# Point-of-Care Microdevices for Blood Plasma Analysis in Viral Infectious Diseases

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**Abstract**—Each year, outbreaks of viral infections cause illness, disability, death, and economic loss. As learned from past incidents, the detrimental impact grows exponentially without effective quarantine. Therefore, rapid on-site detection and analysis are highly desired. In addition, for high-risk areas of viral contamination, close monitoring should be provided during the potential disease incubation period. As the epidemic progresses, a response protocol needs tobe rapidly implemented and the virus evolution fully tracked. For these scenarios, point-of-care microdevices can provide sensitive, accurate, rapid and low-cost analysis for a large population, especially in handling complex patient samples, such as blood, urine and saliva. Blood plasma can be considered as a mine of information containing sources and clues of biomarkers, including nucleic acids, immunoglobulin and other proteins, as well as pathogens for clinical diagnosis. However, blood plasma is also the most complicated body fluid. For targeted plasma biomarker detection or untargeted plasma biomarker discovery, the challenges can be as difficult as identifying a needle in a haystack. A useful platform must not only pursue single performance characteristics, but also excel at multiple performance parameters, such as speed, accuracy, sensitivity, selectivity, cost, portability, reliability, and user friendliness. Throughout the decades, tremendous progress has been made in point-of-care microdevices for viral infectious diseases. In this paper, we review fully integrated lab-on-chip systems for blood analysis of viral infectious disease.

**Keywords**—Point-of-care, Microdevice, Blood, Plasma, Serum, Virus.

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## INTRODUCTION

Infectious diseases are believed to be the cause of approximately 25% of the annual deaths worldwide.[14] Viruses have caused the deadliest pandemics in recorded human history, including the 1918 influenza pandemic (50 million deaths), and the HIV/AIDS pandemic (36 million deaths so far).[14] The emergence of these pathogens and their subsequent spread have made a significant impact on global health, as well as economic losses and interruptions in trade and travel.[5,49,70] Changes in various environmental, biological, socioeconomic, and political factors[49] and expansion of international travel and trade have extended the reach and increased the rate at which viral infectious diseases spread,[29,78] prompting the need for more rapid outbreak detection and efficient monitoring of the spread to minimize the burden on global health and the economy.

Blood is considered to be a mine of information and plays an important role in the immune system. On average, the entire volume of blood circulates throughout the human body every few minutes. It carries nearly real-time information on the status of viral infections.[26,60] It transports blood cells, nutrients, oxygen, metabolic products, and pathogens throughout the body in the cases of many infectious diseases.[73] In addition, blood is a complex mixture as well as a specialized type of connective tissue in which living bloods cells (erythrocytes, leukocytes, and platelets) are suspended in a nonliving fluid matrix, the plasma. Upon centrifugation, the blood will separate into various components based on density: the reddish mass at the bottom consisting of erythrocytes (about 45% of

the whole blood volume), above that a thin whitish layer of buffy coat consisting of leukocytes and platelets (less than 1% of blood volume), and the less dense plasma (makes up remaining 55% of whole blood) at the top. Although blood plasma, a straw-colored, sticky fluid, is mostly water (about 90%), it contains over 100 different dissolved solutes. Plasma proteins, which account for about 8% by weight of plasma volume, are the most abundant plasma solutes. Plasma proteins include albumin (60% of plasma proteins), globulins (36% of plasma proteins), clotting proteins (4% of plasma proteins), and others including metabolic enzymes and hormones.[45] Except for bloodborne hormones and gamma globulins, most plasma proteins are produced by the liver. The composition of plasma varies continuously as substances are added to or removed from the blood by cells. Plasma without clotting proteins, such as fibrinogen, is called serum. Many components drifting along in blood may help to defend the body against foreign invaders, such as bacteria and viruses. Although other samples (e.g., urine, saliva, stool, and sweat) can be used, blood plasma and serum are the most universal and preferred samples for viral infectious disease diagnosis. Despite its complexity, the blood is a stable environment compared to saliva or urine. The human body regulates the balance of blood components to maintain a physiological steady state. However, virus related components can be extremely rare in blood.[4,23] This is the fundamental challenge for virus and viral biomarker detection in blood.[73]

Current diagnostic tests for viral infections include virus isolation and direct detection, identification of viral nucleic acids or antigens, and serological tests involving of the detection of virus-specific antibodies.[22,27,38] Virus can be expanded in a viral culture *in vitro*, where a small sample of tissue or suspicious fluid is inoculated in a special tube along with a monolayer of a relevant cell line. Viral cultures usually require a minimum of 2–10 days to provide statistically significant information.[67] Virus identification can be confirmed by either immunofluorescence detection or hemagglutination assay. Although the diagnostic approach of viral isolation is labor-intensive and time-consuming, it still remains necessary as it is the only technique that provides a viable isolation that can be used for further characterization, such as phenotypic antiviral susceptibility testing.[71] Additionally, rapid viral nuclei acid or immunoglobulin (Ig) protein tests are commercially available. These commercial kits conduct DNA or RNA extraction from viral tissue or blood samples followed by molecular detection by immunofluorescence staining using monoclonal antibodies, polymerase chain reaction (PCR) and gel electrophoresis.[39] Immunoassays, such as enzyme-linked immunosorbent assay (ELISA), are used in the detection of endogenous virus-specific IgM antibodies, which are produced to combat viral infection.[18] Newer diagnostic techniques, such as real-time quantitative PCR (qPCR) assays, are also applied in viral detection. qPCR detection offers high specificity and sensitivity by targeting a particular fragment region of a particular viral genome.[44] However, each of the abovementioned qPCR and ELISA methods consists of series of steps, including sample preparation, incubation, purification, amplification and detection. Thus, in order to avoid false results, not only are highly trained personnel required but also specialized laboratory facilities.

According to the College of American Pathologists, point-of-care testing refers to "tests designed to be used at or near the site where the patient is located, that do not require permanent dedicated space, and that are performed outside the physical facilities of the clinical laboratories." As learned from historical infectious disease outbreaks, death and resource consumption can grow exponentially without effective control.[7,11,16] The World Health Organization (WHO) has developed the ASSURED criteria for a successful point-of-care platform, which is expected to satisfy a set of requirements, including affordability, sensitivity, specificity, user friendliness, rapidity, robustness, equipment-free, and deliverability.[28,43] However, several challenges exist in developing technologies for viral analysis in blood samples. At the early stage of the viral infection, the concentration of the target virions or biomarkers is usually extremely low.[20,64] Thus, high sensitivity and specificity are required for virus detection systems. To improve the signal-to-noise ratio, enrichment is normally needed before detection to capture virus or viral related biomarkers while removing the majority of the interfering components. Also because of the rarity of the targets, a sufficient sample volume is necessary to guarantee an adequate number of targets in the sample (a theoretical limit being one target molecule or particle). The sample volume for a microfluidic device is normally limited to the microliter range. If the required sample volume is large, either the device will be saturated rapidly or the processing time will be unrealistically long. In addition when real-world samples are to be used, the world-to-chip interface problem, including sample loading and on-chip manipulation, is an obviously weak link needed to be strengthened.[46] Analysis speed is also a critical factor for testing infectious diseases. It is ideal to receive answers within minutes after samples are introduced. Automation can be important to improve speed and provide the user with a friendly experience.

Several groups have published review articles on point-of-care infectious disease diagnosis. For example, Lee *et al.*[36] reviewed nano/microfluidics for infectious disease diagnostic techniques with a focus on

on-chip detection, imaging and counting. Chin et al.[8] summarized current point-of-care devices that specialize in fluidic handling in resource-limited settings. The unique needs and design rules in the developing countries were emphasized. Park et al.[56] discussed microfluidics platforms for disease diagnosis using on-chip PCR. Innovations in consumer electronics may have a profound impact on point-of-care diagnosis. Thomas et al.[72] discussed how consumer electronics could affect point-of-care technologies and proposed potential integrations of the two.

In the subsequent sections, we review the current state-of-the-art technologies of point-of-care devices for analysis of several important viral infectious diseases with a focus on fully integrated systems and blood samples.

## LAB-ON-CHIP INTEGRATED SYSTEMS FOR VIRAL ANALYSIS

In general, viruses are small parasites, ranging from 20 to 200 nm in size, that contain either an RNA or DNA genome surrounded by a protective protein coat or capsid.[3] In order to achieve successful transmission, viruses depend on the complex biosynthetic machinery of host cells. The first step in viral invasion is the attachment to host cells via an interaction with functional surface receptor(s).[42] After attaching to host cells, a virus will release its genome into the cytoplasm through endocytosis or direct membrane fusion. Once inside, the viral genome will work as mRNAs to produce viral proteins that will bind with a number of host cell factors to allow for viral replication and transcription. The viruses can then circulate freely in the blood or they may associate with leukocytes, platelets, or erythrocytes and be harbored by them.[3] In response, the host cells will generate an immune response by producing immunoglobulin proteins specific to that particular virus capsule or envelope proteins. Thus, early detection of immune and viral biomarkers within the blood allows for a window of opportunity to establish the risk of disease in a population and take appropriate action.

In this section, we review some of the recent developments of lab-on-chip integrated systems for viral infectious disease diagnosis, which offer the capability of "sample in and answer out." In these examples, the advantages of lab-on-chip are fully demonstrated and these promising systems should be the trend for the future development of point-of-care devices for viral infectious diseases. We focus on several of the most common viral infectious diseases, namely HIV, influenza, hepatitis, dengue, and SARS. Table 1 summarizes the key features and performance parameters of these lab-on-chip integrated systems.

### Human Immunodeficiency Virus (HIV)

HIV remains one of the world's most significant public health challenges, claiming 1.6 million lives in 2012 according to WHO. HIV-1 is transmitted as cell-free virus or directly between cells via cell-to-cell contacts. However, cell-to-cell transmission between CD4+ T cells is more efficiently and rapidly transmitted and predominantly found in lymphoid tissue, in which the majority of virus resides.[69] Viral entry involves direct fusion of viral and plasma cell membrane, which allows the viral core to enter into the cytoplasm of target cells. First, HIV envelope glycoprotein (gp120/gp41, Env) binds to the CD4 receptor first and then to a chemokine receptor (CXCR4 for X4 HIV strains and CCR5 for R5 HIV strains), which is used by HIV particles to activate the gp41-mediated membrane fusion.[12,31] Once inside, HIV particles may follow different pathways: they can be either secreted, as in the case of transcytosis, degraded, or fused with vesicles to inject the viral core into the cytoplasm and initiate the infectious viral cycle.[6] The cycle involves the synthesis of new HIV particles, using the HIV DNA to direct the synthesis of viral RNA and proteins, assembly of HIV particles in the cytoplasm and finally viral escape from the cell by budding through the cell membrane, often killing the cell in the process. Enzyme immunoassay (EIA) or ELISA tests use blood, oral fluid, or urine to detect HIV antibodies and are the most common tests for HIV screening. Results for these tests can take up to 2 weeks. A positive ELISA result is confirmed by another immunologic assay, the Western blot, which can take up to another 2 weeks.[65] A CD4 count of fewer than 200 cells/mm$^3$ is indicative of advanced disease and is one of the qualifications for a diagnosis of AIDS, or Acquired Immune Deficiency Syndrome.[66]

As one of the most serious viral infectious diseases, HIV diagnosis has been investigated for integrated lab-on-chip systems. Western blotting is one of the standard methods to detect proteins. Hughes et al.[24] integrated western blotting into a microfluidic device, including steps of enrichment, separation, immobilization and antibody probing (Fig. 1a). The device not only successfully integrated a conventional blotting technique from benchtop into a handheld device, but also maintained the comparable performance of 50 pM detection limit and dynamic range of 3.6-log within 1 h. A feasibility demonstration of HIV detection directly performed on patient whole blood was reported by Kim et al.[30] The HIV within a finger-prick patient blood sample was transported into a microfluidic device with neutravidin and anti-gp120 surface coating and incubated for 2 min. The immobilized HIV was labeled with two types of quantum dots

TABLE 1. Lab-on-chip integrated system for virus analysis.

| Target | On-chip process | Power | Speed | Performance | Note | Ref. |
|---|---|---|---|---|---|---|
| **1. Influenza** | | | | | | |
| *In vivo* synthesized RNA of influenza A/C in buffer | qPCR | Electricity | 30-35 min | LOD: 5 copies of RNA | Robust | 59 |
| H1N1 in buffer with magnetic beads labeled | Electrochemical detection, PCR | Electricity | 3.5 h | LOD: 10 $TCID_{50}$ | Inexpensive and disposable | 15 |
| Influenza A in buffer | LAMP | Electricity | 30 min, 1 μL | Accuracy: 90.9% (20/22) | Disposable | 1 |
| Swine influenza virus in buffer | Electrophoresis | Electricity | 6 min, 50 μL | DR: $7.5 \times 10^2$ to $4.5 \times 10^4$ TCID: 50 $mL^{-1}$; DOL: 610 TCID50/mL | Repeatable for 100 assays | 63 |
| H5N1 (Swab sample) | qRT-PCR | Magnetism | 28 min | DR: $10-10^7$ (cDNA copies); LOD: 1–20 ng/μL DNA | On-chip qRT-PCR, faster and cheaper than conventional qPCR | 57 |
| Influenza A (PCR product) | Sequencing | Electricity | 2 h | LOD: 375 fM; Accuracy: 91–94% | Self-contained and automated Inexpensive and disposable | 41 |
| **2. HIV** | | | | | | |
| ALP, AST in whole blood | Colorimetric assay | Power free | 30 min, 10 μL | DR: cover clinical relevant range; LOD: ALP (44 U/L), AST (15 U/L), total serum protein (7 g/L) | Low cost; Meets all ASSURED guideline; Whole blood sample | 76 |
| Diluted human sera with HIV immunoreactivity | Western blotting | Electricity | 10 to 60 min | DR: 3.6 log; DOL: 50 pM | $10^3$ fold reduction in antibody consumption, 48 blotting in parallel, high probing efficiency | 24 |
| HIV in whole blood | Immunological capture | Pump (auto pipette) | 10 min, 10 μL | Staining efficiency: 82.4% | Rapid and handheld; Low cost and disposable | 30 |
| HIV in whole blood | Immunoassay (ELISA) | Hand-push (micropipette) | 15 min, 1 μL | Sensitivity: 98.9–100% (HIV); Specificity: 88.7–100% (HIV) | Patient sample tested on-site, low cost | 9 |
| **3. Dengue** | | | | | | |
| IgG, IgM (Dengue biomarker) in serum | On-chip ELISA | Pump and magnetic field | 30 min, 100 μL | DOL: 21 pg | Rapid diagnosis | 37 |
| Dengue virion labeled with magnetic beads | On-chip flow cytometry | Pump | 40 min, 100 μL | DOL: $10^3$ PFU/mL; DR: $10^2–10^7$ PFU/mL | Rapid diagnosis | 79 |
| RNA of dengue virus | Immunological detection | Pump and magnetic field | 15 min | Capture efficiency: 100% (beads) | 100% waste disposal, easy manipulation | 81 |
| **4. Hepatitis** | | | | | | |
| Immunoglobulin of Hepatitis C in serum | On-chip ELISA | Electricity | 20 min | Sensitivity: 96.7%, Specificity: 100%; SD: ±7% | Rapid, cost-effective, user friendly | 32 |
| Antibody and antigen of hepatitis B in whole blood | On-chip ELISA | Centrifuge force | 30 min, 150 μL | DR: 0.51–5 ng/mL (HbsAg), 8.6–480 mIU/mL (Anti-HBs); LOD: 0.51 ng/mL (HbsAg), 8.6mIU/mL (Anti-HBs) | Portable and fully automatic; Reduced ELISA time from 2 h into 30 min | 35 |
| **5. SARS** | | | | | | |
| SARS coronavirus in PCR buffer | PCR and microarray | PCR machine | Several minutes after PCR | LOD: $10^2$ copy/μL; No false negative or positive (86/86) | Also demonstrated detection of influenza A/B and enterovirus | 40 |
| RNA of SARS patient lung tissue in buffer | PCR and electrophoresis | Electricity | 50–60 min | Accuracy: 17 out of 18 of SARS patients | Rapid and labor-saving | 83 |
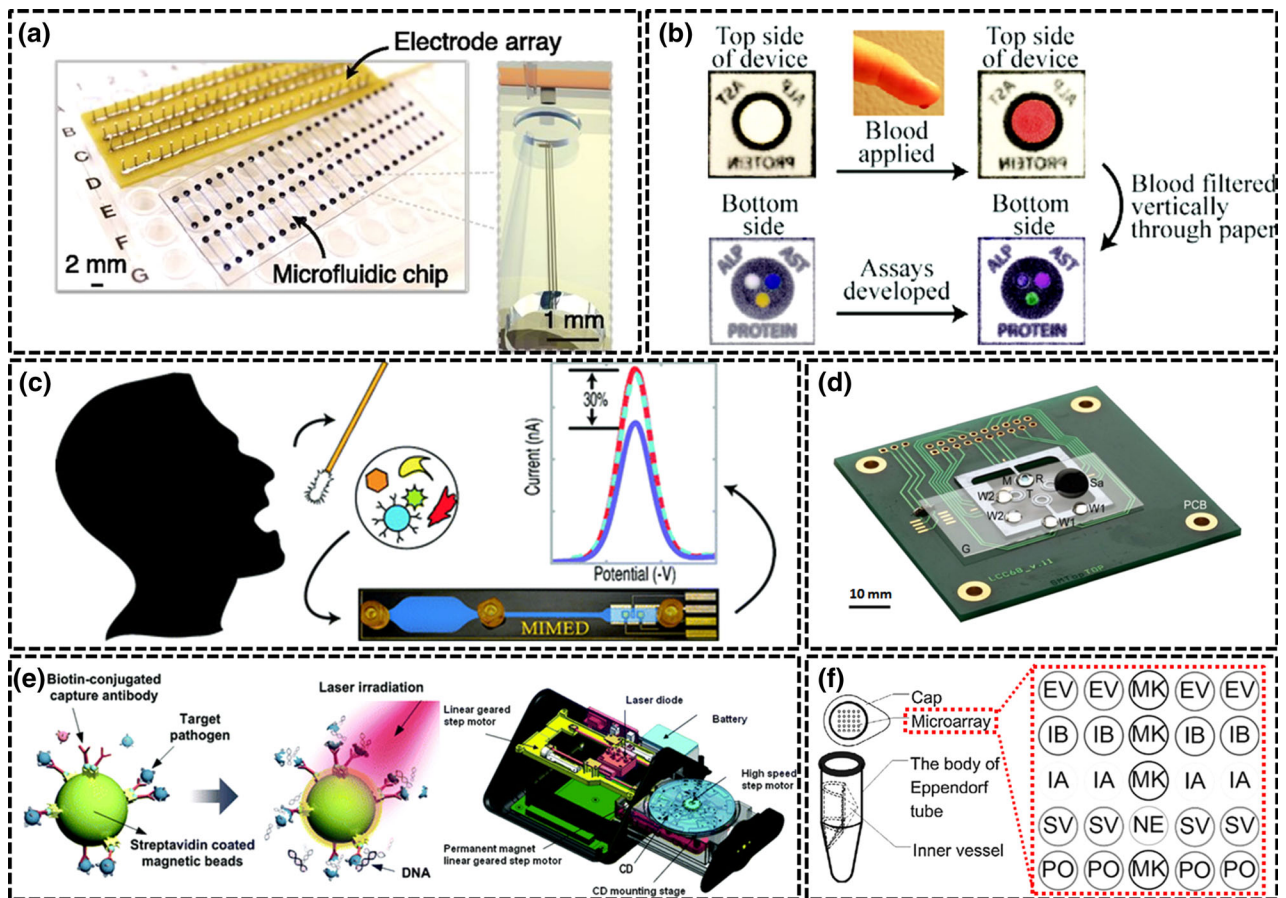
FIGURE 1. Lab-on-chip integrated systems for point-of-care virus analysis. (a) Microfluidics western blotting for HIV diagnosis.[24] (b) Power-free paper-based liver function marker testing assay for HIV and tuberculosis patients.[76] (c) Diagnostic platform for throat swab sample containing H1N1 influenza virus.[15] (d) Droplet manipulation device for real-time PCR virus detection.[57] (e) Hepatitis B DNA detection inside fully automatic CD-format platform.[10] (f) Virus DNA microarray inside engineer-modified Eppendorf tube.[40]

(Qdot525/Qdot655) of different colors by biotinylated antibody and lectin. This dual labeling process made direct observation possible under a fluorescence microscope at 10× magnification. In the paper, the device design made the operation relatively easy by using only an electronic auto-pipette for loading of sample and other reagents. An alternative possibility of a manual process was considered in practical on-site detection. Paper-based microfluidic devices were reported for monitoring of real-time liver function for HIV patients under drug treatment (Fig. 1b).[58,76] Levels of serum transaminases such as, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) can be determined, which are important for patients during anti-viral therapies for tuberculosis and/or HIV. The device is a stack of multiple layers: a top protecting laminated polyester film with a central hole for blood sample introduction, a plasma separation membrane to separate blood plasma from blood cells, and two layers of patterned hydrophilic paper for reconstituting dried reagents and detecting specific

targets. The paper (Whatman No. 1 chromatography paper) is patterned using a wax printer (Xerox 8560DN) for aqueous flow guidance. Three colorimetric assays are created at three different locations on the paper. A pressure-sensitive adhesive is applied to the paper by screen-printing and various layers are bonded with a benchtop laminator. A total of 25–50 $\mu$L blood or serum samples can be loaded and the assays can be finished in 15 min. The performance of the device and the overall assay are comparable to automated instruments. The device is considered a "green" chip, i.e., after use it can be disposed by incineration to avoid any hazardous waste and contamination to the environment. Chin et al.[9] developed an "mChip" assay that performed clinical HIV and syphilis diagnosis in Rwanda. They miniaturized benchtop ELISA into a multi-tasked microfluidic device that was functional for resource-limited settings. By utilizing polystyrene and cyclic olefin copolymer as device materials, the device manufacture can be scaled up to a throughput of about one chip every 40 s, and

material cost as low as \$0.10, while maintaining high fabrication precision (~1 $\mu$m). They developed a bubble-based method to enable automated delivery of multiple reagents for multistep reactions during the on-chip ELISA. The signal amplification and detection took place inside meandering channels where silver ions were reduced onto gold nanoparticles and formed immunosandwich complexes. The density of silver/gold complexes can be measured through light-emitting diodes and photodetectors, which were incorporated into an inexpensive (\$6.50 per unit) and user-friendly compact device. Jordan *et al.*[25] designed another platform for resource-limited settings. By using only a magnet, a heat block, and pipettes, HIV could be diagnosed using dried blood spot (DBS) samples from infants through a helicase-dependent amplification (HDA) assay.

### Influenza (the Flu)

Influenza causes annual epidemics that peak during the autumn and winter seasons in temperate regions. Illnesses result in hospitalizations and deaths mainly among high-risk groups, including the very young, elderly or chronically ill. Worldwide, these annual epidemics result in about three to 5 million cases of severe illness, and about 250, 000–500,000 deaths.[54] An epidemic can take an economic toll through lost workforce productivity due to absent workers and strain on health services. There are three types of seasonal influenza—A–C. Type A influenza viruses pose the most serious threat among the three subtypes and are further categorized into subtypes according to different kinds and combinations of virus surface proteins. Among many subtypes of influenza A viruses, H1N1 and H3N2 are circulating among humans. Two major surface spike glycoproteins, hemagglutinin (HA) and neuraminidase (NA), are present on the surface of the virus. HA is responsible for mediating entry into target cells *via* the host cell receptor, sialic acid (SA) whereas, NA plays a major role during the budding process by releasing progeny virions from the host cell.[47] Although most avian influenzas do not infect humans, the H5N1 strain can cause severe disease in people with the majority of human cases having been associated with direct or indirect contact with infected live or dead poultry (WHO). Studies focusing on seasonally circulating H1 viruses found avian and human viruses differ at two positions in the surface HA protein that is responsible for binding SA.[47] While clinical manifestation serves as a means of tentative diagnosis, confirmative tests such as viral isolation, serological tests, rapid antigen tests and molecular tests[55] are implemented to differentiate the possibilities of other viral or bacterial infections.

With yearly seasonal and global epidemics, influenza is another virus that has been receiving wide attention in point-of-care diagnosis. Prakash *et al.*[59] developed handheld electro-actuation based droplet microfluidics (DMF) with resistive micro-heaters and temperature sensors for influenza virus q-PCR detection. The hydrophobic and circuit-integrated surface was able to manipulate droplets containing RNA and qPCR reaction mixture. Compared with conventional real-time reverse transcription polymerase chain reaction (qRT-PCR) equipment (Applied Biosystems 7500), the device had comparable processing time (30–35 min) and acceptable efficiency (94.4–96.5%) to detect *in vitro* synthesized influenza RNA. The detection limit of the chip-based qRT-PCR technique was about 5 copies of template RNA per PCR reaction. A point-of-care platform was developed by Ferguson *et al.*[15] for influenza (H1N1) detection (Fig. 1c). The sample was prepared with mixing the swab sample with antibody modified magnetic beads and incubating off-chip. Inside the mixture, the targeted H1N1 virus was captured by the magnetic beads modified with an antibody specific for this virus. The mixture was loaded into the microfluidic system and a sequence of procedures including purification, PCR amplification and electrochemical detection were performed. In the upstream region of the device, the virus/magnetic bead complex was concentrated and immobilized at the surface of the substrate by using external magnetic field. Meanwhile, washing media and RT-PCR reagents were added sequentially into the PDMS defined microfluidic device to wash out the unbound supernatant and perform RNA amplification. After 150 min, the products of RT-PCR were transported into the downstream part of the device for electrochemical detection with a detection limit of 10 $TCID_{50}$. Abe *et al.*[1] integrated a portable and easy-to-use influenza detection system. Using loop-mediated isothermal amplification (LAMP), the integrated system was able to detect influenza A within buffer solution in 30 min with 90.9% accuracy. Reichmuth *et al.*[63] concentrated and detected influenza virus on a polyacrylamide gel by performing electrophoresis on-chip. The virus sample was mixed with antibody and loaded into a microfluidic device by the electrophoresis using external platinum electrodes. The virus sample was purified and concentrated inside the polyacrylamide gel and detected by epifluorescence microscopy. With less than 50 $\mu$L sample, the total process was completed in less than 6 min with a detection limit of 610 $TCID_{50}$ mL$^{-1}$. A droplet manipulation platform for avian flu detection was developed by Pipper *et al.*[57] (Fig. 1d). A total volume of only 100 nL was needed, including sample droplets and reagents. A droplet containing RNA magnetic particle complexes was placed on a perfluorinated

surface. By manipulating the droplet with an external magnetic field, the processes, including purifying, washing and thermal cycling, were performed. The RT-qPCR analysis procedures were applied to on-chip target RNA detection. Because the volume of the droplet was only several microliters, the reaction time and reagent consumption could be decreased. Their results showed the H5N1 in a throat swab sample could be detected in 28 min with PCR efficiency of 93%, which was comparable to off-chip procedures, but 400% faster in process time and 2000–5000% cheaper in cost. An on-chip virus sequencing device is integrated by Liu *et al.*[41] A combiMatrix DNA microarray was integrated into a microfluidic device with automatic solution handling, mixing, and flow transport. The device is compatible with a fluorescence scanner. They demonstrated on-chip sequencing of the RNA of influenza A virus (5–20 ng/$\mu$L) in 2 h.

### Hepatitis (HAV, HBV, HCV)

Viral hepatitis is a group of infectious diseases that affects hundreds of millions of people worldwide.[53] Hepatitis A–C (HAV, HBV and HCV, respectively) all directly infect hepatocytes, triggering similar acute disease in symptomatic patients although displaying different modes of infection and levels of pathogenicity.[13] Hepatitis B and C, which can lead to chronic hepatitis, are particularly prevalent with 240 million people chronically infected with hepatitis B and 184 million people with antibodies to hepatitis C.[53] Partly as a result of a successful vaccine, the cell entry mechanism of HAV is poorly understood and cannot be inferred from other picornaviruses due to its atypical characteristics and the diverse entry modes used by other members of the family.[68] Incoming HBV virions are bound by cell-surface receptors, the identity of which still remains unknown.[17] HCV envelope glycoprotein E2 interacts directly with two receptors: CD81 and SR-B1 and requires tight junction components, claudin-1, and occludin to permit HCV entry.[19] Blood samples are tested for hepatitis B surface antigen, HBeAg antibodies against HBeAg, IgM antibody to hepatitis B core antigen (IgM anti-HBc) by ELISA for clinical diagnosis. The presence of hepatitis B surface antigen in the serum for 6 months or longer is indicative of chronic hepatitis B infection.[34] Similarly, hepatitis C core antigen (HCVcoreAg) and anti-HCV antibodies are tested by ELISA.[77] Both HBV and HCV diagnosis may involve a biopsy sample for initial assessment of the severity of the disease.[2]

Complete lab-on-chip systems have been reported for hepatitis diagnosis. A fully automatic system was developed by Lee *et al.*[35] for hepatitis B detection. The

device was capable of ELISA analysis from 150 $\mu$L of whole blood. The CD-format platform generated centrifugal force for transport of both the sample and the required reagents. The device had comparable performance with conventional ELISA in detection limit and detection range, but the processing time was reduced from 2 h to 30 min and required only half the sample (Fig. 1e).[10,35] Kraus *et al.*[32] performed on-chip ELISA for hepatitis C (HCV) detection with signal enhancement technique. The target anti-HCV core immunoglobulin was captured on a gold electrode. The signal amplification was enabled by enzyme labeling with a "Single Electrode Redox Cycling" process. This approach improved the signal-to-noise ratio by 40 fold and processing time by 9 fold compared to standard oxidation and microliter plate based ELISA. The sensitivity was comparable to conventional ELISA with only 30 nL of expensive antigen needed in contrast to 50 $\mu$L in a conventional ELISA.

### Dengue Fever (Breakbone Fever)

Dengue, a mosquito-borne infection, is found in tropical and sub-tropical regions around the world. However, in recent years, transmission has extended to urban areas and has become a major international public health concern.[50] WHO currently estimates that 50–100 million dengue infections occur worldwide every year. Although closely related, four distinct serotypes of the virus can cause dengue (DEN-1–DEN-4). Upon being bitten by an infected female *Aedesaegypti* mosquito, virions attach to the host cell surface receptors and enter the cell *via* receptor-mediated endocytosis.[75] Fusion of the viral and cellular membrane will occur as a result of a conformational change of the E protein on the viral surface triggered by the acidic environment of the endosomal vesicles.[21,80] The viral DNA is then released into the cytoplasm, and the viral genomic RNA is translated. Blood samples are tested for IgM antibodies to dengue virus envelope protein antigen (DENV1-4) by ELISA for clinical diagnosis.[61]

A magnetic bead based approach was developed by Lee et al. for dengue virus detection. Both dengue virion and specific immunoglobulin detection were demonstrated.[37,79] For dengue virion detection, the virus suspension was mixed with magnetic beads inside a micromixer. Then, the unbound substances were washed off while a magnetic field was applied. The virus/magnetic bead complexes were released and transported by the micropump for flow cytometer detection. The immunoglobulin was detected by on-chip ELISA after immobilization by magnetic beads with a detection limit of 21 pg in 30 min. Zaytseva *et al.*[81] developed a device for dengue RNA detection.

Targeted dengue RNA bound to both magnetic beads and liposomes through DNA capture and reporter probe, respectively, inside the microfluidic channel. The liposome served as a fluorescent signal amplifier during detection. The process was operated under a continuous flow of 80 $\mu$L/min and could be completed in 15 min.

### *Severe Acute Respiratory Syndrome (SARS)*

SARS is a viral respiratory illness, which is caused by a coronavirus and infected a total of 8098 people in more than two dozen countries in North America, South America, Europe, and Asia during the 2003 epidemic.[62] The viral envelope glycoprotein Spike (S) binds to the host cell ACE2 receptor and triggers a conformational change in the S protein, which leads to cleavage of the S protein by host cell proteases and viral infectivity.[48] Unlike many other viruses previously discussed, SARS is a new disease in humans. The ELISA test, which is commonly used for detection of most viruses has been reported to inconsistently detect antibodies to SARS before day 10 or 20 after the onset of symptoms.[82] Laboratories test for SARS using reverse transcription (RT)-PCR assays to amplify the replicase 1 AB/polymerase gene of the coronavirus from various samples including blood, stool, respiratory secretions or body tissues.[51,52] The symptoms of the early stages of SARS are similar to those of influenza, which can result in misdiagnosis.[74] Consequently, PCR is the standard method for a rapid and accurate SARS diagnosis, which is necessary based on the high morbidity and mortality rate of the disease.[33]

Zhou *et al.*[83] integrated PCR into a microfluidics device for SARS diagnosis. The sample was manipulated *via* capillary electrophoresis and the thermal cycling was performed by Peltier devices. The PCR product was analyzed off-chip. An engineered Eppendorf tube was developed for SARS detection (Fig. 1f).[40] The tube can perform PCR and DNA microarray in one batch without re-opening the tube cap. The DNA microarray was patterned on the Eppendorf cap. After the PCR was completed, the tube was turned over to start the reaction with the pre-patterned microarray. In addition to SARS, the device also demonstrated detection of influenza A/B and enterovirus. No false positive or false negative diagnosis was identified.

### CONCLUSION AND FUTURE DIRECTIONS

With the possibility of sudden viral infectious disease outbreaks and consequent global spread leading to devastating impacts on both society and the economy,

there is pressing need to develop new methods for early diagnosis of the pathogen, affordable and effective population screening, and real-time treatment monitoring. Point-of-care microdevices demonstrate the potential to fulfill the ASSURED criteria of being sensitive, specific, low-cost, rapid, portable and accessible. In the past decades, lab-on-chip technologies for viral infectious diseases have been developed actively from single device components to complete systems on-chip. They are promising approaches to change clinical practices for viral infectious disease management in the near future.

Although lab-on-chip microdevices have been explored for viral infectious diseases, there are still significant challenges to overcome as well as opportunities that are worthwhile to investigate. Before testing with clinical samples, in addition to pure samples in water or PBS, the devices need to be validated with artificially constructed samples (i.e., target of known concentration spiked into blood plasma/sera of healthy donors) under controlled conditions. Success in artificially constructed samples is a critical milestone in the technology development. A large dynamic range of viral biomarkers in artificially constructed samples should be evaluated to ensure that both clinical sensitivity and specificity can be achieved. Next, clinically derived samples and clinical studies with microdevices are required for translation of the technology into medical practice. When compared with the artificial spike-in sample, clinical patient samples have a higher degree of uncertainty in target concentration and interference species. For example, the differences of the targeted virus/biomarkers or immunoglobulin concentration are less predictable among human subjects because of the variations in immune responses, diet preferences and treatment history among patients. Thus, better performance in sensitivity, specificity and dynamic range might be required for the microdevices to avoid high false-positive and false-negative results in clinical testing. The same argument favors testing with a large number of clinical samples and statistical data analysis. However, there are also non-technical hurdles important for clinical studies, distribution and commercialization of the point-of-care technologies. Most of the current devices are prototypes that are fabricated inside research labs. In order to be fully utilized on a daily basis and widely accepted in most clinical settings, the prototypes must be further developed into consumer products. For example, the fabrication process needs to be transformed into batch process and it is necessary for the device and instrument to be reliable, robust, automatic and user-friendly. It is likely this will be a joint effort of academia, industry and government. The overall process also depends on the medical needs and readiness, commercialization

strategy, and market acceptance, in addition to the technology itself.

In the near future, based on the progresses being made among various research fields, we can imagine more detailed analyses beyond simple viral detection. For example, the genomic sequence of a virus can be determined rapidly on-chip to identify the virus and track its mutation and evolution. Similar to bacteria culture and antibiotic screening, personalized drug response tests for viral infectious diseases may improve the efficacy of the viral drug treatment. On the technological side, new functional materials such as carbon nanomaterials or biodegradable polymers can potentially break the barriers to achieve higher sensitivity, selectivity and speed. In addition, more efficient miniaturization of macroscopic viral detection systems may provide a shortcut to improve performances in the micro and nano scales. Today, the development of point-of-care microdevices for viral infectious diseases is an active field. If its promises are realized, it can potentially prevent virus spread and significantly improve the clinical diagnosis and treatment of viral infectious diseases.

## ACKNOWLEDGMENT

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