Analytical Methods

ROYAL SOCIETY OF CHEMISTRY

TUTORIAL REVIEW

View Article Online
View Journal | View Issue

Cite this: *Anal. Methods*, 2021, **13**, 740

Hepatitis C virus (HCV) diagnosis *via* microfluidics

Vigneswaran Narayanamurthy, *[a] Z. E. Jeroish,[b] K. S. Bhuvaneshwari[c] and Fahmi Samsuri[b]

Received 4th November 2020
Accepted 12th December 2020

DOI: 10.1039/d0ay02045a

rsc.li/methods

Humans are subjected to various diseases; hence, proper diagnosis helps avoid further disease consequences. One such severe issue that could cause significant damage to the human liver is the hepatitis C virus (HCV). Several techniques are available to detect HCV under various categories, such as detection through antibodies, antigens, and RNA. Although immunoassays play a significant role in discovering hepatitis viruses, there is a need for point-of-care tests (POCT). Some developing strategies are required to ensure the appropriate selection of POCT for HCV detection, initiate appropriate antiviral therapy, and define associated risks, which will be critical in achieving optimal outcomes. Though molecular assays are precise, reproducible, sensitive, and specific, alternative strategies are required to enhance HCV diagnosis among the infected population. Herein, we described and assessed the potential of various microfluidic detection techniques and confirmatory approaches used in present communities. In addition, current key market players in HCV chip-based diagnosis and the future perspectives on the basis of which the diagnosis can be made easier are presented in the present review.

Introduction

Hepatitis C virus

A virus is an infectious agent that incorporates into body cells and is programmed to proliferate rapidly. The hepatitis C virus (HCV) is a 50–60 nm long single-stranded RNA virus, as shown in Fig. 1(a and b) that causes liver disease, followed by cell damage. However, the virus itself does not cause the most considerable damage to the liver. A significant damage to the liver is attributable to inflammation, which develops through the body's immune response to fight the virus. When the body begins to fight the disease, it causes cell damage and ultimately leads to fibrosis, where hard but non-functional fibrous tissues replace the liver cells. The HCV infection may be symptomatic or asymptomatic but both lead to chronic infection. In chronic hepatitis C, the damage caused by inflammation is sufficient to harm the liver but unfortunately, it is not adequate to clear the virus entirely. This means that inflammation continues for a long time, engendering ultimate tissue damage and less viral clearance. Hepatitis C may eventually cause the cirrhosis of liver or even hepatocellular carcinoma (HCC), if left unchecked.[13] Fig. 1(c) depicts the progression of HCV infection. Patients with chronic hepatitis C (CHC) are at high risk of life-threatening complications, including cirrhosis in 20% of the cases and HCC at an incidence of 4%–5% per year in cirrhotic patients.[14–18] Epidemiological reports suggested that HCV is related to several extrahepatic manifestations, including resistance to insulin, type 2 diabetes mellitus, glomerulopathies, and oral manifestations.[19–22] However, most patients with HCV infection could advance to chronic hepatitis but about 15%–40% of them spontaneously clear the virus.[27]

Statistics

Globally, 71 million people were estimated to have CHC infection (WHO). In 2016, there were approximately 399 000 notified HCV global mortalities, mostly due to cirrhosis and HCC. Estimates have shown that over the years, somewhere between 435 000 and 500 000 HCV cases have accumulated in Malaysia, accounting for 2.5 percent of the general population (https://www.mac.org.my/v3/the-hidden-epidemic-of-hepatitis-c/, retrieved on March 2019). Acute HCV cases account for about 15%–20% of the total cases. Post-acute HCV infected patients have 50–80% chances of developing chronic infections. Approximately 170 million people across the world have been infected with HCV.[29] The Center for Disease Control and Prevention (CDC) estimated that only 30%–50% of Americans are aware of HCV infection. Despite 3.2 million of the living population with CHCV infection, 50%–70% are unaware of their health status.[33] HCV infection is an increasing problem in Malaysia, as more people are tested for HCV antibodies. In Malaysia, approximately 453 700 people were infected with HCV in 2009 (2.5 percent of the population age of 15–64); 59% of them are infected by injecting drugs. However, there is little

[a]*Fakulti Teknologi Kejuruteraan Elektrik dan Elektronik, Universiti Teknikal Malaysia Melaka, Hang Tuah Jaya, 76100 Durian Tunggal, Melaka, Malaysia. E-mail: vigneswaran@utem.edu.my*

[b]*College of Engineering, Universiti Malaysia Pahang, 26300 Gambang, Pahang, Malaysia*

[c]*Faculty of Electronics and Computer Engineering, Universiti Teknikal Malaysia Melaka, Hang Tuah Jaya, 76100 Durian Tunggal, Melaka, Malaysia*

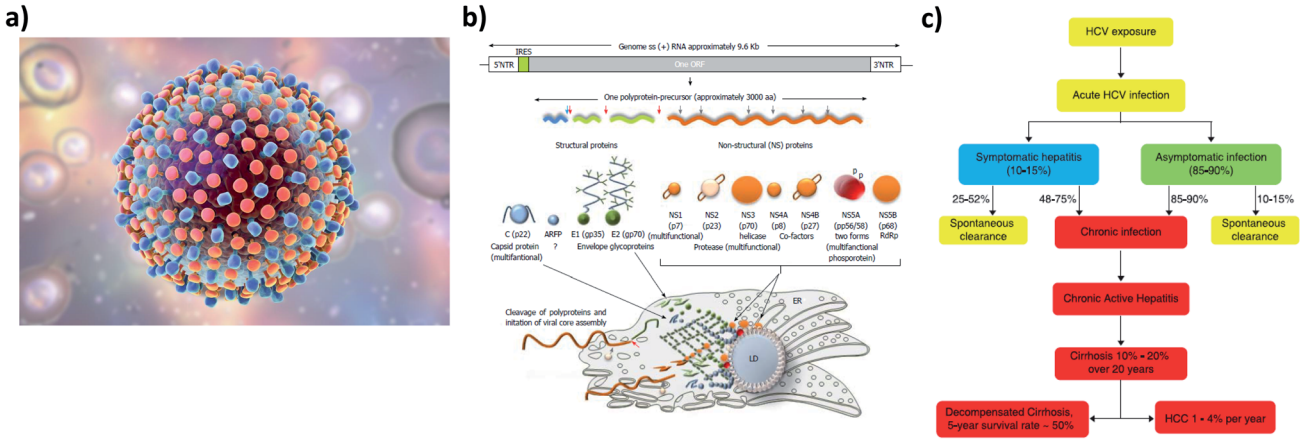

Fig. 1 (a) Structure of HCV (this figure has been adapted from ref. 2 with permission from Healthline Media, copyright: 2017). (b) HCV genome, polyprotein precursor, and initiation of the viral core assembly (this figure has been reproduced from ref. 7 with permission from Baishideng Publishing Group, copyright: 2015). (c) HCV infection history (this figure has been adapted from ref. 12 with permission from Medknow, copyright: 2014).

awareness of hepatitis C in Malaysia.[3,34] In 2000, there were 550 cases of hepatitis C with an occurrence rate of 2.5/100 000 population; in 2004, there were 741 cases with an occurrence rate of 2.9/100 000 population and in the year 2013, the occurrence rate grew to 6.77/100 000, which indicates the rising trend of the disease, as shown in Fig. 2.[3] Genotypes 3 and 1 are the most common genotypes in Malaysia. Many studies have shown that this genotype distribution in Malaysia has remained unchanged in the last 15 years. The prevalence estimated for females is significantly lower than that for males and the estimated number of HCV antibody-positive males in Malaysia is much higher (84 percent) than for the other two ethnic groups. Parenteral drug use (85 percent), blood transfusion (3 percent), and dialysis were identified as the main modes of HCV transmission.[36,37] The other major modes of transmission of HCV are *via* the reuse of medical equipment with inadequate sterilization, therapeutic injections, and blood-exposed sexual practices.[12,39] In the United States, injected drug use is the most common risk factor, accounting for >60% of HCV infections. Unregulated and unlicensed tattoo centers applied by friends or in prison increase HCV acquisition.[41–43] Moreover, non-injecting drug users who sniff or smoke heroin, cocaine, crack, or methamphetamine using pipes that may cause burns in the oral mucosa are susceptible to 2.3–35.3% risk of acquiring HCV.[44] In Malaysia, the National Strategic Plan for hepatitis B and C (NSPHBC) was developed to eliminate hepatitis by 2030 through prevention programs, diagnostic, treatment, and care services.[45–47] The detection of HCV infection at an earlier stage provides several advantages, such early antiviral treatment for the prevention of consequences of the disease and reduction in the risk of infection transmission.[48,49]

Microfluidics

The commonly addressed disadvantage in the conventional technique is that it requires high skill levels and lag portability. The throughputs are low, even when the drawbacks are removed. Trapping and isolation of single cells are the initial steps involved in single-cell investigations. Hence, the intricacies of cells and analytes could be overridden through microfluidics as they can process and analyze the sample (typically between microliter to nanoliter) inside the device and furnish high-throughput.[50] A suitable and specific device dimension is imposed with high precision to accurately manage the sample within the microchannel. In general, the microfluidic channel dimensions are from 10 to 100 μm with promising application in biological and chemical analysis in order to assure every need of research with accurate results.[51–54] The micro-level dimensions of the flow channels used in the biochip equal the size of the biological cell samples.

Microfluidic devices currently play an imperative role in numerous biological, chemical, and engineering applications, where the necessary channel and feature dimensions are fabricated through multiple methods.[55–58] Commercial microfluidic devices are produced through photolithography, injection molding, and hot embossing.[59] There is an obvious attraction in the single-step fabrication of the entire microfluidic device. Henceforth, 3D printing techniques with rapid realization of the model are potentially accepted.[60] As a general rule, choice of the fabrication method is determined by several factors, such as the available technologies and equipment, cost, speed, fabrication capabilities (*e.g.*, desired feature size and profile), and the preferred material substrate.

A variety of materials with properties such as transparency, non-fluorescence, and biocompatibility have been developed to meet the need for microfluidics.[61] A wide range of materials have been employed in the fabrication process, such as silicon, glass, silicone-based elastomers, and PDMS. Hydrogels and plastics are also used in the fabrication of devices for biological assays and commercial implements. Paper, a fabric matrix made of cellulose, is the most recently introduced material in microfluidic chip fabrication.[62] Apart from the quality of the results, the reduction in time, savings in space, reagents, and minimum sample volumes involved (order of microliters) are

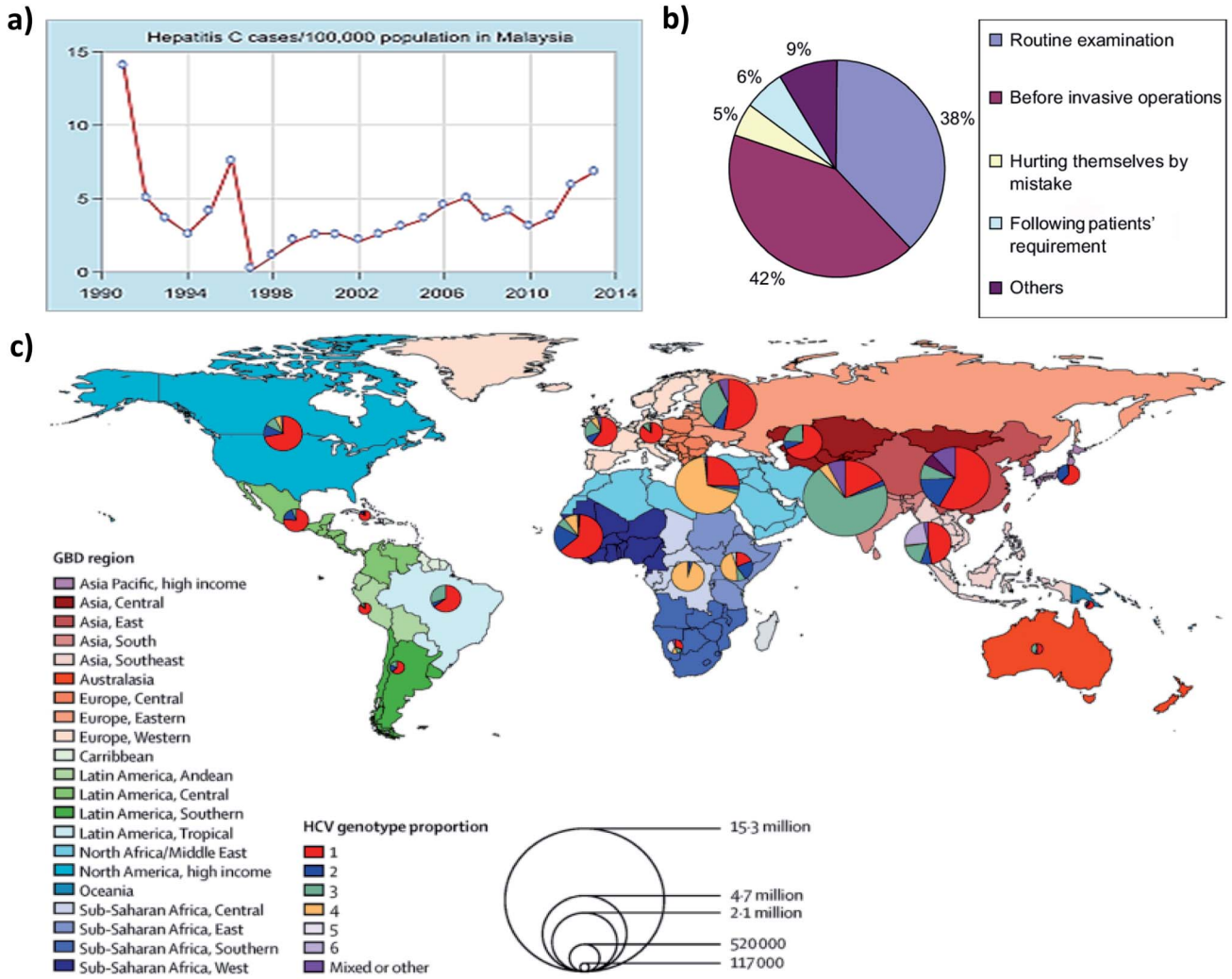

Fig. 2 (a) Graphical illustration of HCV seroprevalence in Malaysia (this figure has been reproduced from ref. 3 with permission from Professional Medical Publications, copyright: 2013). (b) Awareness created for different stages of anti-HCV detection procedures followed in China (this figure has been adapted from ref. 8 with permission from Dove Medical Press, copyright: 2011). (c) Depicts the global distribution of HCV genotypes among people (this figure has been reproduced from ref. 9 with permission from the National Center for Biotechnology Information, copyright 2018).

some of the remarkable advantages of paper-based microfluidics.[63,64] Due to the low fabrication cost, it makes the device easily disposable and eliminates the cross-contamination of the results.[65,66] Microfluidic devices developed to analyze the HCV viral sample loads are antibody cell-based microfluidic systems, a whole-cell pathogen diagnostic approach for detecting hepatitis B virus (HBV), HCV, and human immunodeficiency virus (HIV). Moreover, the microfluidic quantum dot (QD)-based barcodes deliver the multiplex high-throughput detection of HBV, HCV, and HIV.[67] Thus, the microfluidics domain plays a vital role in the development of HCV diagnostic testing kits.

Literature search performed

The literature search was performed using Google Scholar and the investigation was carried out for the terms "hepatitis virus, HCV detection, rapid diagnosis, point-of-care (POC) diagnosis of HCV, and HCV microfluidic chips" for the period 2005–2019. From the search, few relevant articles were handpicked and explained accordingly in the year-wise order. In Fig. 3(a), the bar graph illustrates the widespread publications on HCV detection methods during 2005–2019. The survey outcomes witnessed that more significant research was concentrated on HCV antibody detection than other techniques. In contrast, paper-based detection techniques are only 10% of all the detection methods; hence, further investigations in this domain are essential. This review article will be valuable for researchers who plan to explore the field of HCV diagnosis further.

Patent analysis and current key market players in the field of microfluidic chips for the rapid diagnosis of hepatitis C

A patent analysis was performed in Google Patents search tool, using the keyword hepatitis C virus detection or diagnosis chips, and the key market players are Institute of Basic Medical

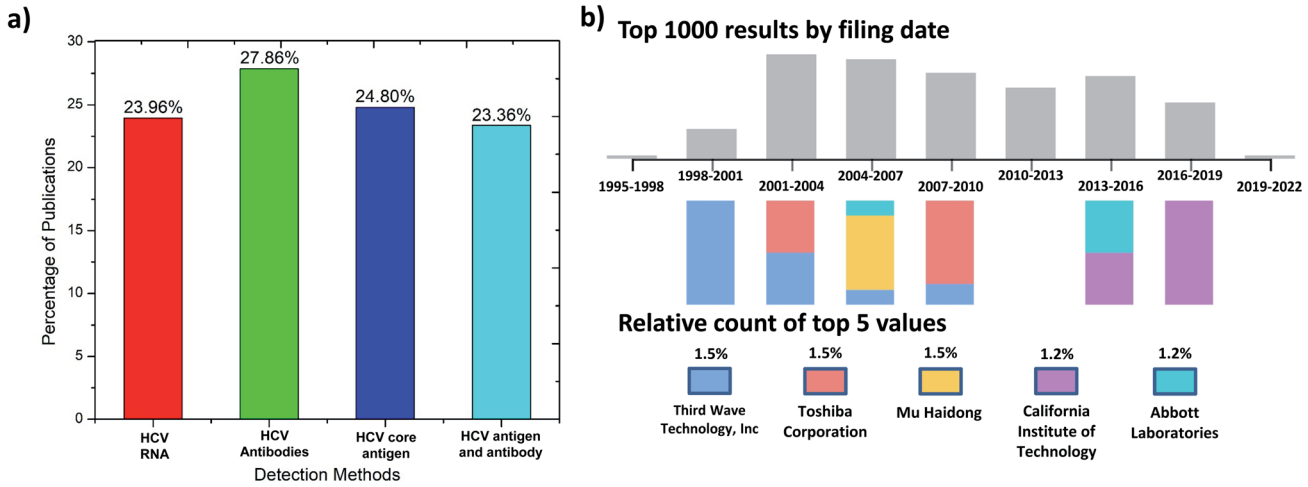

Fig. 3 (a) Percentage of 40 940 publications in HCV detection during 2005–2019; (b) patent analysis on hepatitis C virus diagnostic chips.

Sciences, Chinese Academy of Military Medical Sciences (CN), Shanghai Bohua Gene Chip Technology Co., Ltd. (CN), BioCore Co., Ltd. (US), Fuz Taipu Biological Science CO., Ltd. (CN), Fujirebio (US), Shanghai Fosun Medical Technology Development Co., Ltd. (CN), Shanghai Bohua Gene Chip Technology Co., Ltd. (CN), Bioseum Co., Ltd. (CN), Institut Pasteur (US), Third Wave Technologies, Inc. (US), Eos Biotechnology, Inc. (WO), Fio Corporation (CA,US), ITI Scotland Ltd (CA), Biopico Systems Inc (US), Geneasys Pty Ltd (US), Samsung Electronics Co Ltd (US), Talis Biomedical Corporation (EP), Triad National Security, Llc (US), Nanobiosym, Inc. (US), Credo Biomedical Pte. Ltd (TW), President and Fellows of Harvard College (US), Surnetics, Llc (WO), National Institute of Infectious Diseases (JP), Biological Engineering Co., Ltd. (CN), Abbott Laboratories (JP), Gene Co., Ltd. (CN), Shandong Boke Biological Industry Co., Ltd. (CN), Institute of Chemistry, Chinese Academy of Sciences (CN), Hunan Shengxiang Biotechnology Co., Ltd. (CN), and Shimadzu Research Laboratory Co. Ltd. (CN). The patent analysis of key market players in HCV is shown in Fig. 3(b).

Detection and diagnostic approaches

To diagnose and treat infected persons is the primary intention of viral infection diagnosis. It engenders the prevention of the headway of disease and the virus spread. The onset of HCV infection does not possess any specific indicators since most primary patients are asymptomatic. HCV viremia finds its existence besides the normal level of serum alanine aminotransferase (ALT).[68] Henceforth, various virological methods are practiced to diagnose HCV infection.[69] This article enlightened readers about the detection based on techniques and biomarkers, and has categorized them for better understanding.

Direct and indirect testing methods are the most commonly employed virological approaches to assess viral infections. Fig. 4 depicts the overview of various assays practiced for the diagnosis of HCV infection, which includes microfluidics-based and non-microfluidics-based assays available in recent times.[12,70–75] Indirect detection tests consist of viral-induced antibodies, which include IgM to diagnose recent infections and IgG for current or previous infections. Anti-HCV IgMs are found in 50%–93% of acute hepatitis C patients along with 50%–70% of CHC patients.[76] Hence, in clinical practice, IgM assays are not employed since anti-HCV IgM does not act as a suitable marker of acute HCV infection.[77,78] The protein chip assay (PCA), chemiluminescent microparticle immunoassay (CMIA), enzyme-linked immunosorbent assay (ELISA), lateral flow immunoassay (LFIA), electrochemical immune sensor array, and rapid immune chromatographic assay are some of the assays adapted in antibody detection. The virus isolation, viral antigen detection, and viral nucleic acid detection are the detection parameters of direct tests. The isolation and culture of HCV using clinical specimens is a challenging task at present. However, it can be isolated *via* a traditional single strain isolation methodology. In microfluidics, cost-effective and straightforward single-cell trapping and isolation are available.[79] But currently, it is difficult to isolate and culture HCV using clinical specimens. Hence, HCV viral isolation is in demand for detection, analysis, and research. In RNA detection, magnetic beads, loop-mediated isothermal amplification (LAMP), transcription-mediated amplification (TMA), and reverse transcription-polymerase chain reaction (RT-PCR) are available. Similarly, magnetic microparticle assay, PCR, atomic force microscopy (AFM), and nucleic acid amplification test (NAAT) are adapted in core-Ag detection.

Techniques used for HCV detection

Several techniques are available based on the methods, materials, and equipment used. In this section, the HCV detection techniques based on amplification, rapid immunoassay system, and sensors are enlisted, as shown in Fig. 5. In amplification, non-isothermal amplification and isothermal amplification are available.[80–83] The immunoassay system comprises of lateral flow assays and immuno-filtration assays. Moreover, electrochemical and optical sensors are available for HCV detection.

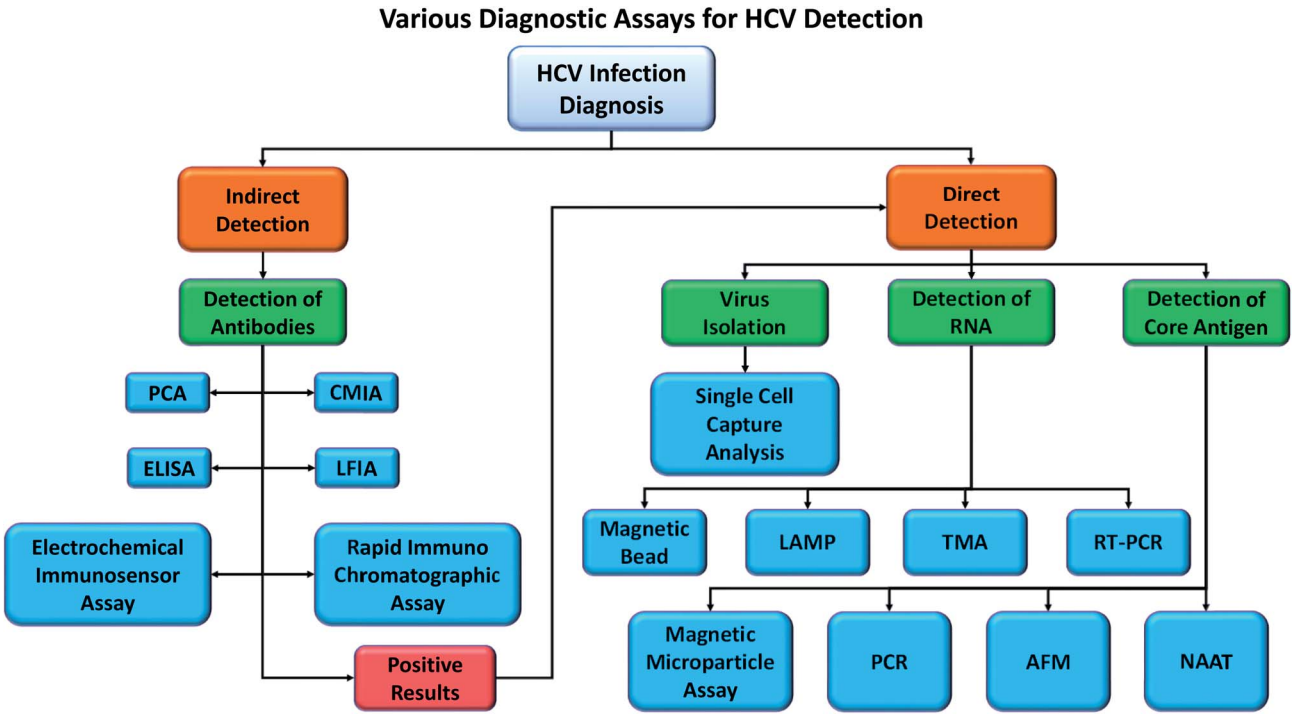

Fig. 4 Overview of various HCV diagnostic assays practiced in recent times.

The illustrations and process of various HCV detection techniques based on microfluidics are shown in Fig. 6.

Non-isothermal amplification

• Reverse Transcription Polymerase Chain Reaction (RT-PCR) is used to convert RNA viral genomes to complementary DNA (cDNA) templates in order to detect RNA expression and clone expressed genes.[84,85] The development of "Digital" single-molecule measurements is attractive for the quantitative measurement of RNA concentration, with typically limited dynamic range. Hence, Shen *et al.* designed and tested two microfluidic rotational slip chip platforms for the quantitative analysis of HCV RNA through multivolume digital RT-PCR with an extensive dynamic range by adding additional wells.[6]

• Real-time PCR is used for the qualitative and quantitative investigation of viral nucleic acids for therapy monitoring.[86] The SYBR Green RT-PCR assay reported by Vázquez-Morón *et al.* showed that the limit of detection (LOD) of HCV is 5000 copies per mL in dried blood spot (DBS) specimens with 100% efficiency. However, it is not an effective screening of HCV due to its very high detection limit (DL).[61] In the field of bedside clinical tests, RT-PCR is not suitable for POCT due to the requirement of a laboratory environment and equipment with trained professionals. Hence, isothermal amplification is brought into existence for addressing these issues.

Isothermal amplification

• Transcription Mediated Amplification (TMA) uses two enzymes, such as RNA polymerase and reverse transcriptase, for rapid RNA/DNA amplification.[87] TMA-based assays are highly sensitive due to their lower DL and can detect the non-detectable HCV RNA by PCR methods to avoid the recurrence of infection.[88]

• Reverse Transcription Loop-Mediated Isothermal Amplification (RT-LAMP) in which LAMP amplifies the DNA with high specificity, efficiency, and rapidity under isothermal conditions, which is combined with the reverse transcription step, is used to detect RNA. Generally, the LAMP reaction necessitates four primers to recognize six different regions of the target DNA.[74]

• Forward inner primer (FIP) comprises of F2 and F1c regions at the 3′-end and 5′-end, respectively.

• Forward outer primer (F3 Primer) contains the F3 region matching the F3c region of the template DNA.

• Backward inner primer (BIP) contains a B2 and B1c region at the 3′-end and 5′-end, respectively.

• Backward outer primer (B3 Primer) contains a B3 region complementary to the B3c region of the template.

Chang *et al.* presented a LAMP-based lab-on-disk optical system that allows the simultaneous detection of viruses through turbidity measurements.[89] It has a wide range of applications in POCT and rapid testing in food and environmental samples.[22]

• Rolling Circle Amplification (RCA) is an isothermal enzymatic amplification method where short DNA or RNA primers are amplified to form a long single-stranded DNA or RNA using a circular DNA template and specific DNA or RNA polymerases.[88] Liu *et al.* observed paper-based RCA with higher efficiency than solution-based RCA. The amplified HCV DNA is detected by conjugating with AuNP for colorimetric visualization.[90] The characteristic feature of amplification techniques for HCV are enlisted in Table 1.

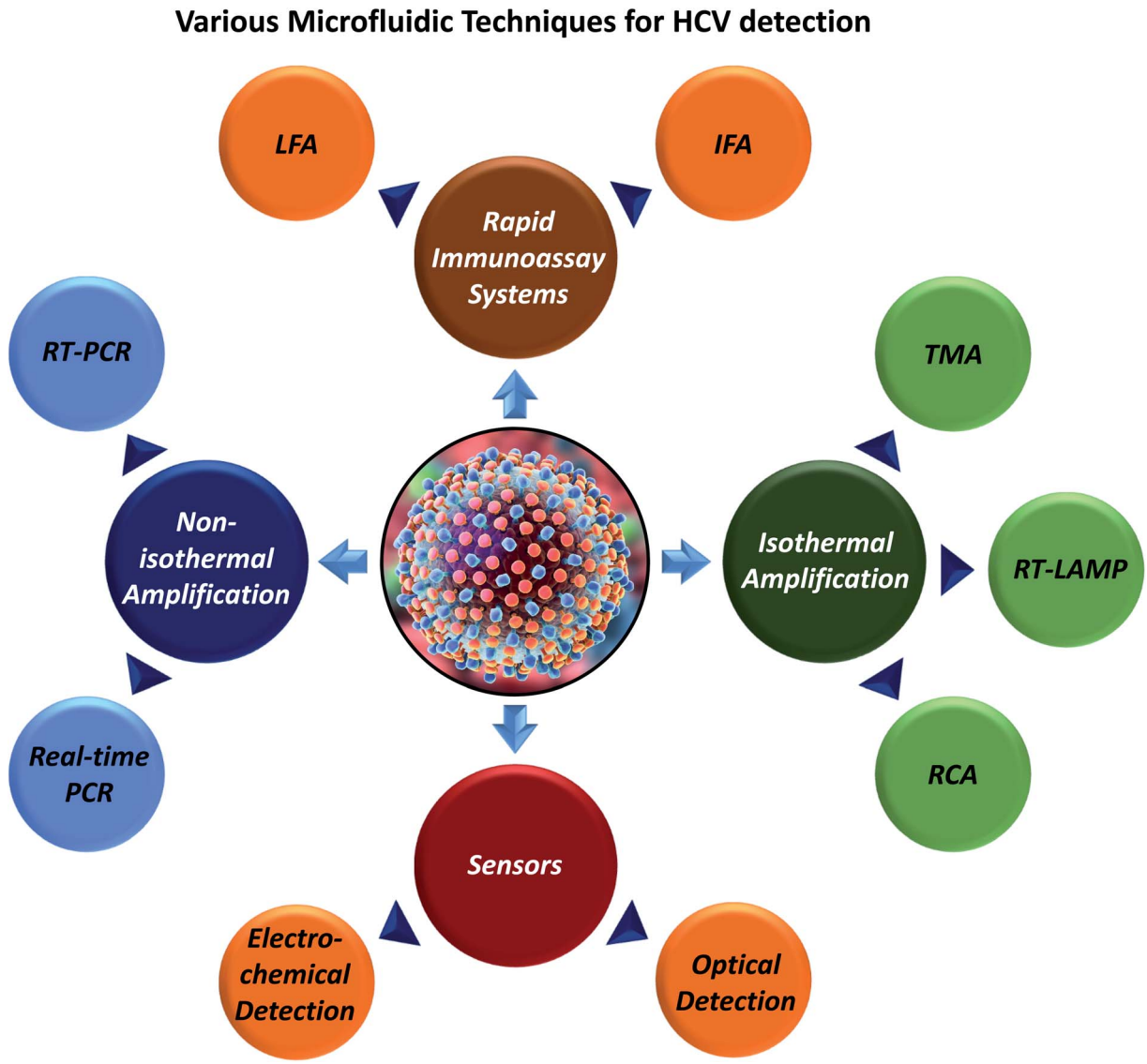

Fig. 5 Various microfluidics-based techniques adapted for HCV detection.

Rapid immunoassay systems

• Lateral Flow Assays (LFA), commonly known as immunochromatographic assays (ICA), are highly adapted for the qualitative analysis of nucleic acids and quantitative analysis of protein for POCT. LFA consist of a sample pad, a membrane on which the antigens/antibodies are immobilized, and an absorption pad over a membrane strip assembled on a plastic backing.[88] The sample containing HCV IgG antibodies binds to protein-A on colloidal gold, which binds to antigens coated on the membrane, thus inducing a color change for detection.[91] In addition to that, Ryu *et al.* integrated a portable fluorescence reader (AFIAS-6 reader) to improve the sensitivity and to carry out multiple simultaneous tests.[1]

• Immuno-Filtration Assay (IFA) is also known as flow-through assay, where specific antigens are immobilized on a porous immuno-filtration membrane for the subsequent filtration and binding of HCV antibodies. The captured antibodies are then combined with antibody-specific IgG to produce distinct colors on the region.[92] Traditional IFAs are commonly qualitative or semi-quantitative and are very restricted for clinical diagnosis. Hence, Zhang *et al.* developed an IFA, based on QDs, capped with both polyethylene glycol (PEG) and glutathione as the fluorescent labels for the quantitative detection of C-reactive proteins, which is a well-known diagnostic marker for acute viral and bacterial infections.[93]

Sensors

• Electrochemical detection is the process of measuring the electrical potential of the sample with the change in the virus concentration.[59] Thereby, Zribi *et al.* integrated electrochemical sensors based on carbon nanotubes with ferrocene as a redox marker for viral pathogenic DNA detection.[94] Later, Aronoff-Spencer *et al.* genetically engineered the yeast cell lines to display HCV core Ag concatenated to the gold binding peptide,

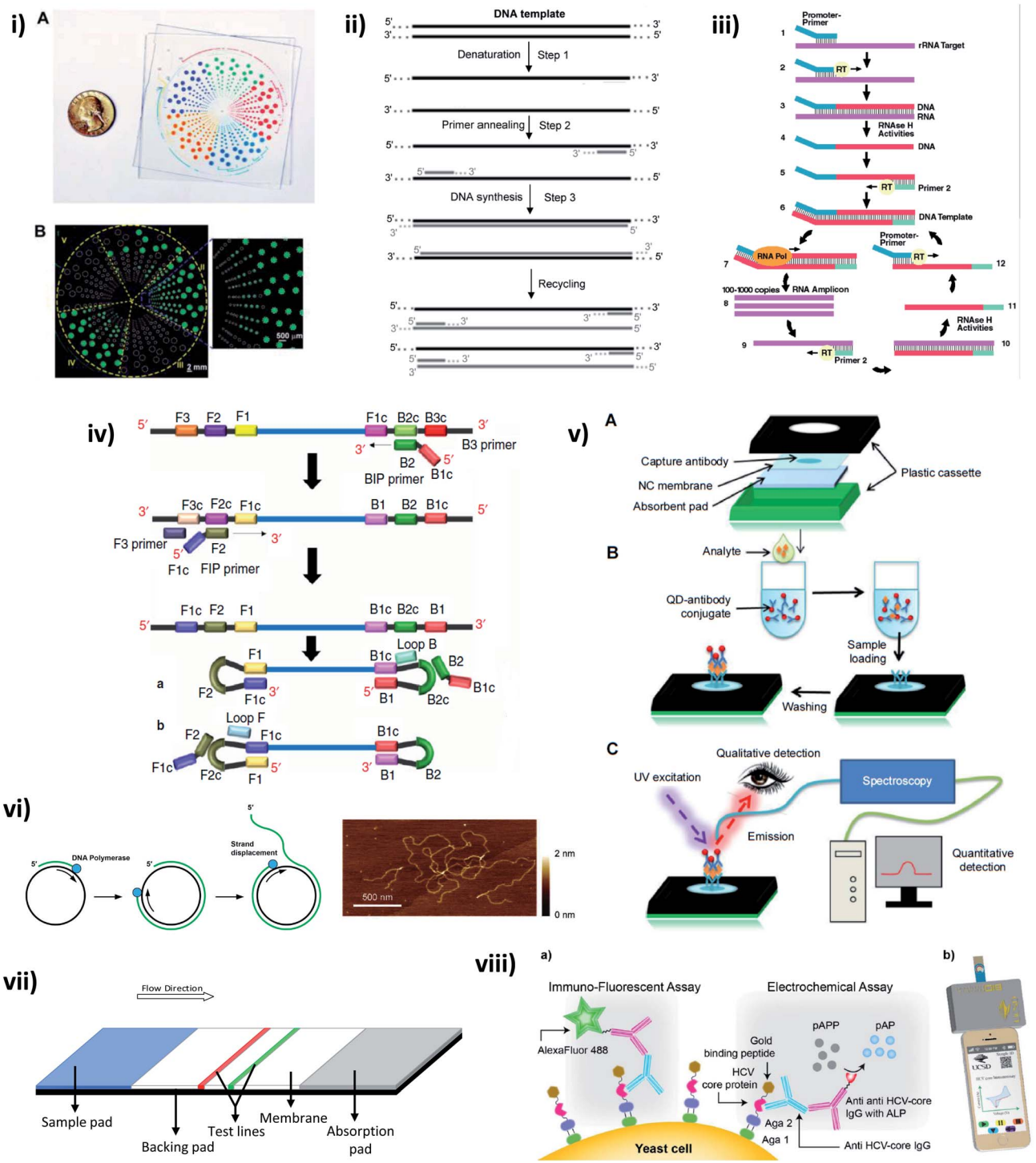

Fig. 6 Illustrations and process of HCV detection techniques. (i) Multivolume Digital RT-PCR (this figure has been adapted from ref. 6 with permission from ACS, copyright: 2011). (ii) Illustrates the process of PCR (this figure has been reproduced from ref. 10 with permission from Taylor & Francis, copyright: 2008). (iii) Portrays the process of TMA (this figure has been adapted from ref. 23 with permission from Oxford University Press, copyright: 2008). (iv) Demonstrates the process of LAMP (this figure has been reproduced from ref. 24 with permission from Wiley-Blackwell, copyright: 2018). (v) Illustration of the PEGylated QDs-based immuno-filtration assay (this figure has been reproduced from ref. 25 with permission from Dove Medical Press, copyright: 2015). (vi) Principle of RCA and the AFM image of the RCA product (this figure has been adapted from ref. 28 with permission from Springer, copyright: 2016). (vii) Illustration of LFA. (viii) Illustration of anti-HCV core antibody detection by fluorescence and electrochemical methods with a smartphone-based potentiostat (this figure has been adapted from ref. 30 with permission from Else, copyright: 2016).

Table 1 Characteristic features of the amplification techniques for HCV detection

Method	Preferred amplicon	Reaction temperature (°C)	No. of primers	Amplification	Time	Disadvantage	Ref.
PCR	DNA	95, 60, 72	2	30 cycles yield 10^9-fold amplification	2–4 h	• Required thermal cycler • High time consumption • Minute contamination leads to misleading results	95
TMA	RNA and DNA	37	2	10^6-fold amplification	Within 1–2 h	• Requires pre-heating • Requires 3 enzymes • Lower temperature increases the non-specific interactions of the primers	10 and 96
LAMP	DNA	65	4–6	10^9-fold amplification	Less than 1 h	• Complicated multiple primer designs • The final product is a complex mixture of stem-loop cauliflower-like DNA structures of various sizes	97
RCA	Circular DNA or RNA	37	1	9000 nucleotides from a 34 nucleotides template	Within 1 h	• Non-specific cross-linking can occur • Only a few DNA aptamers and DNA enzymes have been used • Variability of binding sites for nano species immobilization is rather limited	98 and 99

enabling single-step purification, surface preparation, and deposition for optical imaging to electrochemical sensing.[30]

• Optical detection was carried out by Timurdogan *et al.* using a 5 mW laser diode, a photodetector, lenses, and embedded diffraction gratings at the tip of each cantilever. The entire read-out electronics can be miniaturized and made portable.[4]

Detection based on biomarkers

Diagnosis and HCV management are crucial to enable diagnosis, treatment monitoring, and treatment selection in reducing disease progression. Due to the advancement of easier and earlier detection in protein chip assay (PCA), Chen *et al.* developed a surface-enhanced laser desorption/ionization time-of-flight mass spectrometric (SELDI-TOF-MS) method for the detection of serum biomarkers in liver disease.[100] Later, Mukherjee *et al.* explained the serological assays, rapid diagnostic tests (RDTs), OraQuick HCV rapid antibody test, clinical chemistry assays, molecular assays, and HCV genotyping assays.[40] On the other hand, Kamili *et al.* concentrated on the challenges in HCV detection, the upcoming technologies to distinguish acute and chronic HCV during their assay, and concluded the importance of immunoassays for HCV core antigen (HCV cAg) detection to reduce the cost and labor-intensive NAT.[101] Then, Foudeh *et al.* presented the status of microfluidic devices for pathogen diagnosis and emphasized the innovative designs, strategies, and trends to throw light upon the design and modification of various components to develop lab-on-chip (LOC) devices.[102] Moreover, Khuroo *et al.* assessed the diagnostic accuracy and applicability of POCTs for HCV, which stood as one of the critical analysis for the further implementation of devices in POC diagnostics.[103]

One of the notable works of Li *et al.* was the study of the general properties of HCV RNA and their protein composition along with the screening methods and diagnostic tools, which help in concluding the requirement of the new prophylactic vaccine.[27] Later, Trucchi *et al.* summarized three major unresolved issues: (i) the perspectives for the universal screening of HCV, (ii) the need for direct-acting antiviral (DAA) resistance testing in the future, and (iii) necessary preventive HCV vaccine.[104] Further, Chevaliez *et al.* reported the list of alternatives to the standardized tests for the virological examination of hepatitis B and C in POC testing, DBS, alternatives to nucleic acid testing (NAT), and Food and Drug Administration (FDA) approved RDTs.[105] Later, Mane *et al.* proposed multiple detection techniques of HCV antibodies by considering five different RDTs, namely, Alere Truline, SD Bioline, Flaviscreen, advanced RDTs, and OraQuick, where Alere Truline, Bioline, and OraQuick had high specificity and sensitivity compared to other techniques.[106]

Marwaha *et al.* focused on the currently increasing trends and status of various generation tests involved in screening HCV-infected blood donors from different countries. They also discussed the sensitivity of the antibody, antigen, and combination assays. Furthermore, they recapitulated the importance of combined antigen and antibody assays for HCV screening and concluded that "fourth generation" combined antigen–antibody assays will be the best approach for HCV screening in resource-constrained environments.[107] In recent times, oral POCT was available for self-testing with comparable performance to blood-based tests. This improves treatment initiation

and patient care. Tucker *et al.* explained the importance of POCT in sexually transmitted infections.[108] Duchesne *et al.* summarized the main challenges, advances to overcome the difficulties in the field of viral hepatitis, and the LOD for each method.[109] From these review articles, we are acquainted with a plethora of detection techniques available for the detection of HCV infection, each with a definite DL. We categorized all the probable methods based on the detection modalities, *i.e.*, the detection of HCV RNA, antibodies, core antigen, and antigen and antibody together beyond confronting the intricacies.

HCV RNA. Within the exposure of 2–14 days to HCV, HCV RNA will appear in the bloodstream, which increases the alanine aminotransferase (ALT) enzyme of the liver.[110] Hsieh *et al.* demonstrated a fast DNA sample and mutant detection in the homogeneous liquid phase of the microdroplets through molecular beacon (MB) as a DNA sensing probe. Furthermore, they evaluated dynamic MB-DNA duplex formation using label-free DNA.[111] Later, Roh *et al.* proposed a QDs-supported RNA oligonucleotide technique to detect HCV viral protein using biochip through the immobilization of HCV non-structural protein 5B (NS5B) on a glass chip.[112] In addition, Roh *et al.* developed a protein glass chip to visualize the QDs-based HCV NS3 biomarker through a fluorescent imaging probe using the RNA aptamer coupled with QDs605.[112] For the simultaneous detection of multiple bio-molecules, Sochol *et al.* constructed a microfluidic chip that integrates both the microfluidic mixing of the mobile microbeads and hydrodynamic microbeads arraying, and detected through ssDNA molecular beacon probes immobilized on polystyrene microbeads for fluorescence visualization and signal detection. For evaluation purposes, perfect match (PM), one mismatch (SNP), and mismatch (MM) DNA oligonucleotide sequence corresponding to perfect complementary for MB, a single base-pair mismatch to MB, and with multiple base-pair mismatches to MB, respectively, were used.[113] Subsequently, Ember *et al.* fabricated DNA and protein microarrays, which were directed against universal HCV determinants for the rapid detection of HCV infection in clinical samples that were more sensitive compared to the commercial ones.[114]

The detection of HCV genotype was necessary for antiviral therapy. PCR step, exonuclease digestion, and genotype detection steps were essential to predict the genotype, which could be done in GenMark eSensor. Then, Sam *et al.* validated the LOD, specificity, accuracy, and precision of the GenMark eSensor®.[87] Mukaide *et al.* described the detection of mutations of the HCV core protein gene in codon 70 through next-generation droplet digital polymerase chain reaction (ddPCR) assay. This assay possesses highly sensitive quantitative detection of single nucleotide polymorphisms within the viral genomes and accurately quantitates the total amount of HCV RNA.[115] Later, TaqMan Array Cards (TAC) were developed for the rapid and simultaneous detection of five hepatitis viruses. The microfluidic technology allows sample distribution to individual RT-PCR reactions and thereby, the TAC assay was done through the ViiA7 instrument.[116] Similarly, Chang *et al.* presented a LAMP-based lab-on-disk optical system that allows the simultaneous detection of HBV, HCV, and cytomegalovirus by measuring the turbidity of DNA samples. It consists of a disposable microfluidic disk, a temperature control system, a servo motor, a control unit, and an SPR detection system.[89]

Lu *et al.* developed a powerful homogeneous electronic monitoring platform to eliminate the intricacies of multiple separation and rinsing steps and identify low concentration nucleic acid through a negatively charged screen-printed carbon electrode.[117] When the high flow of fluid passed to the sensor of carbon nanotubes associated with ferrocene as the redox marker, a very thin depletion layer was formed on the surface, engendering a capture rate up to one DNA strand per second.[94] Portable microdevices for HCV-RNA purification and detection lagged in early-stage detection. Thus, Vaghi *et al.* developed a microdevice whose surfaces were treated to bear optimal positive charges in order to purify HCV RNA from the plasma by adsorbing HCV RNA and reverse-transcribed it into cDNA for detection.[118] To make it cheaper and more straightforward, Liu *et al.* successfully developed a fully functional paper device for the detection of DNA or microRNA *via* target-induced rolling circle amplification by capturing colorimetric signals.[90] Fig. 7 shows the different chips used in the detection of HCV RNA.

Digital nucleic acid detection (dNAD) platform was developed by Chen *et al.* through the combination of emulsion microreactors, single-molecule magnetic capture, and on-bead LAMP for the detection of HCV DNA. Furthermore, the use of a LAMP emulsifier instead of PCR forgoes the thermocycler and improves the amplification. Magnetic capture partitioned each DNA with single encapsulated beads, thus enhancing the sensitivity.[119] Thereby, Llibre *et al.* designed an instrument that focused on developing a POC assay for the qualitative detection of HCV RNA through the PCR Genedrive instrument. The Genedrive HCV assay has been evaluated through a case–control study, which results in high sensitivity and specificity for decentralized HCV nucleic acid amplification testing (NAAT).[120] Tu *et al.* provided a digital strategy for HCV RNA detection using glucose-loaded liposomes as the labeling probe. The detached glucose-loaded liposome was dissolved with Triton X-100 after the magnetic separation of the bead to release the glucose molecules, which were detected by a digital glucometer.[121] The sensitivity, DL, and disadvantages of each HCV RNA detection technique in microfluidics are summarized in Table 2.

HCV antibodies. Detection of antibodies is considered to be the simplest when compared to RNA and antigen detection. Hence, more assays with different procedures are clinically available. However, the HCV antibody develops after 30–60 days of exposure to HCV.[110] Hence, within 6–12 weeks of infection, the HCV antibodies are usually detectable.[123] Daniel *et al.* developed a fourth-generation flow-through immunoassay to detect HCV-Ab and compared the outcome with the enzyme immunoassay (EIA) and microparticle enzyme immunoassay (MEIA). The NS3, NS4, and NS5 antigens of HCV were immobilized on a porous immunofiltration membrane, consisting of three test dots, T1, T2, and a quality control or serum control dot. When the serum/plasma sample is loaded on the membrane, HCV-Ab binds to the immobilized antigen. Upon

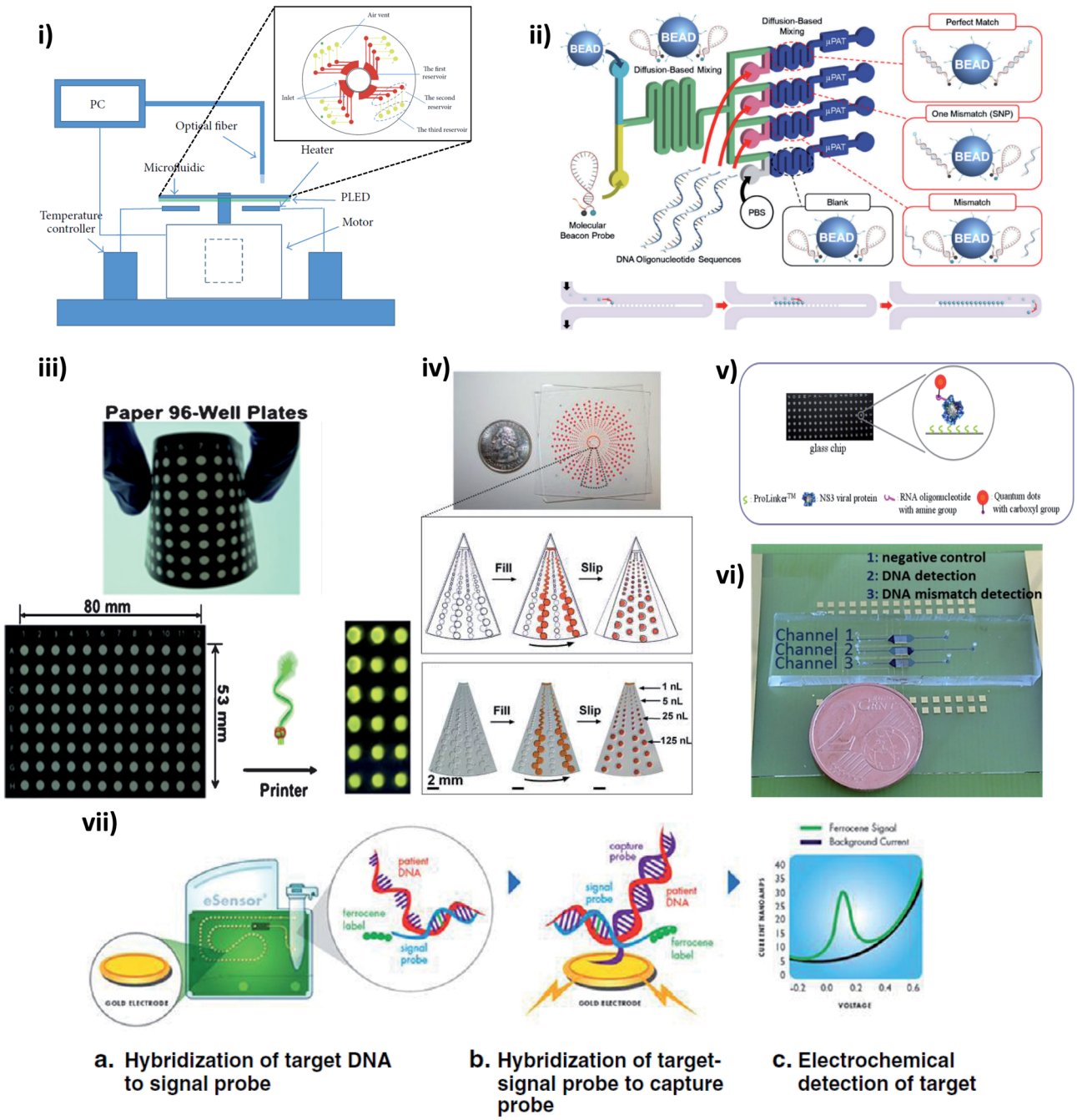

Fig. 7 Various devices used for HCV RNA detection. (i) Microfluidic testing using compact disk microfluidic channels (this figure has been reproduced from ref. 89 with permission from Hindawi, copyright: 2015). (ii) A dynamic bead-based microarray for parallel DNA detection (this figure has been reproduced from ref. 113 with permission from IOP, copyright: 2011). (iii) Image of 96 microzones paper plate (this figure has been reproduced from ref. 90 with permission from Wiley, copyright: 2016). (iv) SlipChip for multiplexed, multivolume digital RT-PCR with high dynamic range (this figure has been adapted from ref. 6 with permission from ACS, copyright: 2011). (v) Protein biochip for viral protein detection (this figure has been adapted from ref. 112 with permission from Wiley, copyright: 2010). (vi) On-chip genetic analysis of HCV (this figure has been adapted from ref. 94 with permission from AIP, copyright: 2016). (vii) Hybridization and electrochemical detection mechanism (this figure has been reproduced from ref. 87 with permission from Elsevier, copyright: 2013).

the addition of protein A conjugate, the Fc portion of HCV-specific immunoglobulin G binds, which results in a pinkish-purple dot in the test region.[124] The preparation, quality control, and clinical evaluation of a protein chip were investigated by Zhang *et al.* for the simultaneous detection of different HCV antibodies by six antigens arrayed onto the aldehyde-coated slides and scanned using the Scanarray 3000 scanner.[125] Later, Duan *et al.* described the rapid and simultaneous detection of HBV and HCV antibodies through PCA using Nano-gold Immunological Amplification and Silver Staining

Table 2 Recent HCV RNA detection techniques in microfluidics

Sl no.	No. of samples (sample type)	Assay	Operation time	Practical implication	DL	Sensitivity (Se), and specificity (Sp)	Application	Detection	Ref.
1	(SynBRCA1 and HepCV DNA sample)	Label-free DNA analysis in the microdroplet	NA	Required excitation source	As low as 500 fM	NA	Pharmacogenomics research, evaluate drug efficacy, toxicity, and metabolism	DNA	111
2	(Purified PCR-amplified HCV gene)	RNA-oligonucleotide nanoparticle assay	NA	Required centrifugation	1 ng mL^{-1}	NA	Multiple virus detection coupled with nanoparticles	HCV viral protein	122
3	329 samples (serum)	TaqMan Array Cards (TAC)	4 h	Lower sensitivity compared to other assays	100 IU per mL	Se: 100%, Sp: 100%	Screening of the donor specimens	HCV RNA	116
4	(PCR amplified HCV gene)	Quantum dots-based RNA aptamer system	NA	Multiple centrifugations are required	5 ng mL^{-1}	NA	Detection and monitoring of human infection and in research	Viral protein	112
5	(PM, SNP, and MM DNA oligonucleotide sequences)	Chemical and biomolecule detection assays	NA	Syringe pumps and vacuum loaded device is necessary	NA	NA	Bio-molecule detection, medical diagnostics, and drug screening	HCV DNA	113
6	2 patients (plasma)	Multivolume digital RT-PCR	NA	Dilution errors and RNA degradation need to considered	40 molecules per mL	NA	POC resource limited settings and cell research	HCV RNA	6
7	48 samples (plasma)	GenMark eSensor® HCV genotyping	NA	Contamination may occur	175 IU per mL	Se: 96.8%	Antiviral therapy	HCV RNA	87
8	87 patients (serum)	Droplet digital polymerase chain reaction (ddPCR) assay	NA	Total quantitation of hepatitis viral sequences has not been reported	2.5 copies per well	Se: 99.9%, Sp: 100%	Quantitation of mutations in other polymorphic viral genomes	HCV RNA	115
9	(Blood)	LAMP-based lab-on-disk system	Less than 1 hour	Complicated equipment	60 copies per mL	NA	Lab-on-disk system	HCV DNA	89
10	5 specimens (serum)	Homogeneous electronic monitoring platform	NA	Abdicate probe labeling and immobilization of the DNA sensing probe	2.3 pM	NA	As a one-step incubation reaction	HCV DNA	117
11	(NH_2-ssDNA)	Electrochemical-based sensors	1.5 h	Operate under high flow	0.1 fM to 1 pM	NA	POC	HCV DNA	94
12	2 patients (plasma or serum)	PCR	4 h	Surface modification of PDMS	9 812 000 UI per mL	NA	Clinical management and POC	HCV RNA	118
13	Circular DNA template (CDT)	Rolling circle amplification (RCA)	NA	RCA reagents will be inactive within 15 days at room temperature	10 pM	NA	Colorimetric bioassays and biomarkers in clinics	HCV-1 DNA	90
14	2 patients (plasma)	Digital nucleic acid detection	NA	Complex workflow and the deliberate design of primers	300 copies per mL	NA	Clinical plasma sample diagnostics	HCV RNA	119
15	925 samples (serum)	Genedrive HCV assay	NA	Semi automotive and require pre-training	2362 IU per mL	Se: 98.6%, Sp: 100%	POC tests	HCV RNA	120
16	15 samples (human serum)	Magnetic bead single-stranded DNA glucose-loaded liposomes	Less than 2 h	Required glucose-loaded nanoliposomes	1.9 pM	NA	Clinical diagnosis	HCV RNA	121

(NIASS) method. The enhancing solution in the assay contains silver ions and a reducing agent, which is buffered to an acidic pH. Intense dark signals were obtained during immunogold silver staining enhancement, where the colloidal nano-gold acts as a nucleation site for metallic silver deposition, thus making it visible to naked eyes.[126]

Similarly, Xu *et al.* described a simple, rapid, and sensitive protein microarray to determine two viral antigens and seven

viral antibodies of human hepatitis viruses in human sera. The result was amplified using a tyramide signal amplification system and assessed directly by the naked eye or analyzed through a quantitative detector.[127] To perform consecutive flow (CF), Corstjens *et al.* developed and constructed a semi-automatic microfluidic module prototype that fits into the existing UPlink-compatible cassettes. The CF format was initially designed to detect human antibodies against HIV-1 and 2. However, for the multiplexed detection of various antibodies from a single specimen, different test lines were placed transversely across the linear flow (LF) strip without disturbing the up-converting phosphor technology (UPT) reporter.[31] Likewise, Desbois *et al.* developed a visual, qualitative, and rapid assay to detect anti-HCV IgG antibodies based on an immunochromatographic test, where the membrane was stripped with recombinant HCV antigens representing the core, NS3, NS4, NS5 proteins, and a reagent control. IgG antibodies in the sample were bound to protein-A on colloidal gold, followed by the binding of antigens coated on the membrane, which implied color change in the specific region of the membrane.[91]

Lee *et al.* compared the new, rapid, non-instrumented POC test of OraQuick® HCV assay for five specimen types with FDA-approved laboratory methods and concluded that the rapid anti-HCV test was a suitable aid in HCV Ab diagnosis.[128] OraQuick® HCV Rapid Antibody Test utilizes an indirect immunoassay method in a lateral flow device. It was a non-invasive procedure while using oral samples. They used a nitrocellulose strip where HCV antigens were immobilized on a single test line. The antibodies bind to give a reddish-purple line using colloidal gold, labeled with protein-A. However, the sensitivity of the oral fluid is slightly lower than the finger-stick blood (FSB) specimens.[129] In addition to that, Jewett *et al.* evaluated the sensitivity and specificity with the other two pre-market rapid POC tests.[130] Similarly, Smith *et al.* evaluated the sensitivity and specificity of the POC tests that utilize FSB and two oral fluid rapid assays from 3 manufactures. Finally, FDA approved the OraSure assay for venous and FSB samples.[131] Some of the FDA-approved antibody assays are Abbott HCV EIA 2.0, Advia Centaur™ HCV Assay, Architect Anti-HCV, AxSYM™ Anti-HCV, Elecsys™ Anti-HCV II, OraQuick™ HCV Rapid Antibody Test, Ortho HCV Version 3.0 ELISA Test System, and Vitros Anti-HCV.[132] Some of the devices used in HCV antibody detection are shown in Fig. 8.

Due to the prolongation of HCV, HCC may occur; thus, Akada *et al.* immobilized the cysteine-tagged recombinant antigenic proteins on maleimide-coated diamond-like carbon (DLC) silicon chips to detect multiple auto-antibodies in HCC individuals.[5] To address the performance gap of high duration confirmatory diagnosis, heterogeneous barcode immunoassay was applied to capture HCV antibodies in the serum *via* the electrophoreses of barcode-patterned gel.[11] Mu *et al.* developed a multiplexed microfluidic paper-based immunoassay to address the diagnostic challenges of HCV infection by integrating the segmented assays rapidly and economically so as to transform the diagnostic pathway for confronting HCV.[35] The main aim of Cha *et al.* was to give a piece of knowledge about the clinical sensitivity and specificity of the OraQuick HCV Rapid Antibody Test, which utilizes lateral flow immunoassay that helps in comparing the performance of the biological samples.[133] Similarly, Scalioni *et al.* analyzed the performance of the three rapid tests such as (1) WAMA Immuno-Rápido HCV, (2) Bioeasy HCV Rapid Test, and (3) OraQuick HCV Rapid Test from the samples that were obtained from different individuals with different endemicity and progress risk factors. In all these tests, recombinant antigens from the core and non-structural regions of the HCV genome were immobilized on a test strip for detection.[134]

Due to the requirement of telediagnosis in resource-limited settings, Zhao *et al.* integrated the paper-based diagnostic platform with multiplexing and telemedicine capabilities. This can be achieved by detecting the HCV core antibodies in an electrochemical immunosensor array and the output was read-out through a handheld potentiostat integrated with a Bluetooth module.[135] Similarly, an electrochemical microfluidic paper-based immunosensor array (E-μPIA) was integrated with a handheld potentiostat for the diagnosis of the HIV/HCV co-infection and remote transmission of the diagnostic results to a host computer or smartphone for telemedicine.[38] Parween *et al.* reported human IgE, the antibody of hepatitis C virus core antigen, and three 20 mer-oligonucleotides were detected by an activated ultra-miniaturized assay plate (AUAP), where the biomolecules were immobilized through covalent binding and were quantified digitally.[32] Robin *et al.* conducted a hospital-based cross-sectional study on the serum samples to detect HIV-1 and HIV-2, and the HCV antibodies in a manually operated, visually interpreted, lateral flow immunochromatographic assay within 15 min irrespective of age and sex. According to the results obtained, the HCV-seropositive sera were positive for HCV RNA, whereas negative results were resolved of infection. The authors also predicted that the triplex detection device has shown very high sensitivity, specificity, as well as excellent concordance with Chemiluminescent Microparticle Immunoassay (CMIA) Abbott results.[136]

For the qualitative detection of the HCV antibody, Ryu *et al.* developed a fluorescent LFIA (Lateral Flow Immunoassay) employing Automated Fluorescent Immunoassay System (AFIAS) through the optical signaling probe with a high signal-to-noise ratio, an Eu(III) fluorescent dye to improve the sensitivity, and an automated fluorescent strip reader to record the fluorescence intensity.[1] Finally, for rapid POC test, Kweon *et al.* developed EuDx-HE (A, B, C), a manually-operated and visually-interpreted kit based on the immunochromatographic assay with lateral flow consisting of a sample pad, a conjugate pad, and a nitrocellulose membrane immobilized with HCV antigens, to capture the antibodies. The architect immunoassay analyzer evaluates the diagnostic accuracy of the kit.[26] Though the HCV antibody detection stepped into the new era of surveillance, it additionally requires epidemiological mapping, testing, and prevention.[137] The sensitivity, DL, and disadvantages of each HCV antibody detection technique in microfluidics are explained in Table 3.

HCV core antigen. During acute HCV infection, HCV cAg develops before antibody production.[138] Hence, the HCV core antigen assay detects the HCV infection between 40 and 50 days

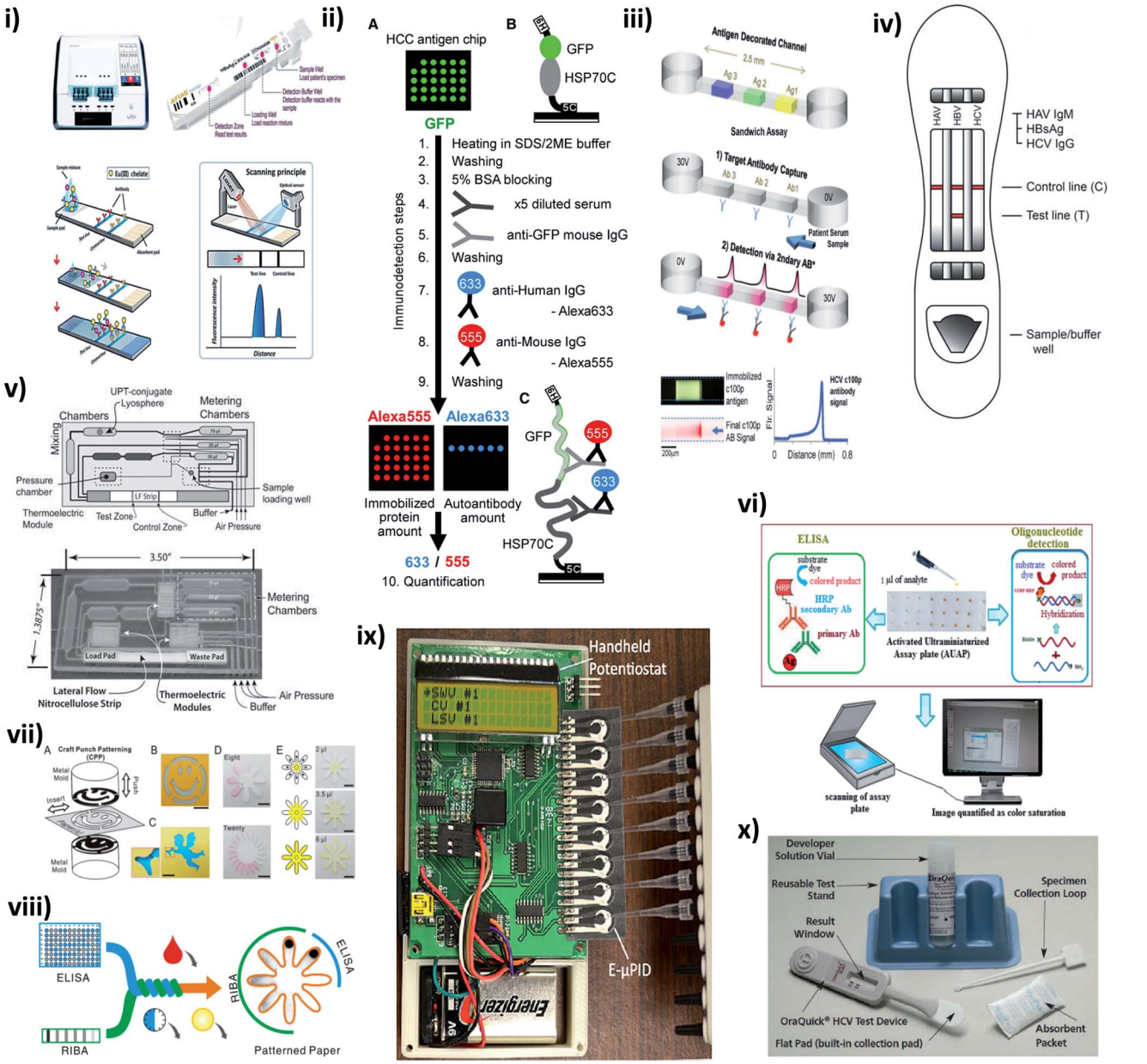

Fig. 8 Various devices used for HCV antibody detection. (i) Design of Automated Fluorescent Immunoassay System (AFIAS) (this figure has been adapted from ref. 1 with permission from Korean Society for Laboratory Medicine, copyright: 2018). (ii) Auto-antibody detection on protein array chips (this figure has been reproduced from ref. 5 with permission from Biomed Central, copyright: 2013). (iii) Schematic of the microfluidic barcode assay and the antibody sandwich assay (this figure has been adapted from ref. 11 with permission from Royal Society of Chemistry, copyright: 2013). (iv) Schematic of EuDx-HE (A, B, C) kit (this figure has been reproduced from ref. 26 with permission from Wiley-Blackwell, copyright: 2019). (v) CF semi-automatic microfluidic device (this figure has been adapted from ref. 31 with permission from Wiley-Blackwell, copyright: 2007). (vi) Illustration of the ultra-miniaturized assay technique on an ultra-miniaturized assay plate (this figure has been reproduced from ref. 32 with permission from Springer, copyright: 2016). (vii) Images of craft punch patterning with different designs; (viii) Paper-based immunoassay for ELISA and RIBA (figures (vii) and (viii) have been adapted from ref. 35 with permission from ACS Publications, copyright: 2014). (ix) Paper-based immunosensor array introduced in a handheld potentiostat (this figure has been adapted from ref. 38 with permission from AAAS, copyright: 2012). (x) OraQuick HCV Test kit (this figure has been reproduced from ref. 40 with permission from EVISA, copyright: 2015).

earlier than the current third-generation HCV antibody screening assays.[139] Bouzgarrou *et al.* described the HCV core antigen testing by Ortho trak-C assay (OTCA) in dialysis patients in developing countries where RT-PCR techniques remain unavailable. It consists of a microplate coated with antibodies that bind the HCV antigens upon the addition of conjugate (Fab fragments of the horseradish peroxidase). The addition of *O*-phenylenediamine (OPD) oxidizes the conjugate and yields a reddish product.[138] Although the current HCV antigen assay techniques are not as sensitive as NAT, however, they are

Table 3 Recent HCV antibody detection techniques in microfluidics

Sl no.	No. of samples (sample type)	Assay	Operation time	Practical implication	DL	Sensitivity (Se) and specificity (Sp)	Application	Detection	Ref.
1	2590 samples (serum)	Rapid assay	5 min	One sample from a high-risk individual indicated as negative	NA	Se: 99.3%, Sp: 99.0%	Screening and diagnosis	HCV antibodies	124
2	490 samples (serum or plasma)	Protein chip assay (PCA)	NA	High time	NA	Se: 97.4%	*In vitro* detection and used in blood banks	HCV antibodies	125
3	305 samples (serum)	PCA	Less than 40 minutes	Preparation of nano-gold particles was tedious	3 ng mL^{-1}	NA	Proteome, clinical diagnostics, and drug discovery	HCV antibodies	126
4	(Serum)	Protein microarray and ELISA	20 min	Complex processes, expense, and sophisticated devices were required	0.1 ng mL^{-1}	NA	Clinical and epidemiological screening	HCV antibodies	127
5	300 (plasma specimens)	UPT–CF	NA	Careful development and optimization of capture lines are required to avoid non-specific binding and antibody cross-reactivity,	NA	NA	Antibody test module	HCV antibodies	31
6	421 sera (serum)	Rapid immuno-chromatographic assay	15 min	False-negative results in HIV positive patients	NA	Se: 95.5%	Reassure health-care workers	HCV antibodies	91
7	572 samples (venous and FSB, serum, plasma, or oral fluid)	OraQuick® HCV assay	20–40 min	Concerns about clinical performance and test quality	NA	Se: 99.8%, Sp: 99.2%	Clinical, physician offices and community outreach centers	HCV antibodies	128
8	2206 samples (blood or oral fluid)	OraQuick® HCV Rapid Antibody Test	20 min	Lower sensitivity in oral fluids and repetition of RNA tests is required to determine the actual state of HCV infection	20 IU per mL	Se: 98.1–99.9%, Sp: 99.6–99.9%	Clinical and non-clinical POC settings	HCV antibodies	129
9	409 specimens (oral fluid and blood)	Chembio and MedMira	Less than 40 min	Sensitivity was low in MedMira FSB tests	NA	Se: 76.6–97.1%, Sp: 99–100%	POC	HCV antibodies	130
10	1861 specimens (blood and oral fluid)	Chembio, MedMira, and OraSure	Less than 40 min	Performance was slightly weaker in field-use	NA	Se: 78.9–97.4%, Sp: 80–100%	Social service and methadone maintenance treatment programs	HCV antibodies	131
11	46 samples (serum)	PCA	NA	Complicated procedure and image analysis	NA	NA	Prediction of the onset of particular cancers	HCV antibodies	5
12	(Serum)	Heterogeneous barcode immunoassay	30 min	Required low power external source	25 ng mL^{-1}	NA	Low-resource laboratory settings	HCV antibodies	11
13	137 samples (oral fluids and sera)	Oraquick antiviral rapid test	NA	Patients were clinically proven to be infected by HCV previously	NA	Se: 94.1%, Sp: 99.5%	POC tests	HCV antibodies	133
14	10 pieces of patient serum	Multiplex microfluidic paper-based immunoassay	NA	Avoid direct contact of	0.15 ng mL^{-1}	NA	Detection of biomarker panel	HCV antibodies	35

Table 3 (*Contd.*)

Sl no.	No. of samples (sample type)	Assay	Operation time	Practical implication	DL	Sensitivity (Se) and specificity (Sp)	Application	Detection	Ref.
	and 193 serum mixtures			patterned paper with fingers			(tens of proteins) of cancer and Alzheimer's		
15	575 people (blood)	WAMA Immuno-Rápido HCV Kit	40 min	Large sample consumption and not utilized for sera and whole-body fluids	NA	Se: 76.03–93.84%, Sp: 93.75–100%	Laboratory and field settings	HCV antibodies	134
16	8 samples (serum)	Paper-based electrochemical immunosensor array	20 min	Decreased adaptability	750 pg mL^{-1}	NA	POCT and telemedicine capabilities	HCV antibodies	38 and 135
18	(Blood)	Activated ultra-miniaturized assay plate (AUAP)	24 min	Biomolecule cannot immobilized through absorption	200 ng mL^{-1}	NA	Clinical diagnosis	HCV antibodies	32
19	250 (sera samples)	Chemiluminescent microparticle immunoassay (CMIA)	NA	Triplex applications require the usage of highly accurate and feasible occupational procedure	1.1 log IU per mL	Se: 96.8–100%, Sp: 99.9–100%	POCTs	HCV antibodies	136
20	3500 samples (blood, oral fluid, serum, and plasma)	LFIA	NA	Stored serum samples are used instead of fresh samples	0.436 cut-off index	Se: 98.8%, Sp: 99.1%	POCTs	HCV antibodies	1
21	1581 (serum samples)	Immunochromatographic strip assay	15 min	Since the kit works on antigen and antibody reaction, interference tests not performed	NA	Se: 94.3–97.96%, Sp: 98.97–99.86%	Helps in the intravenous monitoring of drug users	HCV antigen and antibodies	26
22	(DNA target samples from human serum)	DNA microarray and protein microarray test	14–16 min	Extra handling steps in DNA microarray for sample pre-treatment	10 ng mL^{-1}	NA	POC diagnostics	HCV antibodies	114

a significant improvement than the HCV antibody assay as it detects within the window period.[140] Hence, Lee *et al.* paved the first step toward developing high affinity and specificity RNA aptamer to bind the HCV core antigen for HCV diagnosis by applying the core antigen-specific aptamers to sol–gel-based chips.[141] Timurdogan *et al.* developed a label-free and real-time analyte monitoring and optical biomolecule detection using a 5 mW laser diode through resonant microcantilever arrays as shown in Fig. 9.[4]

Ivanov *et al.* incubated the serum samples through reversible biospecific-fishing and mass spectrometry (MS). They then visualized the immune complexes on the surface of the atomic force microscopy (AFM) chips immobilized with antibodies. These chips show low non-specific adsorption of the serum components due to the aminosilane surface for easy wash from the chip.[142] The LOD in the AFM chip was compared with two proteomic technologies such as the electrophoretic/chromatographic separation of proteins and proteomic microarrays using mass-spectrometric analysis and optical biosensor, respectively. Thereby, the lower LOD of 10^{-1} and 10^{-16} M was obtained using reversible and irreversible AFM fishing, respectively.[143] Further, Fourati *et al.* provided a comprehensive overview of the new simplified approaches for screening, diagnosis, and monitoring HCV infection with different country-specific settings and described the essential tools in future diagnostics. The POC HCV cAg test kits were difficult to develop and they were still in the development phase of research.[144] Pleshakova *et al.* proposed the HCVcoreAg revelation method, which has advantages of AFM and target-protein enrichment to identify the minimum concentration of the detecting antigen in the AFM chip.[145] The sensitivity, DL, and disadvantages of each HCV antigen detection technique in microfluidics are described in Table 4.

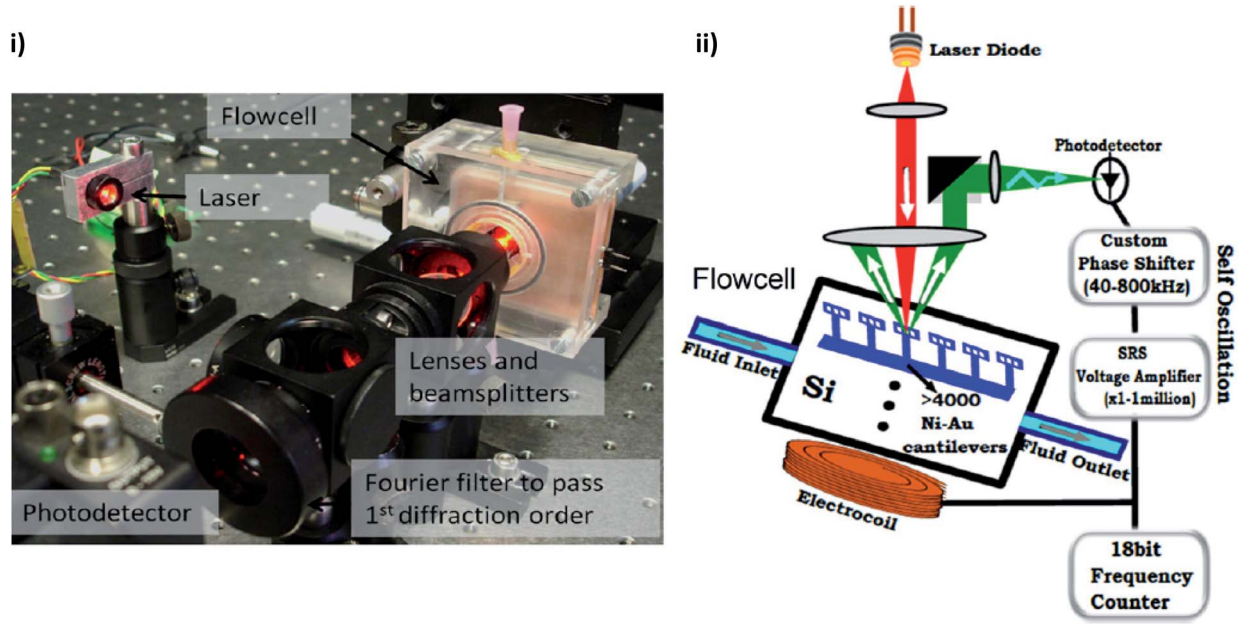

Fig. 9 Devices used for the detection of the HCV antigen. (i) Schematic of the MEMS cantilevers with disposable sensor chips; (ii) illustration of the procedure in the MEMS cantilevers (this figure has been reproduced from ref. 4 with permission from Elsevier, copyright: 2012).

HCV antigen and antibody. Initially, Laperche *et al.* simultaneously detected the HCV core Ag and anti-HCV Ab for the early detection of HCV infection, where the nucleic acid detection technologies were not implemented. Screening involves the addition of serum, control, and specific conjugate in suitable microwells with proper incubation period and washing. The results were analyzed by absorbance measurement.[146] Later, Ansaldi *et al.* developed and evaluated the sensitivity and

Table 4 Recent HCV antigen detection techniques in microfluidics

Sl no.	No. of samples (sample type)	Assay	Operation time	Practical implication	DL	Sensitivity (Se) and specificity (Sp)	Application	Detection	Ref.
1	303 (serum samples)	OTCA and EIA	NA	Lower sensitivity and specificity compared to other methods	10 000 UI per mL	Se: 84%, Sp: 89%	Early viral response (EVR)	HCV core antigen	138
2	500 plasma specimens (serum and plasma)	Magnetic microparticle-based assay	NA	Not as sensitive as NAT	10 000 copies per mL	Se: >97%, Sp: 99%	Diagnostic and transplant settings	HCV core antigen	140
3	(Human sera)	PCA	NA	Weaker signals were obtained	100 nM	NA	Sensitive and specific detection of multiple HCV antigens	HCV antigen	141
4	(Undiluted serum)	Resonant microcantilever arrays	30 min	Flow cell must be wet always	0.1 ng mL^{-1}	NA	Portable diagnostics instrument	HCV antigen	4
5	20 samples (serum)	Reversible bio-specific AFM-fishing and MS	NA	Inability to identify visualized objects, and required repetition for accuracy	NA	NA	Disease diagnostics	HCV core Ag	142
6	(DBS or FSB)	NAAT	Less than 60 min	Too expensive and complexity	500 to 3000 IU per mL	NA	POC	HCV core antigen	144
7	(Buffer solution)	AFM-chip	NA	Fishing experiment must be repeated	10^{-13} M	NA	Development of aptamer-functionalized surface-based ELISA methods	HCV core antigen	145

specificity of a MONOLISA HCV Ag–Ab ULTRA assay to detect both the antibody and antigen in a microplate coated with monoclonal antibodies, two recombinant proteins, one recombinant antigen, and a peptide. Upon the addition of suitable conjugates 1 and 2 with proper incubation and washing, the antigen–antibody complex was revealed.[147] Similarly, Larrat *et al.* evaluated the performance of both combined enzyme immunoassay (cEIA) (MONOLISA HCV Ag–Ab ULTRA assay) and the POC device (OraQuick® HCV) on FSB and oral mucosal transudate (OMT) for the detection of the HCV antibody. As a result, with FSB specimens, cEIA and the POC device exhibited 100% specificity and 98.2% and 97.4% sensitivity, respectively. With OMT specimens, the cEIA sensitivity and specificity of 71.7% and 94.3%, respectively, and the OraQuick® HCV sensitivity of 94.6% and specificity of 100% were obtained.[148] Moreover, Kania *et al.* focused on the screening strategy of DBS sampling and provided the HCV status in a parallel manner, where the series of blood samples with DBS were analyzed for the detailed result progress.[149] Besides, the HCV diagnosis at the early stage with advanced method rather than using standard solutions could provide more keener results. Applegate *et al.* also suggested the requirement for the development of new diagnostic techniques.[150] The sensitivity, DL, and disadvantages of each HCV combined antigen and antibody detection techniques in microfluidics are summarized in Table 5.

Intricacies faced in HCV diagnosis

The viral evolutionary dynamics and host genetic polymorphisms, *e.g.*, the interleukin 28B (IL28B) gene, are vital need for detecting the outcome of the HCV infection.[151,152] Quasis species have been referred to as the most commonly noted viral variants in HCV-infected patients, which were pointed out for the functional diversity of nucleotides between the isolates.[153] In an infected person, the HCV nucleotide sequence is observed to be varied at the rate of 1% to 5%, branching out to different subtypes and genotypes as a result of the accumulation of nucleotide substitutions, which headed to the heterogenous HCV RNA genome sequences. At present, HCV is classified into eleven genotypes (referred to as 1–11) with a nucleotide sequence difference of 30–50%, six of which are the main ones (genotypes 1 to 6).[154,155] Within the HCV genotype, several subtypes (designated as a, b, c, *etc.*) can be defined, which differ by 15–30% in their nucleotide sequence.[156,157] HCV varies geographically in the prevalence of genotypes and subtypes.[70,158] Genotype 1 is currently the most commonly distributed (46%) in the world, followed by genotype 3, genotype 2, and genotype 4. Different genotypes have different infections and pathogenicity, affecting the rate of cirrhosis progression and the risk of HCC. HCV heterogeneity would also lead to different reactions to antiviral therapies.[154,159,160] The development of pan-genotypic antiviral medicines resulted in the challenge of HCV heterogeneity.[29]

The general disadvantage of the conventional technique is that it requires high levels of skill. Moreover, some of the major unresolved issues are (i) the perspectives for the universal screening of HCV, (ii) the need for DAA resistance testing in the future, and (iii) HCV preventive vaccine.[104] The non-invasive detection of viral infections is always preferred compared to invasive testing. Biosamples such as saliva, sweat, urine, and teardrops are extensively used as non-invasive detection samples. However, in HCV diagnosis, the presence of HCV antibody concentration in the saliva is much lower than that in the blood.[161,162] Hence, the testing kit should have a high LOD. During testing, the intricacies of multiple separation and rinsing steps may put forth the challenges of cross-contamination, the requirement of expertise persons, and great time consumption.

Passive microfluidic lab-on-chip devices for viral detections

In general, the need for auxiliary amenities such as a pump or a pressure controlling system for introducing the fluid has been in demand for the microfluidic operational procedure. In some exceptional cases, they also meet with the prerequisite for some

Table 5 Recent HCV antigen and antibody detection techniques in microfluidics

Sl no.	No. of samples (sample type)	Assay	Practical implications	DL	Sensitivity (Se) and specificity (Sp)	Application	Detection	Ref.
1	191 samples (whole blood)	EIA	Mean delay of 30.3 days than HCV RNA assay	260 pg mL^{-1}	NA	Early detection	HCV antigen and antibody	146
2	500 samples (serum)	MONOLISA HCV Ag–Ab ULTRA assay (ELISA)	NA	<850 000 IU per mL	Se: 91.4%	Diagnostic settings	HCV antigen and antibody	147
3	113 samples (FSB, OMT, and serum samples)	cEIA (Monolisa® HCV-Ag–Ab-ULTRA) and OraQuick® HCV	No case study of acute HCV; sensitivity decreased for the co-infected patient and OMT	NA	FSB: Se: 98.2 & 97.4%, Sp: 100% OMT: Se: 71.7 & 94.6%, Sp: 94.3 & 100%	POC assay	HCV antigen and antibody	148
4	218 samples (DBS and paired plasma)	Monolisa HCV antibody-antigen ULTRA assay	Expensive procedure	NA	Se: 100%, Sp: 100%	HCV detection during pregnancy	HCV antibodies and antigens	149

specialized electronics or optical equipment that does not fall into conventional medical or biological laboratory equipment, resulting in further technical hitches. The area density of the analyte arrays has been pulled down significantly due to the increased space occupied by the auxiliary parts. However, different substrates are necessary for different applications. Every substrate has its advantages and disadvantages, and it is solely based on the application of work. Nevertheless, some of these substrates are costly.[163] The various microfluidic chip design structures have tougher fabrications because of the multiple layer requirements along with voluminous channels and valves. These drawbacks have narrowed down the practical applications of these microfluidic amenities in the clinics and general biological laboratories. On the other hand, multiplexed POC testing can be achieved through microfluidic systems that will minimize overtreatment, reduce the resistance, and improve the diagnostic precision and overall quality of care.[164] Moreover, the detection of viruses from whole blood requires plasma separation due to the small size and quantity of viruses. Microfluidic methods draw the basic principles and perform the extraction efficiently at the micro-scale.[165] The simple hydrodynamic trapping technique has been developed due to the evolution that could meet with the increased demand in trapping-based design in microfluidic operating procedures. In this way, the analytes can be well-maintained instead of being wasted by the existence of an external force. Though the process is simple, the natural state of the separated particles has been maintained, which is worthwhile for various research purposes. The hydrodynamic trapping technique uses a mechanical barrier to detach the target particle from the central flow. After the separation process, the hydrodynamic trapping sites maintain hold of the target particles, which paves the way for future research endeavors. The results from the advancements of research technologies obtained in the past decades enable the progress of microfluidic systems for rapid diagnostics.[166]

Critical discussion

During pregnancy and certain emergency trauma situations, rapid blood transfusion is crucial. Hence, rapid testing of any infection in the blood is essential. However, some of the most common sources for the rapid spread of HCV infection are poor blood transfusion methods with insecure injection practices. Sometimes, it can be transmitted through personal belongings such as sharing razors. Thus, a quick diagnosis is obligatory for reducing disease progression and transmission. The development of screening techniques such as EIA, recombinant immunoblot assay (RIBA), NAT assays, and molecular virological methods such as RT-PCR have high specificity and sensitivity in detecting active infection. However, in resource-constrained clinical settings, these methods are assumed to be difficult and time-consuming.[167–169]

People prefer non-invasive modes of diagnosis due to reduced pain, time-consuming, and discomfort but the sensitivity in oral samples is low compared to blood samples. Antibody concentration in the saliva is much lower than that in the blood, which reduces the accuracy.[161] Hence, FDA approval is difficult for diagnostic purposes and it needs further enhancements.[170] The further development of advanced primers for amplification may improve the sensitivity of oral samples. POCT strategies need to be implemented in economically impoverished areas.[103] Due to these requirements, many POCT have been developed. The use of the DBS sample provides overall 95% sensitivity and specificity for HCV Ab detection compared to plasma and serum samples.[171] Reverse transcription-quantitative real-time polymerase chain reaction (RT-qPCR) has been recommended as the alternative method by the U.S. Center for Disease Control and Prevention (CDCP).[172] Besides being more specific and sensitive, RT-qPCR methods require pre-treatment, prior purification of the samples (*i.e.*, urine and blood), and non-isothermal temperatures for amplification.[173] Thus, it forgoes the usage in POCT and resource-limited settings.

Several techniques are available for antibody detection, whereas antibodies develop only after 6–12 weeks of infection. The FDA approves the OraQuick® Rapid HCV Test for antibody detection in blood due to its high sensitivity and specificity. Moreover, false-negative results often occur for co-infected patients while using rapid test kits.[174] However, in oral fluids, the sensitivity is slightly lower.[129] During the initial stage of the disease, the virus cannot be detected in oral samples but can be seen in the blood. In addition to this, even after no active infection, antibodies can be detected. RNA develops within 2–14 days of infection and increases the ALT. However, detecting ALT alone is not sufficient as it increases even for minute liver injury. Thus, RNA and antigen detection modalities are preferred to diagnose an active infection. The detection time, window period, and the benefits of each biomarker detection are illustrated in Fig. 10. The high detection time HCV detection technology enables better accuracy with a decreased window period. Hence, the requirement of POCT in detecting the RNA and antigen is vast, whereas only fewer techniques are available due to the requirement of complex structures and methodologies in detecting HCV Ag. Furthermore, the detection of Ab and Ag plays a vital role in determining the infection progression and treatment plan. From this literature search, the MONOLISA HCV antibody-antigen ULTRA assay provides high sensitivity and specificity compared to other techniques but is expensive. The high sensitivity HCV diagnostic assays for each biomarker are listed in Table 6.

NAT's RNA detection is the gold standard for diagnosing active infections but requires expert specialists, expensive equipment, and dedicated technique.[101] Cheap and stable molecular probes replace costly and unstable antibody aptamers in the AFM chips for target protein enrichment and direct MS identification but require highly sophisticated laboratory equipment.[145] Many of the assays required a thermocycler or thermal equipment due to the requirement of different temperatures in order to bind various components added for detection. Systems such as Genedrive HCV assay are not fully automated but battery-operated and suitable for POCT.[120] Even though Genedrive costs less than molecular methods, the OraQuick® Rapid HCV Test is less expensive than Genedrive. However, to overcome the high morbidity that occurs in

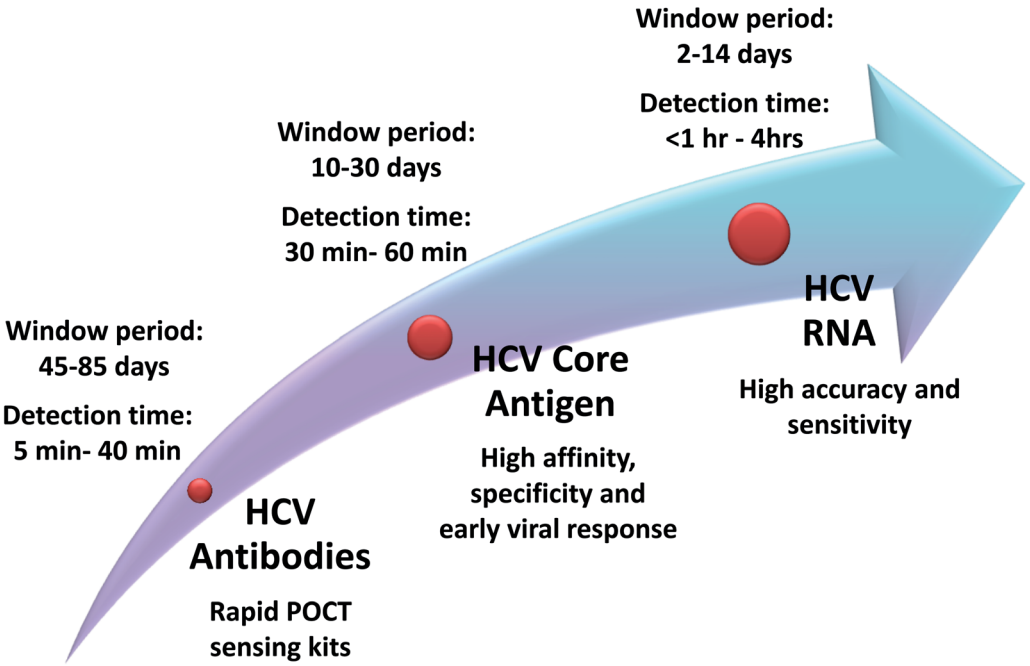

Fig. 10 Characteristic feature of each biomarker detection.

Table 6 HCV diagnostic assays with high sensitivities

Detection	Sample type	Diagnostic tool	Sensitivity	Ref.
HCV RNA	Serum	TAC	100%	116
HCV Ab	Oral fluid	OraQuick® Rapid HCV Test	95.5%	128
	Blood	OraQuick® HCV Rapid Antibody Test	99.9%	129
HCV Ag	Serum and plasma	Magnetic microparticle-based assay	>97%	140
HCV Ag & Ab	DBS	MONOLISA HCV antibody–antigen ULTRA assay	100%	149

multiple hepatitis virus infections, the EuDx-HE (A, B, C) POC test kit was developed to detect anti-HAV IgM, HBsAg, and anti-HCV but the LOD was not evaluated. Even though several POCT are available, filtering unwanted things in the blood or plasma seems problematic. The inexpensive, simple, and low-cost mass production of paper microfluidic enhances blood filtration *via* capillary action and viral detection.[85,175] The current HCV detection and quantification methods are not sensitive enough to detect viral clearance since some individuals show recurrence by the end of treatment.[176] We have listed some of the considerations to be followed while developing any POCT for viral detection.

• LOC design: the design must be simple, flexible, inexpensive, portable, and should provide a long shelf-life period. Furthermore, cross-contamination and evaporation must be reduced.

• Integration: the designed LOC should be in such a way that it is able to be integrated with other thermal sensors, fluoroscopes, or optical detectors for effortless quantization, scaling, or scanning of the viral load.

• Genotype identification: genotype identification LOC devices are obligatory to provide the exact choice of drug for treatment.

• Sensitivity: the diagnostic tool must have lower LOD such that the recurrence of infection after treatment can be reduced.

From these considerations, the ideal design of rapid LOC will pave the way for portable and easy detection in ambient temperature *via* the naked eye or with the help of simple equipment. Furthermore, the integration of microfluidic technology with the Internet-of-Things and artificial intelligence enhances the digital revolution in telediagnosis and improves the automatic quantization of the viral load.[177] This can be achieved only with a teamwork of physicists, chemists, clinical researchers, engineers, materialists, and doctors. Thus, researchers need to focus on developing a novel microfluidic platform to step into the new era of detection modality.

Conclusion and future perspectives

Currently, the costs and complexities of diagnostic algorithms are essential complications in screening and treatment monitoring. With the employment of new treatment procedures, the current pathway for HCV diagnosis can be simplified for randomly-accessible assays or reliable HCV cAg assays to become affordable in high prevalence settings. At present, plenty of detection techniques are available for HCV antibodies.

Besides the availability, the detection can be executed only in the prolonged state of disease. On the other hand, in the case of HCV antigen detection assays, they could be detected within a few listed days. However, the facilities available for this technique are limited. Hence, the researchers must concentrate on developing novel techniques to detect the disease at an early stage. Implementing different diagnostic measures followed in various countries, based on the practice of similar countries who have already taken the initial steps towards HCV prevention and exploring innovative approaches to reduce the cost of these tests through large-scale projects in different contexts, will be the future challenge. Similarly, detection through rapid assay and POC techniques are not full-fledged. Therefore, researchers can focus on these areas to efficiently detect the disease before getting at the CHC stage.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

The authors would like to thank the support from Universiti Teknikal Malaysia Melaka, University Malaysia Pahang, and acknowledge the research grant RDU1803183.

References

1 J. H. Ryu, M. Kwon, J. D. Moon, M. W. Hwang, J. M. Lee, K. H. Park, S. J. Yun, H. J. Bae, A. Choi, H. Lee, B. Jung, J. Jeong, K. Han, Y. Kim and E. J. Oh, *Ann. Lab. Med.*, 2018, **38**, 578–584.
2 Hepatitis C Organ Transplant Program Expands, 2018, https://consultqd.clevelandclinic.org/hepatitis-c-organ-transplant-program-expands/.
3 N. A. Mohamed, Z. Zainol Rashid, K. K. Wong, S. A. Abdullah and M. M. Rahman, *Pak. J. Med. Sci.*, 2013, **29**, 1142–1146.
4 E. Timurdogan, B. E. Alaca, I. H. Kavakli and H. Urey, *Biosens. Bioelectron.*, 2011, **28**, 189–194.
5 J. Akada, S. Kamei, A. Ito, M. Ito, T. Kitagawa, H. Furumoto, Y. Kato, M. Tamesa, M. Takashima, M. Shirai, H. Yamano, M. Oka, Y. Kuramitsu and K. Nakamura, *Proteome Sci.*, 2013, **11**, 33.
6 F. Shen, B. Sun, J. E. Kreutz, E. K. Davydova, W. Du, P. L. Reddy, L. J. Joseph and R. F. Ismagilov, *J. Am. Chem. Soc.*, 2011, **133**, 17705–17712.
7 V. A. Morozov and S. Lagaye, *World J. Hepatol.*, 2018, **10**, 186–212.
8 B. Feng, J. Zhang and L. Wei, *Adv. Med. Educ. Pract.*, 2011, **2**, 209–214.
9 S. S. Hamid, K. Thornton and R. Chou, *Guidelines for the Care and Treatment of Persons Diagnosed with Chronic Hepatitis C Virus Infection*, National Center for Biotechnology Information, Geneva, 2018.
10 P. Gill and A. Ghaemi, *Nucleosides, Nucleotides Nucleic Acids*, 2008, **27**, 224–243.
11 M. K. Araz, A. A. Apori, C. M. Salisbury and A. E. Herr, *Lab Chip*, 2013, **13**, 3910–3920.
12 E. Gupta, M. Bajpai and A. Choudhary, *Asian J. Transfus. Sci.*, 2014, **8**, 19–25.
13 D. Moradpour, F. Penin and C. M. Rice, *Nat. Rev. Microbiol.*, 2007, **5**, 453–463.
14 S. L. Chen and T. R. Morgan, *Int. J. Med. Sci.*, 2006, **3**, 47–52.
15 J. H. Hoofnagle, *Hepatology*, 2002, **36**, S21–S29.
16 G. M. Lauer and B. D. Walker, *N. Engl. J. Med.*, 2001, **345**, 41–52.
17 J. Bukh, *J. Hepatol.*, 2016, **65**, S2–S21.
18 T. Pietschmann and R. J. P. Brown, *Trends Microbiol.*, 2019, **27**, 379–380.
19 M. Carrozzo and K. Scally, *World J. Gastroenterol.*, 2014, **20**, 7534–7543.
20 A. Ozkok, *World J. Gastroenterol.*, 2014, **20**, 7544.
21 S. Grimbert, P. Valensi, C. Levy-Marchal, G. Perret, J. P. Richardet, C. Raffoux, J. C. Trinchet and M. Beaugrand, *Gastroenterol. Clin. Biol.*, 1996, **20**, 544–548.
22 L. Montenegro, A. De Michina, G. Misciagna, V. Guerra and A. Di Leo, *Am. J. Gastroenterol.*, 2013, **108**, 1108–1111.
23 D. Payne, N. L. Zitterkopf and A. Muthukumar, *Lab. Med.*, 2008, **39**, 430–436.
24 Y. P. Wong, S. Othman, Y. L. Lau, S. Radu and H. Y. Chee, *J. Appl. Microbiol.*, 2018, **124**, 626–643.
25 P. Zhang, Y. Bao, M. S. Draz, H. Lu, C. Liu and H. Han, *Int. J. Nanomed.*, 2015, **10**, 6161–6173.
26 O. J. Kweon, Y. K. Lim, H. R. Kim, T. H. Kim and M. K. Lee, *J. Med. Virol.*, 2019, **91**, 1056–1062.
27 H. C. Li and S. Y. Lo, *World J. Hepatol.*, 2015, **7**, 1377–1389.
28 N. I. Goo and D. E. Kim, *BioChip J.*, 2016, **10**, 262–271.
29 S. A. McDonald, R. Mohamed, M. Dahlui, H. Naning and A. Kamarulzaman, *BMC Infect. Dis.*, 2014, **14**, 564.
30 E. Aronoff-Spencer, A. G. Venkatesh, A. Sun, H. Brickner, D. Looney and D. A. Hall, *Biosens. Bioelectron.*, 2016, **86**, 690–696.
31 P. L. Corstjens, Z. Chen, M. Zuiderwijk, H. H. Bau, W. R. Abrams, D. Malamud, R. Sam Niedbala and H. J. Tanke, *Ann. N. Y. Acad. Sci.*, 2007, **1098**, 437–445.
32 S. Parween and P. Nahar, *Biomed. Microdevices*, 2016, **18**, 33.
33 B. D. Smith, J. Drobeniuc, A. Jewett, B. M. Branson, R. S. Garfein, E. Teshale, S. Kamili and C. M. Weinbaum, *J. Infect. Dis.*, 2011, **204**, 825–831.
34 R. Raihan, *Euroasian J. Hepatogastroenterol.*, 2016, **6**, 52–55.
35 X. Mu, L. Zhang, S. Chang, W. Cui and Z. Zheng, *Anal. Chem.*, 2014, **86**, 5338–5344.
36 M. Sinniah and B. G. Ooi, *Singapore Med. J.*, 1993, **34**, 132–134.
37 M. Rau, K. Baur and A. Geier, *Viruses*, 2012, **4**, 3281–3302.
38 C. Zhao and X. Liu, *Biomicrofluidics*, 2016, **10**, 024119.
39 T. van de Laar, O. Pybus, S. Bruisten, D. Brown, M. Nelson, S. Bhagani, M. Vogel, A. Baumgarten, M. L. Chaix, M. Fisher, H. Gotz, G. V. Matthews, S. Neifer, P. White, W. Rawlinson, S. Pol, J. Rockstroh, R. Coutinho, G. J. Dore, G. M. Dusheiko and M. Danta, *Gastroenterology*, 2009, **136**, 1609–1617.

40 R. Mukherjee, A. Burns, D. Rodden, F. Chang, M. Chaum, N. Garcia, N. Bollipalli and A. Niemz, *J. Lab. Autom.*, 2015, **20**, 519–538.
41 I. T. Williams, B. P. Bell, W. Kuhnert and M. J. Alter, *Arch. Intern. Med.*, 2011, **171**, 242–248.
42 R. A. Tohme and S. D. Holmberg, *Clin. Infect. Dis.*, 2012, **54**, 1167–1178.
43 K. Carney, S. Dhalla, A. Aytaman, C. T. Tenner and F. Francois, *Hepatology*, 2013, **57**, 2117–2123.
44 R. Scheinmann, H. Hagan, C. Lelutiu-Weinberger, R. Stern, D. C. Des Jarlais, P. L. Flom and S. Strauss, *Drug Alcohol Depend.*, 2007, **89**, 1–12.
45 M. Sulkowski, W. H. Cheng, S. Marx, Y. Sanchez Gonzalez, J. Strezewski and N. Reau, *Adv. Ther.*, 2020, DOI: 10.1007/s12325-020-01535-3.
46 S. A. McDonald, M. Dahlui, R. Mohamed, H. Naning, F. H. Shabaruddin and A. Kamarulzaman, *PLoS One*, 2015, **10**, e0128091.
47 B. Vicknasingam, S. Narayanan and V. Navaratnam, *Drug Alcohol Rev.*, 2009, **28**, 447–454.
48 N. Hayashi and T. Takehara, *J. Gastroenterol.*, 2006, **41**, 17–27.
49 L. Platt, S. Minozzi, J. Reed, P. Vickerman, H. Hagan, C. French, A. Jordan, L. Degenhardt, V. Hope, S. Hutchinson, L. Maher, N. Palmateer, A. Taylor, J. Bruneau and M. Hickman, *Addiction*, 2018, **113**, 545–563.
50 V. Narayanamurthy, T. Lee, A. a. Khan, F. Samsuri, K. Mohamed, H. Hamzah and M. Baharom, *Fluids*, 2018, **3**, 51.
51 A. J. Tudos, G. J. Besselink and R. B. Schasfoort, *Lab Chip*, 2001, **1**, 83–95.
52 A. M. Streets and Y. Huang, *Biomicrofluidics*, 2013, **7**, 11302.
53 X. Z. Niu, S. L. Peng, L. Y. Liu, W. J. Wen and P. Sheng, *Adv. Mater.*, 2007, **19**, 2682–2686.
54 Z. E. Jeroish, K. S. Bhuvaneshwari, V. Narayanamurthy, R. Premkumar and F. Samsuri, *J. Eng. Sci. Technol.*, 2020, **15**, 1840–1854.
55 D. B. Weibel and G. M. Whitesides, *Curr. Opin. Chem. Biol.*, 2006, **10**, 584–591.
56 E. K. Sackmann, A. L. Fulton and D. J. Beebe, *Nature*, 2014, **507**, 181–189.
57 N. Blow, *Nat. Methods*, 2007, **4**, 665–670.
58 N. Vigneswaran, F. Samsuri, B. Ranganathan and Padmapriya, *Procedia Eng.*, 2014, **97**, 1387–1398.
59 K. Ren, J. Zhou and H. Wu, *Acc. Chem. Res.*, 2013, **46**, 2396–2406.
60 S. Waheed, J. M. Cabot, N. P. Macdonald, T. Lewis, R. M. Guijt, B. Paull and M. C. Breadmore, *Lab Chip*, 2016, **16**, 1993–2013.
61 S. Vázquez-Morón, P. Ryan, H. Ardizone-Jiménez, D. Martín, J. Troya, G. Cuevas, J. Valencia, M. A. Jimenez-Sousa, A. Avellón and S. Resino, *Sci. Rep.*, 2018, **8**, 1858.
62 P. N. Nge, C. I. Rogers and A. T. Woolley, *Chem. Rev.*, 2013, **113**, 2550–2583.
63 H. Liu and R. M. Crooks, *J. Am. Chem. Soc.*, 2011, **133**, 17564–17566.
64 A. W. Martinez, S. T. Phillips, G. M. Whitesides and E. Carrilho, *Anal. Chem.*, 2010, **82**, 3–10.
65 D. M. Cate, J. A. Adkins, J. Mettakoonpitak and C. S. Henry, *Anal. Chem.*, 2015, **87**, 19–41.
66 C. F. Fronczek, T. S. Park, D. K. Harshman, A. M. Nicolini and J.-Y. Yoon, *RSC Adv.*, 2014, **4**, 11103–11110.
67 J. M. Klostranec, Q. Xiang, G. A. Farcas, J. A. Lee, A. Rhee, E. I. Lafferty, S. D. Perrault, K. C. Kain and W. C. W. Chan, *Nano Lett.*, 2007, **7**, 2812–2818.
68 E. B. Tapper, E. B. Cohen, K. Patel, B. Bacon, S. Gordon, E. Lawitz, D. Nelson, I. A. Nasser, T. Challies and N. Afdhal, *Clin. Gastroenterol. Hepatol.*, 2012, **10**, 932–937.
69 S. Chevaliez and J. M. Pawlotsky, *Liver Int.*, 2009, **29**(suppl. 1), 9–14.
70 S. Chevaliez and J. M. Pawlotsky, *World J. Gastroenterol.*, 2007, **13**, 2461–2466.
71 G. Albertoni, M. J. Castelo Girao and N. Schor, *Int. J. Infect. Dis.*, 2014, **25**, 145–149.
72 D. Campos-Outcalt, *J. Fam. Pract.*, 2012, **61**, 744–747.
73 S. Chevaliez and J. M. Pawlotsky, *Best Pract. Res., Clin. Gastroenterol.*, 2008, **22**, 1031–1048.
74 S. Chevaliez and J. M. Pawlotsky, *Ann. Hepatol.*, 2009, **8**, 7–12.
75 H. L. Tillmann, *World J. Gastroenterol.*, 2014, **20**, 6701–6706.
76 E. Sagnelli, N. Coppola, C. Marrocco, G. Coviello, G. Rossi, M. Battaglia, C. Sagnelli, V. Messina, A. Tonziello, C. Scolastico and P. Filippini, *J. Biol. Regul. Homeostatic Agents*, 2003, **17**, 207–210.
77 P. Farci, H. J. Alter, S. Govindarajan, D. C. Wong, R. Engle, R. R. Lesniewski, I. K. Mushahwar, S. M. Desai, R. H. Miller and N. Ogata, *Science*, 1992, **258**, 135–140.
78 N. Yuki, N. Hayashi, K. Ohkawa, H. Hagiwara, M. Oshita, K. Katayama, Y. Sasaki, A. Kasahara, H. Fusamoto and T. Kamada, *Hepatology*, 1995, **22**, 402–406.
79 J. James, M. Sushmitha, R. Premkumar, V. Narayanamurthy and R. Kalpana, *presented in part at the 2017 International Conference on Microelectronic Devices, Circuits and Systems (ICMDCS)*, 2017.
80 Y. Zhao, F. Chen, Q. Li, L. Wang and C. Fan, *Chem. Rev.*, 2015, **115**, 12491–12545.
81 P. J. Asiello and A. J. Baeumner, *Lab Chip*, 2011, **11**, 1420–1430.
82 C. M. Chang, W. H. Chang, C. H. Wang, J. H. Wang, J. D. Mai and G. B. Lee, *Lab Chip*, 2013, **13**, 1225–1242.
83 J. Li and J. Macdonald, *Biosens. Bioelectron.*, 2015, **64**, 196–211.
84 C. M. Cheng, A. W. Martinez, J. Gong, C. R. Mace, S. T. Phillips, E. Carrilho, K. A. Mirica and G. M. Whitesides, *Angew. Chem., Int. Ed. Engl.*, 2010, **49**, 4771–4774.
85 V. Narayanamurthy, Z. E. Jeroish, K. S. Bhuvaneshwari, P. Bayat, R. Premkumar, F. Samsuri and M. M. Yusoff, *RSC Adv.*, 2020, **10**, 11652–11680.
86 B. Pozzetto, T. Bourlet, F. Grattard and L. Bonnevial, *Nephrol., Dial., Transplant.*, 1996, **11**(suppl. 4), 2–5.
87 S. S. Sam, H. B. Steinmetz, G. J. Tsongalis, L. J. Tafe and J. A. Lefferts, *Exp. Mol. Pathol.*, 2013, **95**, 18–22.

88 Y. Chander, J. Koelbl, J. Puckett, M. J. Moser, A. J. Klingele, M. R. Liles, A. Carrias, D. A. Mead and T. W. Schoenfeld, *Front. Microbiol.*, 2014, **5**, 395.
89 H.-C. Chang, Y.-T. Chao, J.-Y. Yen, Y.-L. Yu, C.-N. Lee, B.-C. Ho, K.-C. Liu, J. Fang, C.-W. Lin and J.-H. Lee, *Adv. Mater. Sci. Eng.*, 2015, **2015**, 1–8.
90 M. Liu, C. Y. Hui, Q. Zhang, J. Gu, B. Kannan, S. Jahanshahi-Anbuhi, C. D. Filipe, J. D. Brennan and Y. Li, *Angew. Chem., Int. Ed. Engl.*, 2016, **55**, 2709–2713.
91 D. Desbois, P. Vaghefi, J. Savary, E. Dussaix and A. M. Roque-Afonso, *J. Clin. Virol.*, 2008, **41**, 129–133.
92 S. D. Warkad, K. S. Song, D. Pal and S. B. Nimse, *Sensors*, 2019, **19**, 4257.
93 P. Zhang, Y. Bao, M. S. Draz, H. Lu, C. Liu and H. Han, *Int. J. Nanomed.*, 2015, **10**, 6161–6173.
94 B. Zribi, E. Roy, A. Pallandre, S. Chebil, M. Koubaa, N. Mejri, H. Magdinier Gomez, C. Sola, H. Korri-Youssoufi and A. M. Haghiri-Gosnet, *Biomicrofluidics*, 2016, **10**, 014115.
95 M. Kubista, J. M. Andrade, M. Bengtsson, A. Forootan, J. Jonak, K. Lind, R. Sindelka, R. Sjoback, B. Sjogreen, L. Strombom, A. Stahlberg and N. Zoric, *Mol. Aspects Med.*, 2006, **27**, 95–125.
96 R. Sooknanan, L. T. Malek and B. van Gemen, in *Molecular Methods for Virus Detection*, 1995, pp. 261–285, DOI: 10.1016/b978-012748920-9/50013-3.
97 T. Notomi, H. Okayama, H. Masubuchi, T. Yonekawa, K. Watanabe, N. Amino and T. Hase, *Nucleic Acids Res.*, 2000, **28**, E63.
98 A. Fire and S. Q. Xu, *Proc. Natl. Acad. Sci. U. S. A.*, 1995, **92**, 4641–4645.
99 W. Zhao, M. M. Ali, M. A. Brook and Y. Li, *Angew. Chem., Int. Ed. Engl.*, 2008, **47**, 6330–6337.
100 L. Chen, S. Fatima, J. Peng and X. Leng, *Protein Pept. Lett.*, 2009, **16**, 467–472.
101 S. Kamili, J. Drobeniuc, A. C. Araujo and T. M. Hayden, *Clin. Infect. Dis.*, 2012, **55**(suppl. 1), S43–S48.
102 A. M. Foudeh, T. Fatanat Didar, T. Veres and M. Tabrizian, *Lab Chip*, 2012, **12**, 3249–3266.
103 M. S. Khuroo, N. S. Khuroo and M. S. Khuroo, *PLoS One*, 2015, **10**, e0121450.
104 C. Trucchi, A. Orsi, C. Alicino, L. Sticchi, G. Icardi and F. Ansaldi, *J. Immunol. Res.*, 2016, **2016**, 1412840.
105 S. Chevaliez and J. M. Pawlotsky, *J. Hepatol.*, 2018, **69**, 916–926.
106 A. Mane, J. Sacks, S. Sharma, H. Singh, A. Tejada-Strop, S. Kamili, K. Kacholia, R. Gautam, M. Thakar, R. S. Gupta and R. Gangakhedkar, *PLoS One*, 2019, **14**, e0210556.
107 N. Marwaha and S. Sachdev, *World J. Gastroenterol.*, 2014, **20**, 2948–2954.
108 J. D. Tucker, C. H. Bien and R. W. Peeling, *Curr. Opin. Infect. Dis.*, 2013, **26**, 73–79.
109 L. Duchesne and K. Lacombe, *J. Viral Hepatitis*, 2018, **25**, 108–117.
110 B. Hajarizadeh, J. Grebely and G. J. Dore, *Nat. Rev. Gastroenterol. Hepatol.*, 2013, **10**, 553–562.
111 A. T.-H. Hsieh, P. J.-H. Pan and A. P. Lee, *Microfluid. Nanofluid.*, 2009, **6**, 391–401.
112 C. Roh, H.-Y. Lee, S.-E. Kim and S.-K. Jo, *J. Chem. Technol. Biotechnol.*, 2010, **85**, 1130–1134.
113 R. D. Sochol, B. P. Casavant, M. E. Dueck, L. P. Lee and L. Lin, *J. Micromech. Microeng.*, 2011, **21**, 054019.
114 S. W. Ember, H. Schulze, A. J. Ross, J. Luby, M. Khondoker, G. Giraud, J. G. Terry, I. Ciani, C. Tlili, J. Crain, A. J. Walton, A. R. Mount, P. Ghazal, T. T. Bachmann and C. J. Campbell, *Anal. Bioanal. Chem.*, 2011, **401**, 2549–2559.
115 M. Mukaide, M. Sugiyama, M. Korenaga, K. Murata, T. Kanto, N. Masaki and M. Mizokami, *J. Virol. Methods*, 2014, **207**, 169–177.
116 M. Kodani, T. Mixson-Hayden, J. Drobeniuc and S. Kamili, *J. Clin. Virol.*, 2014, **61**, 260–264.
117 M. Lu, L. Xu, X. Zhang, R. Xiao and Y. Wang, *Biosens. Bioelectron.*, 2015, **73**, 195–201.
118 V. Vaghi, C. Potrich, L. Pasquardini, L. Lunelli, L. Vanzetti, E. Ebranati, A. Lai, G. Zehender, D. Mombello, M. Cocuzza, C. F. Pirri and C. Pederzolli, *Biophys. Chem.*, 2016, **208**, 54–61.
119 J. Chen, X. Xu, Z. Huang, Y. Luo, L. Tang and J. H. Jiang, *Chem. Commun.*, 2018, **54**, 291–294.
120 A. Llibre, Y. Shimakawa, E. Mottez, S. Ainsworth, T. P. Buivan, R. Firth, E. Harrison, A. R. Rosenberg, J. F. Meritet, A. Fontanet, P. Castan, A. Madejon, M. Laverick, A. Glass, R. Viana, S. Pol, C. P. McClure, W. L. Irving, G. Miele, M. L. Albert and D. Duffy, *Gut*, 2018, **67**, 2017–2024.
121 H. Tu, K. Lin, Y. Lun and L. Yu, *Anal. Bioanal. Chem.*, 2018, **410**, 3661–3669.
122 C. Roh, H. Y. Lee, S. E. Kim and S. K. Jo, *Int. J. Nanomed.*, 2010, **5**, 323–329.
123 D. M. Netski, T. Mosbruger, E. Depla, G. Maertens, S. C. Ray, R. G. Hamilton, S. Roundtree, D. L. Thomas, J. McKeating and A. Cox, *Clin. Infect. Dis.*, 2005, **41**, 667–675.
124 H. D. Daniel, P. Abraham, S. Raghuraman, P. Vivekanandan, T. Subramaniam and G. Sridharan, *J. Clin. Microbiol.*, 2005, **43**, 1977–1978.
125 W. Zhang, J. Huang, M. F. Zhou, L. Y. Chen, Y. P. Ding, H. J. Cao, Y. Y. Geng and S. Q. Wang, *Mol. Diagn.*, 2005, **9**, 81–87.
126 L. Duan, Y. Wang, S. S. Li, Z. Wan and J. Zhai, *BMC Infect. Dis.*, 2005, **5**, 53.
127 R. Xu, X. Gan, Y. Fang, S. Zheng and Q. Dong, *Anal. Biochem.*, 2007, **362**, 69–75.
128 S. R. Lee, G. D. Yearwood, G. B. Guillon, L. A. Kurtz, M. Fischl, T. Friel, C. A. Berne and K. W. Kardos, *J. Clin. Virol.*, 2010, **48**, 15–17.
129 S. R. Lee, K. W. Kardos, E. Schiff, C. A. Berne, K. Mounzer, A. T. Banks, H. A. Tatum, T. J. Friel, M. P. Demicco, W. M. Lee, S. E. Eder, A. Monto, G. D. Yearwood, G. B. Guillon, L. A. Kurtz, M. Fischl, J. L. Unangst, L. Kriebel, G. Feiss and M. Roehler, *J. Virol. Methods*, 2011, **172**, 27–31.
130 A. Jewett, B. D. Smith, R. S. Garfein, J. Cuevas-Mota, E. H. Teshale and C. M. Weinbaum, *J. Clin. Virol.*, 2012, **54**, 213–217.

131 B. D. Smith, E. Teshale, A. Jewett, C. M. Weinbaum, A. Neaigus, H. Hagan, S. M. Jenness, S. K. Melville, R. Burt, H. Thiede, A. Al-Tayyib, P. R. Pannala, I. W. Miles, A. M. Oster, A. Smith, T. Finlayson, K. E. Bowles and E. A. Dinenno, *Clin. Infect. Dis.*, 2011, **53**, 780–786.
132 L. M. Villar, H. M. Cruz, J. R. Barbosa, C. S. Bezerra, M. M. Portilho and P. Scalioni Lde, *World J Virol.*, 2015, **4**, 323–342.
133 Y. J. Cha, Q. Park, E. S. Kang, B. C. Yoo, K. U. Park, J. W. Kim, Y. S. Hwang and M. H. Kim, *Ann. Lab. Med.*, 2013, **33**, 184–189.
134 P. Scalioni Lde, H. M. Cruz, V. S. de Paula, J. C. Miguel, V. A. Marques, C. A. Villela-Nogueira, F. A. Milagres, M. S. Cruz, F. I. Bastos, T. M. Andrade, A. R. Motta-Castro, L. L. Lewis-Ximenez, E. Lampe and L. M. Villar, *J. Clin. Virol.*, 2014, **60**, 200–205.
135 C. Zhao and X. Liu, *presented in part at the 2015 Transducers – 2015 18th International Conference on Solid-State Sensors, Actuators and Microsystems (TRANSDUCERS)*, Anchorage, AK, USA, 2015.
136 L. Robin, R. S. Mboumba Bouassa, Z. A. Nodjikouambaye, L. Charmant, M. Matta, S. Simon, M. Filali, S. Mboup and L. Belec, *J. Virol. Methods*, 2018, **253**, 1–4.
137 F. Ansaldi, A. Orsi, L. Sticchi, B. Bruzzone and G. Icardi, *World J. Gastroenterol.*, 2014, **20**, 9633–9652.
138 N. Bouzgarrou, I. Fodha, S. B. Othman, A. Achour, F. Grattard, A. Trabelsi and B. Pozzetto, *J. Med. Virol.*, 2005, **77**, 502–508.
139 K. Seme, M. Poljak, D. Z. Babic, T. Mocilnik and A. Vince, *J. Clin. Virol.*, 2005, **32**, 92–101.
140 T. P. Leary, R. A. Gutierrez, A. S. Muerhoff, L. G. Birkenmeyer, S. M. Desai and G. J. Dawson, *J. Med. Virol.*, 2006, **78**, 1436–1440.
141 S. Lee, Y. S. Kim, M. Jo, M. Jin, D. K. Lee and S. Kim, *Biochem. Biophys. Res. Commun.*, 2007, **358**, 47–52.
142 Y. D. Ivanov, A. L. Kaysheva, P. A. Frantsuzov, T. O. Pleshakova, N. V. Krohin, A. A. Izotov, I. D. Shumov, V. F. Uchaikin, V. A. Konev, V. S. Ziborov and A. I. Archakov, *Int. J. Nanomed.*, 2015, **10**, 1597–1608.
143 A. Archakov, Y. Ivanov, A. Lisitsa and V. Zgoda, *Proteomics*, 2009, **9**, 1326–1343.
144 S. Fourati, J. J. Feld, S. Chevaliez and N. Luhmann, *J. Int. AIDS Soc.*, 2018, **21**(suppl. 2), e25058.
145 T. O. Pleshakova, A. L. Kaysheva, C. Bayzyanova Jcapital Em, C. Anashkina capital A, V. F. Uchaikin, V. S. Ziborov, V. A. Konev, A. I. Archakov and Y. D. Ivanov, *J. Virol. Methods*, 2018, **251**, 99–105.
146 S. Laperche, N. Le Marrec, A. Girault, F. Bouchardeau, A. Servant-Delmas, M. Maniez-Montreuil, P. Gallian, T. Levayer, P. Morel and N. Simon, *J. Clin. Microbiol.*, 2005, **43**, 3877–3883.
147 F. Ansaldi, B. Bruzzone, G. Testino, M. Bassetti, R. Gasparini, P. Crovari and G. Icardi, *J. Viral Hepatitis*, 2006, **13**, 5–10.
148 S. Larrat, C. Bourdon, M. Baccard, C. Garnaud, S. Mathieu, J. L. Quesada, A. Signori-Schmuck, R. Germi, M. Blanc, P. Leclercq, M. N. Hilleret, V. Leroy, J. P. Zarski and P. Morand, *J. Clin. Virol.*, 2012, **55**, 220–225.
149 D. Kania, A. M. Bekale, N. Nagot, A. M. Mondain, L. Ottomani, N. Meda, M. Traore, J. B. Ouedraogo, J. Ducos, P. Van de Perre and E. Tuaillon, *Clin. Microbiol. Infect.*, 2013, **19**, E533–E541.
150 T. L. Applegate, E. Fajardo and J. A. Sacks, *Infect. Dis. Clin. North. Am.*, 2018, **32**, 425–445.
151 N. Kato, M. Hijikata, Y. Ootsuyama, M. Nakagawa, S. Ohkoshi and K. Shimotohno, *Mol. Biol. Med.*, 1990, **7**, 495–501.
152 J. Gomez, M. Martell, J. Quer, B. Cabot and J. I. Esteban, *J. Viral Hepatitis*, 1999, **6**, 3–16.
153 K. Chayama and C. N. Hayes, *J. Gastroenterol. Hepatol.*, 2011, **26**(suppl. 1), 83–95.
154 Y. Wang, *World J. Gastroenterol.*, 2013, **19**, 7910–7921.
155 J. Bukh, R. H. Miller and R. H. Purcell, *Semin. Liver Dis.*, 1995, **15**, 41–63.
156 P. Simmonds, *J. Gen. Virol.*, 2004, **85**, 3173–3188.
157 E. Gower, C. Estes, S. Blach, K. Razavi-Shearer and H. Razavi, *J. Hepatol.*, 2014, **61**, S45–S57.
158 J. P. Messina, I. Humphreys, A. Flaxman, A. Brown, G. S. Cooke, O. G. Pybus and E. Barnes, *Hepatology*, 2015, **61**, 77–87.
159 A. El-Shamy and H. Hotta, *World J. Gastroenterol.*, 2014, **20**, 7555–7569.
160 H. Scheiblauer, M. El-Nageh, S. Nick, H. Fields, A. Prince and S. Diaz, *Transfusion*, 2006, **46**, 708–718.
161 P. L. Corstjens, W. R. Abrams and D. Malamud, *J. Natl. Dent. Assoc.*, 2012, **143**, 12S–18S.
162 G. B. Menezes, F. A. Pereira, C. A. Duarte, T. M. Carmo, H. P. Silva Filho, M. A. Zarife, M. A. Krieger, E. A. Reis and M. G. Reis, *Mem. Inst. Oswaldo Cruz*, 2012, **107**, 680–683.
163 A. M. Kocharekar and M. S. Dukhande, *Int. J. Sci. Res.*, 2017, **6**, 1909–1914.
164 N. Pant Pai and J. Daher, *Expert Rev. Mol. Diagn.*, 2015, **15**, 463–469.
165 G. L. Damhorst, M. Murtagh, W. R. Rodriguez and R. Bashir, *Proc. IEEE*, 2015, **103**, 150–160.
166 V. Narayanamurthy, S. Nagarajan, A. Y. Firus Khan, F. Samsuri and T. M. Sridhar, *Anal. Methods*, 2017, **9**, 3751–3772.
167 M. Thursz and A. Fontanet, *Nat. Rev. Gastroenterol. Hepatol.*, 2014, **11**, 28–35.
168 R. Firdaus, K. Saha, A. Biswas and P. C. Sadhukhan, *World J Virol.*, 2015, **4**, 25–32.
169 R. A. Ponde, *Med. Microbiol. Immunol.*, 2011, **200**, 7–11.
170 L. De Cock, V. Hutse, E. Verhaegen, S. Quoilin, H. Vandenberghe and R. Vranckx, *J. Virol. Methods*, 2004, **122**, 179–183.
171 A. Soulier, L. Poiteau, I. Rosa, C. Hezode, F. Roudot-Thoraval, J. M. Pawlotsky and S. Chevaliez, *J. Infect. Dis.*, 2016, **213**, 1087–1095.
172 A. M. Nicolini, K. E. McCracken and J. Y. Yoon, *J. Biol. Eng.*, 2017, **11**, 7.

173 J. Hedman and P. Radstrom, *Methods Mol. Biol.*, 2013, **943**, 17–48.
174 J. van der Helm, R. Geskus, C. Sabin, L. Meyer, J. Del Amo, G. Chene, M. Dorrucci, R. Muga, K. Porter and M. Prins, *Gastroenterology*, 2013, **144**, 751–760.
175 B. Gao, X. Li, Y. Yang, J. Chu and B. He, *Analyst*, 2019, **144**, 6497–6511.
176 S. D. Warkad, S. B. Nimse, K. S. Song and T. Kim, *Sensors*, 2018, **18**, 3423.
177 V. Narayanamurthy, K. S. Bhuvaneshwari, Z. E. Jeroish and F. Samsuri, *J. Phys.: Conf. Ser.*, 2020, **1502**, 012023.