone are inadequate if not sustained by health promotion policies to boost the consciousness a propos the significance of early detection of infectious viral diseases outbreak and its spread. In conclusion, good quality diagnosis has a cost that only developed countries can afford in regular practice so far, and this is delaying the execution of new-fangled methods in developing world and in disease endemic areas. Conversely, it is anticipated that asserted efforts may persist toward developing new high-quality tests inexpensive in low-income countries, which would considerably reinforce disease control strategies.

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