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Detection of RNA viruses from influenza and HIV to Ebola and SARS-CoV-2: a review

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RNA-based viruses likely make up the highest pandemic threat among all known pathogens in about the last 100 years, since the Spanish Flu of 1918 with 50 M deaths up to COVID-19. Nowadays, an efficient and affordable testing strategy for such viruses have become the paramount target for the fields of virology and bioanalytical chemistry. The detection of the viruses (influenza, hepatitis, HIV, Zika, SARS, Ebola, SARS-CoV-2, *etc.*) and human antibodies to these viruses is described and tabulated in terms of the reported methods of detection, time to results, accuracy and specificity, if they are reported. The review is focused, but not limited to publications in the last decade. Finally, the limits of detection for each representative publication are tabulated by detection methods and discussed. These methods include PCR, lateral flow immunoassays, LAMP-based methods, ELISA, electrochemical methods (*e.g.*, amperometry, voltammetry), fluorescence spectroscopy, AFM, SPR and SERS spectroscopy, silver staining and CRISPR-Cas based methods, bio-barcode detection, and resonance light scattering. The review is likely to be interesting for various scientists, and particularly helpful with information for establishing interdisciplinary research.

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1. Introduction

Viruses are small, nanometer-scale carriers of genetic material. Unable to replicate by themselves, they live inside host cells and use their hosts for replication and assembly. Hence, they are intracellular parasites. In order to exist, viruses must be able to have multiple hosts. Viruses of bacteria, fungi, plants, and animals exist. Furthermore, a single virus can have hosts belonging to different species (*e.g.*, rhinolophid bats and humans). Such viruses can jump from one host species to another. Viruses are not "interested" in killing their host instantaneously because the host's death would lead to the termination of viral spread. Therefore, the majority of viruses do not cause lethality. Some viruses, however, are infectious agents capable of causing illnesses with lethal outcomes. According to the CDC, the flu caused 12 000–61 000 deaths in the USA each year since 2010.

Depending on the type of nucleic acid they carry, viruses are classified into DNA-containing and RNA-containing viruses. DNA-containing viruses can be single-stranded or double-stranded; they are typically more benign than RNA-viruses and mutagenize to a lesser degree. RNA-viruses can be positive-strand, negative-strand or ambisense. Positive-strand RNA viruses contain genomic RNA, which is identical to viral mRNA, and can be translated by a host cell. Negative-strand viruses contain RNA in their genome, which is complimentary to mRNA and is used as a template for transcription by RNA-dependent RNA polymerase. Ambisense viruses contain genomic RNA, part of which behaves as positive-strand, while another part possesses negative-strand characteristics. For their replication, RNA-containing viruses rely on polymerases without stringent proofreading abilities. Contrarily, DNA-containing viruses rely on DNA-polymerase, which has proof-reading properties. Therefore, the genome of RNA-containing viruses is subject to mutations at a much higher rate. This makes RNA-containing viruses rapidly evolving viruses that outnumber DNA-containing viruses. In addition, RNA-containing viruses are more pathogenic as compared to DNA-containing viruses. Examples of RNA-containing viruses that cause diseases include SARS-coronaviruses, influenza, hepatitis A, C, D, E, dengue, Ebola, HIV and other viruses.

Viruses are a diverse group of infectious agents, with more than 100 families characterized to date. Within the current review, we will focus on the laboratory diagnostics of RNA-containing viruses, which are causative agents of diseases with a high mortality rate or with a tendency for pandemic spread.

A multitude of different diagnostics tools exists. They include the detection of viruses, nucleic acids, and antibodies against viruses. The criteria that diagnostics tests should satisfy include a low limit of detection, high sensitivity, high specificity, high accuracy, and rapid speed of diagnosis. The limit of detection refers to the lowest concentration of analyte detected

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by a particular test. Depending on the test, the limit of detection can span a few RNA species in a reaction (for assays that detect nucleic acids) or a few ng mL^{-1} of analyte (for assays that detect antibodies). The sensitivity refers to the ability of a particular test to detect a virus when the virus is present in a sample, and is expressed in % (100% – % of false negative results). Specificity refers to the ability of a particular test to show a negative result when the virus is absent from a sample, and is expressed in % (100% – % of false positive results). Accuracy denotes the percentage of times at which the performed test results are correct. Thus, a high accuracy indicates low percentages of false-positive and false-negative results. The time it takes for a particular test to produce results depends on the test. Antibodies-detecting tests take less time to produce results than nucleic-acid-based tests (such as quantitative real-time PCR). The present review is focused on comparing different viral diagnostic tests through a quantitative perspective.

Cheng *et al.*'s review in *Analytical and Bioanalytical Chemistry* gave a detailed picture of viral diagnostics in 2009,[1] where the detection was described using a method-to-method approach. Since the publication of that review, new lethal viruses have appeared (Ebola and SARS-coronaviruses), and many tests were developed and validated for their detection and diagnostics. The present review is focused on the quantitative analytical parameters in viral diagnostics as well, but it covers the subject from virus to virus. We selectively covered viruses that are lethal to humans, which contain RNA, and which have at least some pandemic potential. Most attention is directed towards the COVID-19 pandemic virus. The papers described in this review typically reported the limit of detection or some other analytical parameters describing the test accuracy (selectivity, sensitivity) (Fig. 1). Moreover, the orthogonal approach of viral detection classified by detection method is briefly shown as a table at the end of the review.

2. Detection of influenza viruses

Influenza is a respiratory disease of viral origin. Two types of viruses, influenza A and influenza B, are causative agents[2] of the disease. Both viruses are negative-strand single-strand RNA viruses. The viral genome is segmented and contains 8 segments. They code for RNA-dependent RNA polymerase (needed to convert negative-strand into positive-strand RNA), haemagglutinin (HA, glycoprotein, required for viral entry), neuraminidase (NA, glycoprotein, needed for viral release), nucleoprotein (NP), matrix protein, membrane protein, nuclear export protein (NEP), and nonstructural proteins (NS). HA and NA proteins have high antigenic variability, contributing to the great diversity of viral subtypes. Influenza is characterized by seasonal epidemics, with an ability to transform into pandemics. The occurrence of pandemics is due to the zoonotic origin of the influenza A virus. It is of particular significance that the influenza A virus can spread among both animals and birds. The pandemics of 1918 and of 2009 (Spanish flu and swine flu, respectively) were caused by the H1N1 viral strain. The pandemic of 1918 resulted in more than 40 million deaths

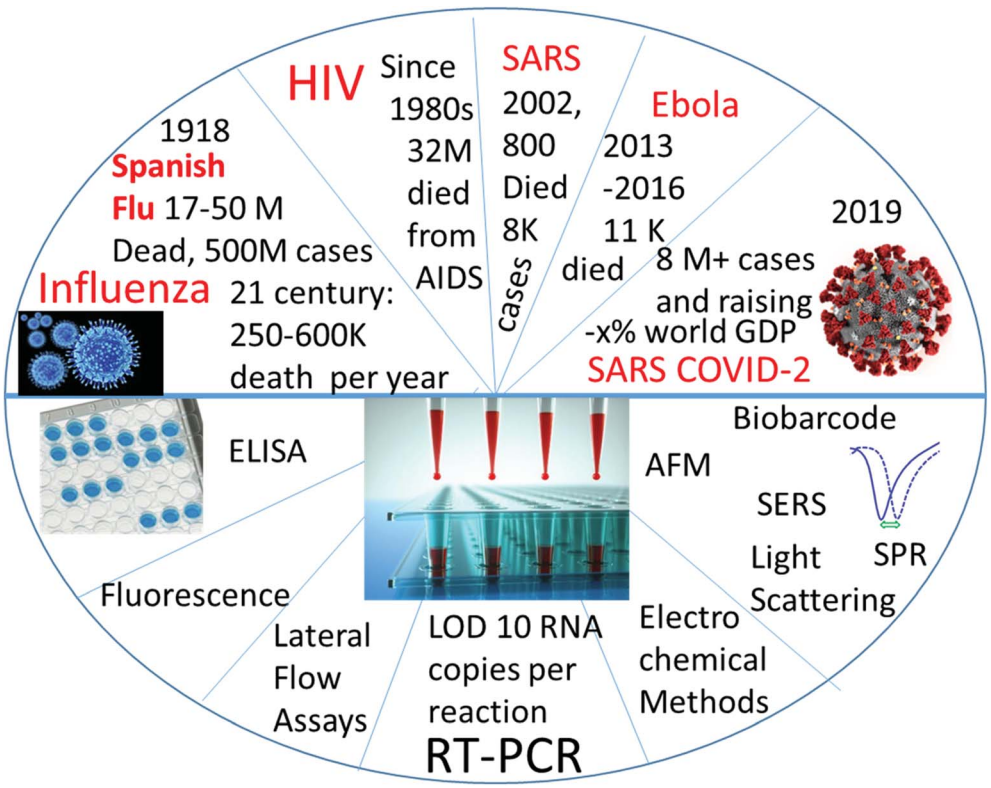

Fig. 1 Graphic Content of the review: detection of RNA-containing pandemic-prone viruses by virus (top half) and by method of detection (bottom half).

worldwide. The 2009 swine flu pandemic was due to the H1N1 influenza A virus, and caused an estimated 201 200 respiratory deaths, together with 83 300 cardiovascular deaths; only 18 500 deaths were laboratory-confirmed.[3] The WHO estimates that 290 000 to 650 000 deaths occur annually worldwide due to influenza-associated respiratory diseases.[4] The CDC estimates that influenza has resulted in between 9 million and 45 million illnesses, between 140 000 and 810 000 hospitalizations, and between 12 000 and 61 000 deaths annually, since 2010 in the USA.[5] The representative test for the detection of the H5N1 influenza virus is shown in Fig. 2, while different methods are presented in Table 1.

3. Detection of RNA-containing viruses of health concern

HIV/AIDS is prevalent mostly in East and Southern Africa, with a prevalence of 7.4% in the region in 2014, which is over 3 times higher than the prevalence in Western and Central Africa. Eastern Europe and Central Asia are the only regions with a rising HIV prevalence, at 0.8% in 2014. The global prevalence was 0.8% in 2014. The morbidity rate is currently at 1.1 million per year (2015).[15] A variety of methods exist for HIV diagnostics. Two such methods are presented in Fig. 2 and 3. Both methods detect the p24 antigen by colloidal gold immunochromatography, and by ELISA with the use of AFM, respectively. In 2014, 10 000 people were infected with Ebola, with 4922 fatalities. The mortality rate for Ebola can reach 90%.[16]

In 2016, more than 85 countries and territories had the Zika virus infection transmitted by mosquitos. Brazil had the greatest impact, with more than 200 000 cases of the Zika virus disease.[17] The Zika virus had a mortality rate of 8.3% in Brazil.[18]

Cases of viral hemorrhagic fever were seen in Zimbabwe, Uganda, the Democratic Republic of Congo, Kenya, and Angola, with a fatality rate of up to 15%.[19]

The number of dengue cases reported to the WHO has increased over 15 fold over the last two decades, from 505 430 cases in 2000 to over 2 400 138 in 2010 and 3 312 040 in 2015. Deaths increased from 960 in 2000 to more than 4032 in 2015.[20]

There are an estimated 1.4 million cases per year of hepatitis A, with 0.5% of mortalities due to viral hepatitis.[21] Globally, an estimated 71 million people have chronic hepatitis C virus infection. The WHO estimated that approximately 399 000 people died from hepatitis C in 2016, mostly from cirrhosis and hepatocellular carcinoma (primary liver cancer).[22] Hepatitis D virus (HDV) globally affects nearly 5% of people who are chronically infected with the hepatitis B virus (HBV). The superinfection of HDV on chronic hepatitis B accelerates the progression to a more severe disease in all ages and in 70–90% of persons.[23] Every year, there are an estimated 20 million HEV infections worldwide. The WHO estimates that hepatitis E caused approximately 44 000 deaths in 2015 (accounting for 3.3% of the mortality due to viral hepatitis).[24] The summary of different methods to detect the outlined viruses of health concern is presented in Table 2.

4. Detection of coronaviruses

Coronaviruses are single-stranded, plus-strand, enveloped RNA-containing viruses, with a relatively large genome averaging at 30 kb pairs. Human coronaviruses (HCoV) 229E, NL63, OC43,

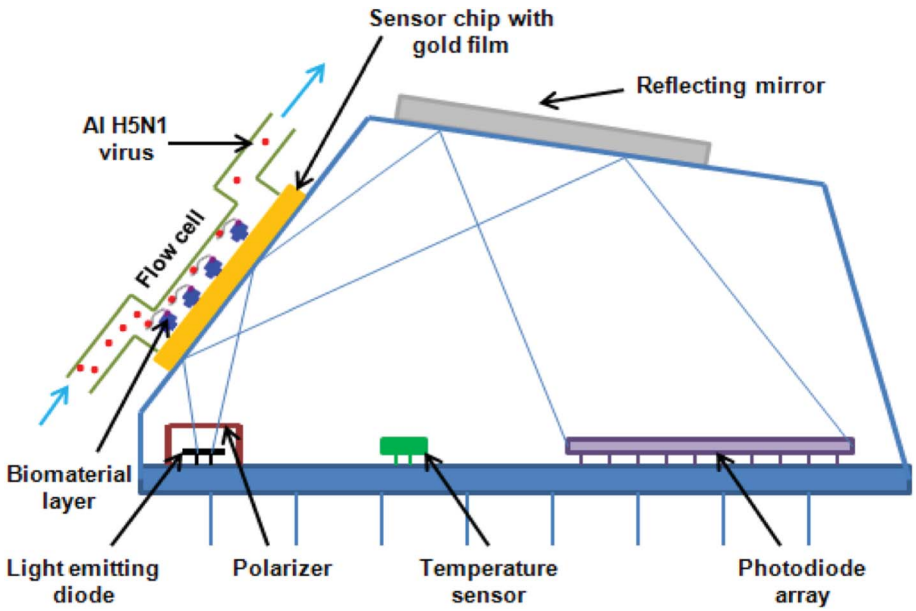

Fig. 2 SPR sensor for the detection of H5N1 avian influenza virus. The sensor is based on the changes of the refractive index of the plasmon as a result of binding the virion to the aptamer. The aptamer is bound to the gold surface of the sensor *via* biotin–streptavidin linkage. The portable sensor is capable of detecting 0.128 HAU, which is equal to $0.17 \times 10^{3.2}$ ELD_{50} per mL. The image is reprinted with permission from H. Bai, R. Wang, B. Hargis, H. Lu and Y. Li, A SPR Aptasensor for Detection of Avian Influenza Virus H5N1, *Sensors*, 2012, **12**(9), 12506–12518.

and HKU1 are known endemic human coronaviruses that cause mild respiratory infection with such symptoms as rhinorrhea and mild cough. Two other coronaviruses – Severe-Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS) coronaviruses – are more virulent, and lead to a severe respiratory disease with symptoms similar to influenza. The SARS coronavirus caused a global epidemic in 2002–2003,[85–87] killing 774 people out of the 8096 infected, and thus having a 9.56% mortality rate. MERS-CoV caused an epidemic in the Middle East that started in 2012.[88] By January 2020, MERS-CoV had killed 866 people out of the 2519 that were sick, and so it has a 34.4% death rate.[89]

Diagnostic tests that use Polymerase Chain Reaction (PCR) allow for the detection of viral nucleic acid. With DNA-containing viruses, the PCR technique is more straightforward, as it depends on the amplification step of isolated viral DNA. The detection of RNA-containing viruses requires an additional step of converting RNA into DNA by reverse transcription. PCR utilizes synthetic single-stranded DNA primers and probes, and depends on the hybridization. Because primers and/or probes might bind nonspecifically to a region different from the intended one, PCR tests can result in false-positive outcomes. On the other hand, false-negative results might result from improperly collected material. The focus of the present review is to compare the key parameters of different test assays, such as specificity and selectivity, to get a quantitative outlook on the test systems.

There are different modalities of PCR, which are useful in viral detection. Among them are quantitative real-time PCR (qPCR), which allows for rapid detection with the identification of relative amounts of the nucleic acid. The quantitative real time RT-PCR (RT-qPCR) method is an efficient method of diagnostics, which allows for the rapid detection of viral nucleic acid. Many RT-qPCR methods are developed for the detection of coronaviruses. The range of methods for the detection of SARS and MERS coronaviruses is summarized in Table 3.

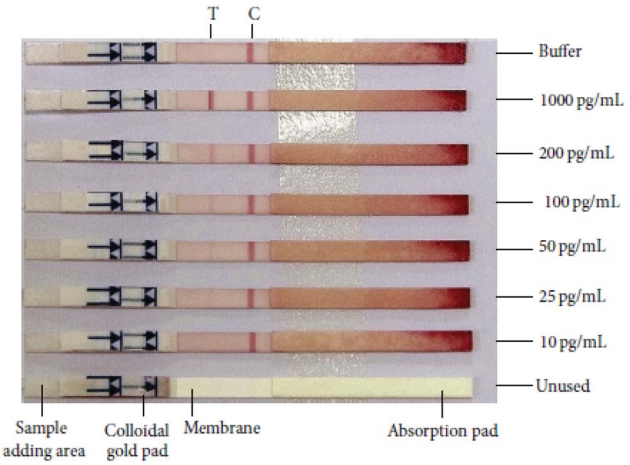

Fig. 3 Colloidal gold immunochromatographic assay to detect the recombinant p24 protein of HIV-1. The reported limit of detection for this method is 25 pg mL^{-1}. It is completed in 20 minutes with an accuracy of 98.03%. The image is reprinted with permission from Ma, Ni *et al.*, Development of Monoclonal Antibodies against HIV-1 p24 Protein and Its Application in Colloidal Gold Immunochromatographic Assay for HIV-1 Detection, *BioMed Research International*, 2016, **2016**, 1–6.

Table 1 Detection of influenza viruses

#	Method	Target/analyte	Volume	Accuracy	Time for test	Ref.
1	Commercial antigen detection tests and RT-qPCR	Swine-origin influenza virus (S-OIV) and seasonal influenza A (H1N1) isolate: antigens and M genes	N/A	N/A	N/A	6
2	– Rapid antigen test – Direct immunofluorescence (DFA) – R-Mix culture – Respiratory Virus Panel (RVP)	H1N1 influenza A antigen	200 μL of respiratory sample	Rapid antigen: 93.6% DFA: 94.5% R-Mix culture: 100% RVP: 100%	N/A	7
3	SPR aptasensor	Avian influenza virus H5N1 virions	N/A	N/A	1.5 hours	8
4	ELISA	Anti-*influenza A virus* nucleoprotein antibodies	15 μL of porcine sera	99.3% Sensitivity – 96.6%	N/A	9
5	A double-antigen sandwich ELISA	Antibodies to influenza A viruses	50 μL of serum sample	97.3% Sensitivity – 98%	N/A	10
6	RT-LAMP on an integrated centrifugal disc	Influenza A (subtypes H1, H3, H5, H7, and H9) and influenza B RNA	25 μL	N/A	45 min	11
7	A nanocomposite of AuNPs and polyols with a dual response	Influenza A virions	N/A	N/A	N/A	12
8	Aptamer-based field-effect transistor	H5N1 avian influenza virus hemagglutinin (HA) protein	3 μL	N/A	5 hours	13
9	Magnetic particle spectroscopy	Influenza A virus subtype H1N1 nucleoprotein	100 μL	N/A	10 s	14

Table 2 Detection of different RNA-containing viruses with health concern

#	Method summary	Analyte	Sample volume	Accuracy	Time	Ref.
1	Colloidal gold immunochromatographic assay (GICA)	p24 (viral protein of HIV-1)	75 μL r-p24 and 75 μL detector mAb	Specificity – 98.03% (1.96% – false-positive)	20 min	25
2	Chemiluminescent magnetic microparticle-based immunoassay (ARCHITECT HIV Ag/Ab Combo)	HIV-1 p24 antigen	N/A	N/A	N/A	26
3	Fluorescent microparticle enzyme immunoassay (AxSYM HIV Ag/Ab Combo)	HIV p24 antigen	N/A	N/A	N/A	26
4	Enzyme-linked fluorescent assay (VIDAS HIV DUO Quick)	HIV p24 antigen	N/A	N/A	N/A	26
5	Enzyme-linked fluorescent assay (VIDAS HIV DUO Ultra)	HIV p24 antigen	N/A	N/A	120 min	26
6	Quantitative enzyme-linked fluorescent immunoassay (VIDAS HIV p24 II)	HIV p24 antigen	N/A	N/A	N/A	26
7	Nanometer-scale antibody array-based analysis with AFM detection	HIV p24 antigen	1 μL	N/A	6 hours; 2–3 hours before measurement	27
8	Ultrasensitive capacitive immunosensor functionalized by anti-HIV-1 p24 mAb	HIV p24 antigen	250 μL	N/A	20 min	28
9	Boosted ELISA based on immune complex dissociation and amplified signal	HIV p24 antigen	100 μL	N/A	>120 min	29
10	Nanoparticle-based biobarcode amplification assay	HIV p24 antigen	100 μL	100%	>120 min	30
11	Colorimetric lateral diffusion immunochromatography	Antibody against HIV	Finger prick blood	N/A	3–30 min	31
12	Electrochemical ELISA	Antibody against HIV-1 and HIV-2 peptides	20 μL	N/A	N/A	32
13	Electrochemical sensor, where antibodies bind to polypeptide epitopes	anti-HIV antibodies	200 μL	N/A	8 min	33
14	Reverse transcription loop-mediated isothermal amplification (RT-LAMP) products are visualized using a lateral flow immunoassay (LFIA): microfluidic rapid and autonomous analysis device (microRAAD)	HIV-1 RNA	12 μL of human whole blood	N/A	90 min	34
15	Luminescence assay	Ebola virus oligonucleotide (RNA)	N/A	N/A	2 days	35
16	Reverse transcription loop-mediated isothermal amplification (RT-LAMP)	Ebola RNA	1 μL of RNA	N/A	1 hour	36
17	ELISA	Ebola virus nucleoprotein (NP)	100 μL rNP	N/A	N/A	37
18	Fluorescence signal for quantitative detection and colorimetric signal for visual detection	Ebola virus glycoprotein	50 μL	N/A	20 min	38

Table 2 (*Contd.*)

#	Method summary	Analyte	Sample volume	Accuracy	Time	Ref.
19	Chemiluminescent ELISA	Antibodies against ebola virus strains Zaire and Sudan	75 μL	Low cross-reactivity	N/A	39
20	Electroluminescent nanospheres and immunomagnetic separation	Ebola virions	200 μL	N/A	2 hours	40
21	Fluorescence assay on a micromagnetic platform	Ebola virions	200 μL EBOV sample	Intra-assay CV = 4.9%	N/A	41
22	SERS immunoassay	Zika virions	10 μL	N/A	N/A	42
23	Aptamer-based ELISA	Zika NS1 protein	100 μL	N/A	N/A	43
24	Field effect biosensing	Zika viral antigen ZIKV NS1	75 μL	1 : 10 dilution: CV = 19.89% 1 : 100 dilution: CV = 9.17%	N/A	44
25	Motion-based immunological detection	Zika virions	10 μL of ZIKV	Correlation: 89.11% with the light microscopy; 100% with CDC Zika MAC-ELISA; 80% with Aptima Zika virus assay	>40 min for assay	45
26	Determination of isothermally Amplified Zika Virus RNA using a Universal DNA-Hairpin Probe	Zika Virus RNA	50 μL	N/A	1 hour	46
27	Advanced strand exchange amplification (ASEA)	Zika Virus RNA	N/A	N/A	25 min	47
28	ELISA	anti-ZIKA IgM antibody	N/A	87.5% positive agreement between CDC and InBios MAC-ELISAs	N/A	48
29	Single particle interferometric reflectance imaging sensor (SP-IRIS) cartridge	HFV virions (model – Ebola virions)	100 μL	N/A	20 min	49
30	Real-time reverse transcription-PCR	HFV RNA	2 μL of RNA	No amplification of HIV-1, hepatitis B and C, herpes simplex type 1, cytomegalovirus, and Modoc viruses	>1 hour	50
31	qRT-PCR	HFV RNA	5 μL of viral RNA	CV < 5%, no cross-reactivity	>1 hour	51
32	RT-LAMP	Crimean-Congo hemorrhagic fever (CCHF) RNA	2 μL of the target RNA	100% agreement between RT-LAMP and the nested PCR	60 min	52
33	Fiber-optic biosensor with chemiluminescence	Crimean-Congo hemorrhagic fever (CCHF) IgG antibodies	200 μL	N/A	90 min	53
34	Loop-mediated isothermal amplification, LAMP	Dengue virus RNA	2 μL of prepared RNA	The area under the ROC curve (AUC) = 0.95	<1 hour	54
35	Biosensor with isothermal nucleic acid sequence-based amplification (NASBA)	Dengue virus RNA, serotypes 1, 2, 3, and 4	2 μL of amplicon (amplified dengue virus RNA)	Serotype 3 displayed low cross reactivity with biosensors designed for the detection of serotypes 1 and 4	15 min	55
36	Tandem toehold-mediated displacement reactions (tTMDR) with fluorescence	Dengue virus RNA	100 μL	N/A	35 min	56
37	Magnetic paper-based ELISA	Dengue immunoglobulin M (IgM) antibodies	5 μL	N/A	N/A	57
38	Long-range surface plasmon polariton (LRSPP) gold (Au) waveguides	Dengue IgM antibody	~10 μL plasma	N/A	N/A	58

Table 2 (*Contd.*)

#	Method summary	Analyte	Sample volume	Accuracy	Time	Ref.
39	Stacking flow immunoassay	Dengue-specific immunoglobulin antibody	100 μL	N/A	N/A	59
40	Separative extended gate field-effect transistor (SEGFET) as an immunosensor	Dengue virus nonstructural protein 1 (NS1)	N/A	N/A	<1 hour	60
41	Lateral flow immunoassay (LFIA)	Dengue NS1 protein	10 mL	N/A	<1 hour	61
42	Magnetic separation and fluorescence detection	Dengue-2 virus virions	20 mL	High specificity in the presence of yellow fever virus	30–60 min	62
43	Electrochemical membrane-based nanobiosensor	Dengue-2 virus virions	5 μL	RSD = 5.9%	N/A	63
44	RT-PCR	Hepatitis A virus (HAV) RNA	5 μL of RNA extracts	N/A	>1 hour	64
45	qRT-PCR	HAV RNA	5 μL of RNA extract	Regression coefficient of 0.9999	>1 hour	65
46	Indirect competitive electrochemical immunosensor	HAV antigen	100 μL	RSD < 3%	N/A	66
47	Electrochemical immunosensor	HAV antigen	1.0 mL min^{-1}	RSD = 3.1–5.7%	5 min	67
48	Solid-phase radioimmunoassay, HAVAB®-M	anti-HAV antibodies	100 μL	RSD = 22%	N/A	68
49	Immunochromatographic assay (ICA)	anti-HAV IgM antibodies	5 μL	Specificity = 100%	<20 min	69
50	Resonance light scattering (RLS) sensor	HAV virions	N/A	RSD = 1.3%	N/A	70
51	Multifunctional molecularly imprinted fluorescence sensor	HAV virions	200 μL	RSD < 2.7%	20 min	71
52	A reduced graphene oxide-assisted hybridization chain reaction + fluorescence	Hepatitis C virus (HCV) RNA	N/A	RSD = 3–6.4%	>8 hours	72
53	Capture of RNA with probes and paramagnetic particle separation	HCV RNA	300 μL of serum	93% sensitivity and 100% specificity	>1 hour	73
54	Electrochemical immunosensor	HCV antigen	1.0 mL min^{-1}	RSD = 2.3–5.3%	5 min	67
55	Chemiluminescent magnetic particle-based immunoassay	HCV core Antigen	A few hundred μL	99% specificity; 97.4% sensitivity	200 assays per hour	74
56	Sandwich electrochemical immunosensor	HCV core antigen	≈10 μL	RSD = 3.1%	30 min	75
57	Nano-gold immunological amplification and silver staining (NIASS)	anti-HCV antibodies	10 μL	N/A	<40 min	76
58	Antibody-induced DNA strand displacement and rolling circle amplification (RCA)	anti-HCV antibodies	1 μL	"High specificity"	30 min	77
59	Immunogold electron microscopy	HCV virions	3 μL	N/A	>3 hours	78
60	Dual-targeting real-time RT-PCR	Hepatitis D Virus (HDV) RNA	140 μL viral sample	N/A	>1 hour	79
61	Real-time PCR	HDV RNA	200 μL specimen	N/A	>20 min	80
62	Electrochemical immunosensor	HDV antigen	1.0 mL min^{-1}	RSD = 3.4–6.8%	5 min	67

Table 2 (*Contd.*)

#	Method summary	Analyte	Sample volume	Accuracy	Time	Ref.
63	IgM capture enzyme immunoassay (EIA)	HDV antibodies IgM anti-HD	100 μL	No cross-reactivity with other antibodies	2 days	81
64	Real-time RT-PCR	Hepatitis E virus (HEV) subtype 3b RNA	5 μL of extracted RNA	N/A	>1 hour	82
65	Array-based nano-amplification and silver stain enhancement	HEV RNA	100 μL	N/A	20–30 min	83
66	Electrochemical immunosensor	HEV antigen	1.0 mL min^{-1}	RSD = 3.4–6.9%	5 min	67
67	Enzyme immunoassay (EIA)	Antibody to the hepatitis E virus (anti-HEV)	250 μL	N/A	N/A	84

5. Detection of SARS-CoV-2 (COVID-19 pandemic virus)

On December 31, 2019, the WHO Chinese Country Office was notified about pneumonia cases of an unknown nature, epidemiologically linked to the seafood market in Wuhan, Hubei province.[97] At the same time, the Chinese CDC conducted an investigation in the field.[98] The viral nature of the disease was identified, 3 viral genomes from the bronchoalveolar lavage of three disease-affected individuals were sequenced, and the sequences were submitted to GISAID (accession ID: EPI_ISL_402119; EPI_ISL_402120; EPI_ISL_402121). According to the sequencing data, the novel virus belongs to the *Coronaviridae* family, *Orthocoronaviridae* subfamily, *Betacoronavirus* genus, *Sarbecovirus* subgenus. The viral genome contains a 5′ untranslated region (5′-UTR), replicase gene (orf1ab), Spike gene (S gene), Envelope gene (E gene), M gene, Nucleocapsid gene (N gene), and open reading frames 3, 7, 8, 10b, 13, and 14. The virus was named novel coronavirus 2019-nCoV, and the disease it causes was named novel coronavirus-infected pneumonia (NCIP) by the Chinese CDC.[99] Later, the WHO renamed the virus into SARS-CoV-2, and the disease into COVID-19.[100] The virus is highly contagious with easy person-to-person transmission, has a variable incubation period (from 4 to 24

Table 3 Detection of SARS and MERS coronaviruses

#	Method summary	Analyte	Sample volume	Accuracy	Time	Ref.
1	RT-PCR and indirect immunofluorescence serologic testing	SARS-CoV RNA	2 mL nasopharyngeal aspirates and 2 g feces	RT-PCR: 60% for positive cases; 99.4% for negative cases Serologic testing: 92% for positive cases; 92% for negative cases	RT-PCR: N/A Serologic testing: >1 hour	90
2	Real time RT-PCR (2 assays: for upE and (ORF)1b)	SARS-CoV RNA: upstream of the E gene (upE) or within open reading frame (ORF)1b	5 μL of extracted RNA	100% specificity for both for upE and (ORF)1b	36+ hours	91
3	Chemiluminescence immunosorbent assay with nanoarray RNA aptamer	SARS-CoV nucleocapsid protein (SARS-CoV N protein)	N/A	C-terminal domain or dimer form N protein is specifically recognized by the aptamer	N/A	92
4	Biosensor assay based on an optical QDs-based RNA aptamer	SARS-CoV nucleocapsid protein (SARS-CoV N protein)	N/A	QDs-conjugated RNA aptamer is selective against the SARS-CoV N protein	1 hour	93
5	Asymmetric five-primer reverse transcription loop-mediated isothermal amplification (RT-LAMP) assay	MERS-CoV RNA (3 genetic loci: ORF1a, ORF1b and E)	4 μL RNA	N/A	30–50 minutes	94
6	ELISA	MERS-CoV nucleocapsid protein (NP)	50 μL of sample	100% specificity	N/A	95
7	ELISA and plaque-reduction neutralization test (PRNT)	Antibodies against MERS-CoV	N/A	No serotypic discrimination between the MERS-CoV strains	N/A	96

Table 4 Detection of SARS-CoV-2

#	Method summary	Analyte	Sample volume	Accuracy	Time	Ref.
1	Real time RT-PCR Commercial kit from Altona diagnostics, Hamburg, Germany	E-gene RNA and S-gene RNA	30 μL	N/A	>1 hour	111 and 112
2	ePlex-based (DNA hybridization and electrochemical detection) SARS CoV-2	cDNA coding for nucleocapsid (N)	200 μL of nasopharyngeal swab	Detection of positive – 94.4%; 95% CI – 74.2–99% Detection of negative – 100%; 95% CI – 92.4–100%	>1 hour	111 and 113
3	Real-time RT-PCR	RNA coding for RdRp gene	N/A	N/A	>1 hour	114
4	Real-time RT-PCR	SARS-CoV-2 RNA coding for nucleocapsid gene (N1 probe)	20 μL	N/A	>1 hour	111 and 114
5	Real-time RT-PCR	SARS-CoV-2 RNA coding for nucleocapsid gene (N2 probe)	20 μL	N/A	>1 hour	114
6	Real-time RT-PCR (E-gene assay, and RdRp gene assay)	SARS-CoV-2 RNA coding for envelope (E) gene and RNA-dependent RNA polymerase (RdRp) gene	25 μL	No reactivity on human coronaviruses	>1 hour	105
7	Real-time RT-PCR	Co-V2 viral RNA; probe against nucleocapsid gene (N and N2 assays)	N/A	N/A	>1 hour	115
8	Real-time RT-PCR	SARS-CoV-2 RNA	5 μL RNA	No cross-reactivity with other human-pathogenic coronaviruses and respiratory pathogens	>1 hour	116
9	RT-PCR: Cepheid Xpert Xpress and Roche cobas assays	SARS-CoV-2 RNA	N/A	Both systems have agreement of 99%	45 min (Cepheid); 90 min (Roche)	117
10	RT-LAMP (reverse transcription loop-mediated isothermal amplification assay)	Viral RNA coding orf1ab gene and S gene	25 μL	Sensitivity – 100% (95% CI 92.3–100%) Specificity – 100% (95% CI 93.7–100%)	26.28 ± 4.48 min	107
11	Colorimetric LAMP	SARS-CoV-2 RNA	3 μL RNA	100% agreement with RT-PCR	30 min	118
12	RT-LAMP	Viral RNA coding for conserved region within nucleocapsid gene	N/A	Sensitivity – 100% Specificity – 98.7%	30 min (colorimetric visualization)	108
13	RT-LAMP	Viral RNA within RdRp gene	25 μL	100% consistency with RT-qPCR on positive samples	50 min real-time monitoring	109
14	DETECTR (SARS-CoV-2 DNA endonuclease-targeted CRISPR trans reporter) – CRISPR-Cas12-based assay	Viral RNA coding for nucleoprotein and envelope genes	N/A	95% for positive samples, 100% for negative samples	30–40 min (sample-to-result)	110
15	ELISA	SARS-CoV-2 neutralizing, spike- and nucleocapsid-specific antibodies	N/A	87–100% specificity	2 days	119
16	COVID-19 IgG/IgM rapid test Cassette	SARS-CoV-2-specific IgM and IgG	5 μL serum	Specificity 100% for IgM and 99.2% for IgG Sensitivity 69% for IgM and 93.1% for IgG	15 min	120
17	Colloidal gold Antibodies test	SARS-CoV-2-specific IgM and IgG	10 μL of serum	Specificity: IgM, 50.0%; IgG, 87.5%	N/A	121
18	Single molecule array (Simoa) immunoassay	SARS-CoV-2 nucleocapsid protein (N-protein)	20 μL of whole blood	100% specificity and 97.4% sensitivity	N/A	122
19	Pulse-controlled amplification (PCA)	SARS-CoV-2 RNA (E gene)	45 μL	100% agreement with RT-qPCR	20 min	123
20	LAMP	SARS-CoV-2 RNA	N/A	N/A	<30 min	124

Table 4 (*Contd.*)

#	Method summary	Analyte	Sample volume	Accuracy	Time	Ref.
21	Reverse-transcription recombinase-aided amplification (RT-RAA)	SARS-CoV-2 RNA	140 μL nasopharyngeal swabs and sputum	100% agreement with RT-PCR	5–15 min	125

Table 5 Detection of RNA-containing lethal viruses: classification by method of detection

PCR-based nucleic acid detection				
Analyte	Ref.	Analytical parameters	Ref.	Analytical parameters
Viral hemorrhagic fever (HF) viruses: Ebola, Marburg, Lassa, Crimean-Congo HF, Rift Valley fever, dengue, yellow fever viruses	50, 2002	LOD = 1545 to 2835 viral genome equivalents per mL of serum (8.6 to 16 RNA copies per assay)	51, 2014	LOD = between 45 and 150 cRNA/rxn
Hepatitis A	64, 2009	LOD = 1 PFU/1.5 L	65, 2010	LOD = 10 PFU/1.5 L of bottled water, 100 PFU/1.5 L of tap water
Hepatitis C	73, 2000	LOD = 33 cRNA per mL; ($\sim 1.74 \times 10^{-4}$ pg mL^{-1})		
Hepatitis D	79, 2018	LOD = 575 IU mL^{-1}	80, 2013	LOD = 7500 HDV cRNA per mL; 190 cRNA/rxn (~0.28 pg mL^{-1})
Hepatitis E	82, 2013	LOD = 25 UI mL^{-1}		
Seasonal influenza A	6, 2009	LOD = $\log_{10}$ 6.5–7.1 of M gene copies		
Swine-origin influenza A	6, 2009	LOD = $\log_{10}$ 6.5–7.3 of M gene copies		
SARS-CoV	90, 2004	LOD = 10 cRNA/rxn; Ac = 60% for positive cases, 99.4% for negative cases	91, 2012	LOD = 3.4 cRNA/rxn for upstream of the E gene (upE) ($\sim 1.11 \times 10^{-2}$ pg mL^{-1}) and 64 cRNA/rxn for within open reading frame (ORF)1b, Sp = 100%
SARS-CoV-2	116, 2020	LOD = 11.2 cRNA/rxn ($\sim 3.67 \times 10^{-2}$ pg mL^{-1})	111, 2020	LOD = 24 cRNA/rxn ($\sim 1.97 \times 10^{-2}$ pg mL^{-1})
	105, 2020	LOD = 3.8 cRNA/rxn ($\sim 2.49 \times 10^{-3}$ pg mL^{-1}) – RdRp-gene assay; 5.2 cRNA/rxn ($\sim 3.41 \times 10^{-3}$ pg mL^{-1}) – E-gene assay	115, 2020	LOD = 25 and 250 cRNA/rxn
Lateral flow immunoassays				
HIV-1 p24 antigen	25, 2016	LOD = 25 pg mL^{-1}		
Ab against HIV	31, 2006	LOD = sub pmol L^{-1} range		
HIV-1 RNA	34, 2019	LOD = 3×10^5 HIV-1 viral particles, or 2.3×10^7 virus copies per mL of whole blood		
Dengue NS1 protein	61, 2020	LOD = 5 ng mL^{-1}		
ELISA				
HIV p24 antigen	29, 2003	LOD = 0.5 pg mL^{-1}		
Ab to HIV-1, HIV-2	32, 2013	LOD = 1 ng mL^{-1} (6.7 pM)		
Ebola virus nucleoprotein (NP)	37, 2001	LOD = 30 ng of purified recombinant NP (rNP) ($\sim 3.00 \times 10^5$ pg mL^{-1})		
Zika NS1 protein	43, 2017	LOD = 0.1 ng mL^{-1}		
IgM-dengue antibodies	57, 2017	LOD = 0.04 μg mL^{-1}		
MERS-CoV nucleocapsid protein (NP)	95, 2015	LOD = 10 $TCID_{50}$/0.1 mL		

Table 5 (*Contd.*)

PCR-based nucleic acid detection				
Analyte	Ref.	Analytical parameters	Ref.	Analytical parameters
AFM				
HIV p24 antigen	27, 2004	LOD = 25 fg mL^{-1}		
Electrochemical detection				
HIV p24 antigen	28, 2010	LOD = 7.9×10^{-8} pg mL^{-1}		
Anti-HIV antibodies	33, 2012	LOD = 1–10 nM (~1.50×10^5 pg mL^{-1})		
Ebola virions	40, 2017	LOD = 5.2 pg mL^{-1}		
Zika viral antigen ZIKV NS1	44, 2018	LOD = 450 pM		
Zika Virus RNA	46, 2019	LOD = 1.11 fg μL^{-1} (~0.3 fM)		
Dengue virus nonstructural protein 1 (NS1)	60, 2014	LOD = 0.25 μg mL^{-1}		
Dengue-2 virions	63, 2012	LOD = 1 PFU mL^{-1}		
H5N1 avian influenza virus hemagglutinin (HA) protein	13, 2020	LOD = 5.9 pM		
Hepatitis A antigen	66, 2017	LOD = 26×10^{-5} IU/mL	67, 2010	LOD = 0.5 ng mL^{-1}
Hepatitis C antigen	67, 2010	LOD = 0.8 ng mL^{-1}	75, 2017	LOD = 3 fg mL^{-1}
Hepatitis D antigen	67, 2010	LOD = 0.5 ng mL^{-1}		
Hepatitis E antigen	67, 2010	LOD = 1 ng mL^{-1}		
Chemiluminescence detection				
HIV p24 antigen	26, 2011	LOD = 18–25 pg mL^{-1} or 1.24 IU mL^{-1}		
Hepatitis C Virus (HCV) core antigen	74, 2006	LOD = viral concentration equivalent to the lowest titer of 2415 cRNA/mL		
Fluorescence detection				
HIV p24 antigen	26, 2011	LOD = 22–77.4 pg mL^{-1} or 1.94–2.25 IU mL^{-1}	26, 2011	LOD = 13 pg mL^{-1} or 0.43 IU mL^{-1}
	26, 2011	LOD = 11.5–25 pg mL^{-1} or 0.66 IU mL^{-1}	26, 2011	LOD = 11.2 pg mL^{-1} or 0.73–1.15 IU mL^{-1}
Ebola RNA	35, 2016	LOD = femtomolar level		
Ebola glycoprotein	38, 2017	LOD = 0.18 ng mL^{-1}		
Ebola virions	41, 2018	LOD = 2.6 pg mL^{-1}		
Dengue virus RNA	56, 2018	LOD = 6 cRNA per sample (~3.62×10^{-4} pg mL^{-1})		
Dengue-2 virions	62, 2008	LOD = 10 PFU mL^{-1}		
HAV virions	71, 2019	LOD = 3.4 pmol L^{-1}		
HCV RNA	72, 2019	LOD = 10 fM		
anti-HCV Ab	77, 2019	LOD = 0.998 pM		
SARS-CoV nucleocapsid protein (SARS-CoV N protein)	92, 2009	LOD = 2 pg mL^{-1}		
LAMP-based nucleic acid detection				
Ebola	36, 2017	LOD = 100 cRNA (~1.04 pg mL^{-1})		
Crimean-Congo hemorrhagic fever	52, 2013	LOD = 0.1 fg of viral RNA (equivalent to 50 viral particles; ~0.05 pg mL^{-1})		
Dengue	54, 2020	LOD = 10^2 PFU per 200 μL of whole blood		
Influenza A	11, 2020	LOD of subtypes H1: 50 copies H3: 20–50 copies H5: 50 copies H7: 20–50 copies H9: 50–100 copies		

Table 5 (*Contd.*)

PCR-based nucleic acid detection				
Analyte	Ref.	Analytical parameters	Ref.	Analytical parameters
Influenza B	11, 2020	LOD = 50 copies		
MERS-CoV	94, 2015	LOD = 0.02 to 0.2 PFU (5 to 50 PFU mL^{-1})		
SARS-CoV-2	118, 2020	LOD = 120 cRNA/rxn (or 4.8 copies per μL); ~7.87×10^{-2} pg mL^{-1}	107, 2020	LOD = 20 cRNA/rxn – ORF1ab gene (~1.31×10^{-2} pg mL^{-1}) and 200 cRNA/rxn – S gene (~0.131 pg mL^{-1})
	109, 2020	LOD = 3 cRNA/rxn (~1.97×10^{-3} pg mL^{-1})		
CRISPR-Cas based				
SARS-CoV-2	110 and 128, 2020	LOD = 10 cRNA/rxn (~0.164 pg mL^{-1})		
Biobarcode detection				
HIV p24 antigen	30, 2007	LOD = 0.1 pg mL; Ac = 100%		
Motion-based detection				
Zika virions	45, 2018	LOD = 1 particle/μL		
NASBA-based nucleic acid detection				
Zika	46, 2019	LOD = 1.11 fg μL^{-1} (~0.3 fM)		
Dengue	55, 2002	LOD = 10 PFU mL^{-1}		
Advanced strand exchange amplification-based nucleic acid detection				
Zika	47, 2018	LOD = 1.0×10^{-15} M; (~33 pg mL^{-1})		
Interferometric reflectance imaging				
HFV virions	49, 2017	LOD = 10 viruses per spot		
Surface plasmon polariton waveguides				
Dengue-specific immunoglobulin M (IgM) antibody	58, 2014	LOD = ~22 pg mm^{-2}		
SERS (Surface Enhanced Raman Spectroscopy)				
Zika virions	42, 2018	LOD = 10 ng mL^{-1}		
FCV virions	129, 2005	LOD = 10^6 viruses per mL		
Radioimmunoassay				
Anti-HAV Ab	68, 1993	LOD = 10 mIU mL^{-1}		
Resonance light scattering				
HAV virions	70, 2017	LOD = 8.6 pmol L^{-1}		
Silver staining				
HCV antibodies	76, 2005	LOD = 3 ng mL^{-1}		
HEV RNA	83, 2006	LOD = 100 fM; (~237.6 pg mL^{-1})		
Electron microscopy				
HCV virions	78, 2006	LOD = 10^7 virions per mL		
SPR				
AIV H5N1 virions	8, 2012	LOD = 0.128 HAU		
Confocal laser scanning microscopy				
SARS-CoV nucleocapsid protein	93, 2011	LOD = 0.1 pg mL^{-1}		

Table 5 (*Contd.*)

PCR-based nucleic acid detection				
Analyte	Ref.	Analytical parameters	Ref.	Analytical parameters
Magnetic particle spectroscopy				
H1N1 nucleoprotein molecule	14, 2020	LOD = 4.4 pmoles		
Nanocomposite-based optical and mechanical detection				
Influenza A virions	12, 2020	LOD = 5×10^7 PFU mL^{-1}		

days),[101] and leads to the development of respiratory diseases with variable symptoms, from a mild cough to pneumonia.[102] Sometimes, the infected person does not show any symptoms whatsoever.[103] Soon after the disease outbreak, close monitoring of the epidemiological situation around the globe started with a real-time count of new cases in the world.[104] Strict quarantine measures were implemented in the countries most affected by the disease, and on March 11th, the WHO declared a state of pandemic. Accurately monitoring the virus and its spread is not possible without reliable diagnostic tools. So, soon after the outbreak, the development of tests detecting SARS-CoV-2 itself or the immune response in the affected person was initiated.

A sample for the test includes material from the upper and lower respiratory tracts, and can include aspirates, oropharyngeal and nasopharyngeal swabs, bronchoalveolar lavage, and sputum, as well as nasal and nasopharyngeal aspirate. Test results depend on the quality of the collected material, as well as on the type of material.

Multiple assays were developed to test the presence of viral nucleic acid. The tests for SARS-CoV-2 detection employ a probe to detect the sequence within the RNA-dependent RNA polymerase viral gene (RdRp), as well as the nucleocapsid gene (NP), envelope protein gene (E), and spike protein gene (S). Some tests rely on the usage of fluorescently labeled TaqMan probes with a fluorescent reporter and a quencher attached to the 5′- and 3′-ends of the probe, respectively. Examples of such probes include probes with 6-carboxyfluorescein (FAM) or HEX dye at their 5′-end, and with a blackberry quencher (BBQ) or blackhole quencher (BHQ) at the probe's 3′-end.[105] The 5′-exonuclease ability of DNA-polymerase removes the fluorescently-labeled 5′-end of the hybridized probe, which leads to probe degradation and unquenching of the fluorescent reporter. There are 14 probes provided by the WHO, and one described by Zhu *et al.*[98] Most of them use TaqMan probes, with one exception from Japan that relies on nested PCR instead. Information about the limit of detection and the accuracy of the real-time reverse-transcription tests is gathered in Table 4.

Alongside real-time RT-PCR, isothermal hybridization is a method of choice for viral RNA detection in a shorter period of time, as compared to real-time RT-PCR. Originally invented by Notomi,[106] the so-called LAMP (loop-mediated isothermal amplification) coupled to reverse transcription is also used for SARS-CoV-2 detection,[107–109] and allows for the rapid detection of viral RNA within a time frame of less than 1 hour. The method relies on the strand-displacing ability of the Bst polymerase, and needs 4 to 6 primers, which increases the target selectivity of this method, as compared to regular PCR with only 2 primers.[106] The method can be run on a regular thermostat at 65 °C. The sensitivity of the RT-LAMP method is slightly lower (~10-fold) than that of real-time RT-PCR[108,109] for SARS-CoV-2 detection.

The most novel method of viral detection, and possibly the most intriguing one, uses CRISPR-Cas 12 endonuclease and isothermal amplification.[110] This method, named DETECTR, is very rapid, allowing for the detection of the viral RNA within minutes. It is a very promising point-of-care test that does not require expensive equipment and can be used in developing countries.

6. Summary of detection methods

Nucleic acid detection methods including PCR and LAMP

Comparing the different methods of viral detection, PCR-based methods can detect a few copies of RNA per reaction, which places them on the high-sensitivity spectrum, corresponding to a fg mL^{-1} concentration range. PCR detection sensitivity is comparable between different viruses, with a range of detection from a few copies per reaction to a few hundreds of copies per reaction. PCR-based methods require trained personnel and expensive equipment, such as the PCR thermocycler. They are also time-consuming and can take several hours. In order to facilitate PCR-based techniques without compromising selectivity and specificity, alternatives to RT-qPCR exist. These include the LAMP technique. LAMP has a limit of detection comparable to that of RT-PCR, spanning from 3 copies of RNA per reaction to 100 copies of RNA per reaction, as evidenced from 5 sources presented in Table 5.

LAMP-based diagnostic tests allow for the rapid detection of the analyte, without a need for expensive equipment. Results can be obtained rapidly (within an hour), which makes these tests a suitable platform to be used for the development of point-of-care diagnostics tests. Alongside LAMP, the NASBA-based nucleic acid detection,[46,55] as well as the advanced strand exchange amplification-based nucleic acid detection, are used and demonstrate a low limit of detection. A standard PCR test can also be incorporated into the assay, with other methods of separation such as laser-irradiated DNA extraction, paramagnetic particle separation, and others. Today, stationary PCR-based methods requiring expensive and complicated

equipment are accompanied by bench scale PCR detection with portable devices and automated procedure, where only one step of sample loading is necessary, such as with the "Cepheid Xpert Xpress" and "Roche cobas" assays.[117]

Immunoassay-based methods

The average limit of detection for ELISA-based methods is within the pg mL^{-1} range, but has been demonstrated to be as low as a fraction of pg mL^{-1}.[29] ELISA-based methods are routinely used and have demonstrated their applicability for an extended period. Despite this, they require trained personnel and are time-consuming. Therefore, alternative methods are being developed to make diagnostics tests relatively cheap and affordable without compromising their sensitivity and selectivity. One of these methods is lateral flow immunoassay. This method has shown a limit of detection at the pg mL^{-1} level, which is comparable to fine ELISA-based methods, but lateral flow immunoassays are faster. For example, a standard ELISA can take anywhere from 2 hours[29] to 2 days,[119] as studies show. However, lateral flow immunoassays take from 20 minutes[47] to 90 minutes,[34] on average. This makes lateral flow immunoassays a good alternative to ELISA, especially because they can be performed at a small scale. The selectivity of immunoassay-based methods allows for the distinguishing of viruses with 100% specificity,[95] but not for making distinctions between different strains of the same virus.[96] Like PCR-based methods, immunoassay-based methods are now commercially available in bench scale, portable, fully automated devices, suitable for point-of-care testing.[120] However, these are currently qualitative, and their sensitivity is unknown. Immunoassay-based methods can detect a viral antigen or antibodies to the virus in the sample. The latter is more widespread for diagnostic testing, but can only be conclusive after the onset of symptoms (on average, a week after infection).

Electrochemical detection methods

Electrochemical detection methods provide sensitive detection of a wide range of analytes: virions,[40] viral antigen,[44] antibodies to the virus,[33] and viral nucleic acids.[46] The main advantage of these detection methods is that they are relatively inexpensive and not limited by a diffraction limit (like optical methods). The lowest limit of detection was reported by Teeparuksapun *et al.*[28] to be at a subattogram per milliliter concentration. Other studies show a pg mL^{-1} to fg mL^{-1} limit of detection range, which is comparable to PCR and immunoassays. The specificity of these methods is also high.[33] Notably, these detection methods also provide reproducible results with RSD < 5%, such as the voltammetry method of Tang *et al.*,[67] which allows the multiplex detection of five analytes (viral antigens). Multiplex analyte detection has been reported in many research papers, which gives electrochemical methods an advantage over PCR and immunoassays. Electrochemical methods can be tuned to perform wash-free analysis[33] as well. Electrochemical detection methods usually use one of three approaches: voltammetry, amperometry, and detection of the change in capacitance. All three can be used in fast analysis,[28,33,67] as the reported time of experiments ranged between 5 and 20 minutes. This is much faster than the majority of PCR tests and immunoassays. However, these electrochemical methods require complicated equipment (for example, the use of gold nanoparticles with TEM and AFM imaging), highly trained operators, solution media, and are not suitable for point-of-care testing. Currently, the potential use of these methods in diagnostic testing is improbable because they require the fabrication of electrochemical sensors, often on a nanoscale level.

Fluorescence

Fluorescence spectroscopy is a well-established technique of sensitive detection. Its limit of detection is in the pg mL^{-1} range, on average, as can be seen in Table 5. Fluorescence signal detection can be incorporated in other procedures, such as immunoassays for the detection of antibodies or antigens.[26] Immunoassays with fluorescence signals have already been commercialized, and are even available in portable devices for home and point-of-care testing.[26] Fluorescence is reported as a reproducible method with RSD < 5%.[41] Other fluorescence-based methods use nanospheres, micromagnetic platforms, or upconversion materials for creating luminescence, which is further absorbed by nanoparticles conjugated to the target analyte. This method allows for more sensitive detection at the femtomolar level.[35] However, it is complicated and requires the fabrication of assay components, as well as highly trained personnel. Fluorescence signal acquisition is fast (around 20 minutes, on average), but the whole assay would take much longer to complete if the assay components need to be fabricated for fluorescence-based detection. Fluorescence-based immunoassays are faster (the whole assay takes around 2 hours), but their limit of detection is higher.[26] Fluorescence is

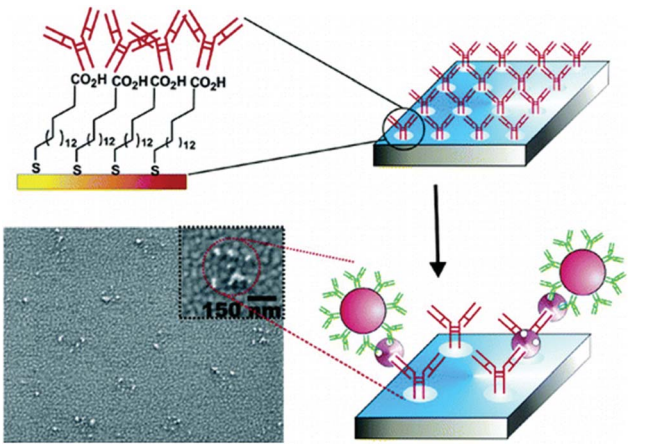

Fig. 4 AFM-based three-component ELISA using dip-pen nanolithography for HIV p24 antigen detection. The current method allows for detection of 25 fg mL^{-1}, which corresponds to 50 RNA copies per mL. The method shows at least 200-fold higher sensitivity compared to the 5 pg mL^{-1} limit of detection of conventional ELISA. The image is reprinted with permission from K. B. Lee, *et al.* The Use of Nanoarrays for Highly Sensitive and Selective Detection of Human Immunodeficiency Virus Type 1 in Plasma, *Nano Letters*, 2004, **4**(10), 1869–1872. Copyright 2004 American Chemical Society.

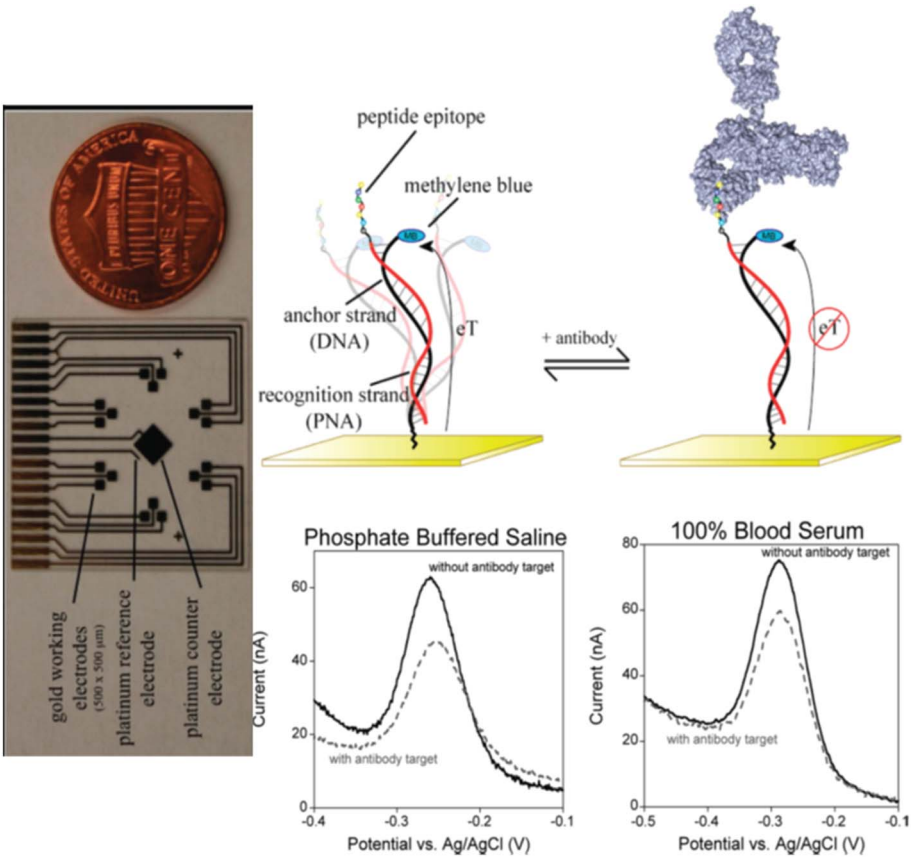

Fig. 5 E-DNA antibody sensor. The sensor (top) comprises an electrode-bound, redox-reporter-modified DNA strand, termed the "anchor strand," that forms a duplex with a complementary "recognition strand" (here composed of PNA) to which the relevant recognition element is covalently attached. In the absence of antibody binding (top middle), the flexibility of the surface attachment chemistry supports relatively efficient electron transfer between the redox reporter and the electrode surface. Binding to the relevant target antibody (top right) decreases electron transfer, presumably by reducing the efficiency with which the reporter collides with the electrode. Binding can thus be measured as a decrease in the peak current as observed *via* square wave voltammetry (bottom). As shown, sensors in this class are highly selective and perform equally well in buffered saline (bottom middle), undiluted blood serum (bottom right), or 1 : 4 diluted whole blood. The electrochemical E-DNA antibody sensor readily supports multiplexed detection. Here, (left, perpendicular orientation) a microfabricated chip containing eighteen 500 × 500 μm sensors, arranged in six three-pixel clusters, was employed. Each cluster is directed against a different antibody. Thus, the device supports the simultaneous, triplicate measurement of six different targets. Copyright 2012 American Chemical Society. Reprinted with permission from ref. 33, R. J. White, H. M. Kallewaard, W. Hsieh, A. S. Patterson, J. B. Kasehagen, K. J. Cash, *et al.* Wash-free, electrochemical platform for the quantitative, multiplexed detection of specific antibodies. *Anal Chem.*, 2012, **84**(2), 1098–1103.

subject to challenges such as photobleaching, autofluorescence, and dissociation of organic dyes used in live cells.

Other methods

There are other promising methods of detection: for example, silver staining, surface plasmon resonance (SPR), surface-enhanced Raman spectroscopy (SERS), confocal laser scanning microscopy, resonance light scattering, and radioimmunoassay. As can be seen in Table 5, silver staining and confocal laser scanning microscopy have the lowest limits of detection, in the pg to ng mL^{-1} and pg mL^{-1} ranges, respectively. They are also suitable for the detection of different analytes, such as viral nucleic acids, antigens, and antibodies to the virus.

Radioimmunoassay is another highly sensitive and inexpensive procedure, as can be seen in Table 5. The selectivity of this method is also very high, and the experimental procedure is similar to that of a standard ELISA. However, special precautions must be taken during the experiment because radiolabeled reagents are used. Resonance light scattering is another valuable technique, whose convenience and sensitivity make it a potential diagnostic tool in health care.

SPR is mainly used to track the binding dynamics of biologically important molecules. It can also be used for the quantitative detection of analytes, as was demonstrated in ref. 8. This method has an advantage over other surface spectroscopy techniques because it does not need a vacuum, and is able to produce a linear dependence of resonant energy on the analyte concentration. SPR spectroscopy has potential for multiplexing, particularly when it is integrated with multichannel microfluidic devices (Fig. 4).[126]

SERS is a technique that allows for producing an enhancement in the order of millions and billions over standard Raman spectroscopy, which makes it useful for sensitive detection (in

ng mL^{-1} range) of various analytes based on the characteristic Stokes shifts. This method requires a skilled operator and the use of a Raman spectrometer, which is now available as a portable, bench-size or even handheld device.[127] In comparison with fluorescence, Raman has great potential for multiplex detection of various analytes. Overall, this makes SERS a potential diagnostic test method. All abovementioned methods are listed in Table 5.

7. Post-COVID-19 trends and future perspectives

Comparing different methods, nucleic acid detection following amplification (real-time RT-PCR and LAMP) has higher sensitivity than ELISA, with a fg mL^{-1} *vs.* pg mL^{-1} average detection limit, respectively. The average limit of detection for real-time RT-PCR is 5 fg mL^{-1} ($n = 7$), and 49 fg mL^{-1} ($n = 6$) for the LAMP method. Having a detection limit higher than that for the ELISA method, the PCR-based tests are not free of drawbacks. Their disadvantages include the need for expensive equipment, trained personnel, and time. It takes several hours from the time of sample collection for a real-time RT-PCR test to produce results. The LAMP technique overcomes this limitation and enables a shorter (less than 1 hour) time until results, without the need for expensive equipment.

The limit of detection of electrochemical methods is comparable to the one for amplification-based methods of nucleic acid detection. The highest limit of detection is demonstrated by electrochemical methods with a capacitive immunosensor[28] for the detection of the HIV p24 antigen, which has a limit of detection of 7.9×10^{-5} fg mL^{-1}, and is accomplished in 20 minutes. The electrochemical methods are diverse in their principle, with some relying on capacitance measurements,[28] while others employ voltammetry[46] or amperometry.[60] They are also less restrictive in terms of the detected analyte, and are designed for the detection of viral particles, whether RNA[46] or protein[28], as well as antibodies (Fig. 5). The electrochemical methods are rapid and diverse. Some of them could be developed into low-cost point-of-care tests.

Amplification-based methods for the detection of nucleic acids, as well as ELISA-based methods for the detection of immune response in the form of antibodies, are two methods that are routinely used and demonstrate high sensitivity and specificity. However, they require time and proper equipment. The future of viral diagnostics lies in the point-of-care methods that can produce results within minutes, and do not require special equipment. The recent development of rapid diagnostics of SARS-CoV-2 in saliva[130] demonstrates the possibility of such a method. Other possible methods that can be used in point-of-care testing include portable antibodies/antigen test kits, fluorescence immunoassay devices, as well as portable RT-PCR and RT-LAMP devices.

Comparing the time it takes for different tests to produce results, lateral flow immunoassays offer a faster time to results, as compared to other methods (usually several minutes). Electrochemical detection is another rapid diagnostics method, which takes less than 1 hour to produce results. Real-time RT-PCR takes the most time out of all test methods.

Enormous interest in virus detection on the wake of the COVID-19 pandemic is likely to reshape this area, directing efforts towards creating detection methods that have a fast time to results, high simplicity, high throughput, and are relatively low-cost. However, the specificity and selectivity of those tests should remain at least on par with well-established methods, such as PCR and ELISA.

Express testing for COVID-19 is necessary to contain the infection, and is particularly important for testing in airports, places of public gathering, transportation hubs and malls. During the pandemic, fast point-of-care tests on the scale of millions per day are required to minimize the mass quarantining of people, and to avert the harsh effects of lockdowns and travel bans on the economy and the wellbeing of people. Tests that take several days, such as the PCR test of the nasopharyngeal swabs, can result in the spread of infections since tested people will not know about their infection status until 5–7 days have passed. Today, there is a focus on developing express COVID-19 detection methods, especially using portable devices. There are several potential methods that provide test results within minutes or hours. One of them is the express RT-PCR test kit. These kits were developed by Cepheid ("Cepheid Xpert Xpress"), Roche ("Roche cobas")[117] and Abbott ("ID NOW").[131] These are already used in hospitals and ERs. According to published research, their accuracy matches that of the regular RT-PCR.[117] This fact is a benefit of portable PCR devices, because with a sensitivity and specificity equal to that of stationary PCR, and with a significantly shorter time to results, they can be used for point-of-care testing. Some of these tests, such as "ID NOW", are approved by the FDA. Another express COVID-19 testing method is RT-LAMP. RT-LAMP uses DNA polymerase with the capability of separating double-stranded DNA, which eliminates the need for cycling temperature and simplifies the procedure. Portable and rapid testing devices using this method have also been developed, such as "Talis One" by Talis Biomedical.[131] This device provides results in 30 minutes, a time comparable to that of portable RT-PCR devices. Published research on the detection of RNA from SARS-COV-2 by RT-LAMP shows 100% agreement between the RT-LAMP and RT-PCR methods.[118] Both RT-LAMP and RT-PCR can be used for the detection of viral RNA in the saliva from a person. Currently, the main method of detection is the same as with nasopharyngeal swabs – RT-PCR. Saliva was reported to be a suitable sample for COVID-19 testing by the University of Illinois. Researchers at the Yale School of Public Health and Hokkaido University even commented that PCR testing of saliva provides more accurate results than testing of nasopharyngeal swabs. Collecting saliva does not need medical staff and is more comfortable for a patient. This allows for more frequent testing without putting pressure on medical staff. Several procedures have been developed to pretreat saliva before PCR because saliva is more of a chemically complex matrix than nasopharyngeal swabs. Some of these procedures were proven to be successful, such as the protocols from the University of Illinois

and Fluidigm's "Advanta Dx SARS-CoV-2 RT-PCR".[130] If the current saliva testing methods obtain governmental permission, they can replace the testing of nasopharyngeal swabs. The detection of viral antigens in blood or nasopharyngeal swabs is also a promising method. Benefits include it being relatively cheap, fast, and portable. One such example is the "Sofia SARS Antigen Fluorescent Immunoassay" by Quidel, which was approved for use in healthcare organizations. Detection of antibodies to the virus is also available in portable devices as a rapid test (*e.g.*, "COVID-19 IgG/IgM Rapid Test Cassette" by Zhejiang Orient Gene Biotech Co., Ltd.). The main benefits are the ease of use and speed (results are available in 10 minutes, and the only requirement is the addition of a drop of blood and the provided buffer on a test slide).[120] Antibody detection, however, is efficient only after several days have passed since the onset of symptoms. Both antibody and antigen tests are easier to use and cheaper, but they are less sensitive than RT-PCR. They can be used after the onset of symptoms to avoid a false negative during early testing. There are other rapid tests, such as pulse-controlled amplification (20 minutes),[123] reverse-transcription recombinase-aided amplification (5–15 minutes),[125] and other methods. However, these approaches are all new research studies requiring proficient scientists, and are confined to the laboratory, so they are not the main focus of today's trends.

Abbreviations

AFM	Atomic-force microscopy
Ab	Antibodies
CDC	Center for Disease Control
CMV	Cytomegalovirus
CoV	Coronavirus
CRISPR	Clustered regularly interspaced short palindromic repeats
cRNA	RNA copies
DNA	Deoxyribonucleic acid
ELD_{50}	50% egg lethal dose
ELISA	Enzyme-linked immunosorbent assay
HA	Haemagglutinin
HAU	Hemagglutination unit
HAV	Hepatitis A virus
HCV	Hepatitis C virus
HDV	Hepatitis D virus
HEV	Hepatitis E virus
HFV	Hemorrhagic fever virus
HIV	Human immunodeficiency virus
LAMP	Loop-mediated isothermal amplification
LOD	Limit of detection
MERS	Middle east respiratory syndrome
NA	Neuraminidase
NP	Nucleoprotein
NS	Nonstructural
PCR	Polymerase chain reaction
PFU	Plaque-forming unit
RNA	Ribonucleic acid
RSD	Relative standard deviation
RT-PCR	Reverse transcription polymerase chain reaction
Rxn	Reaction
SARS	Severe acute respiratory syndrome
SERS	Surface-enhanced Raman spectroscopy
SPR	Surface-plasmon resonance
$TCID_{50}$	50% tissue culture infective dose
WHO	World Health Organization

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Conflicts of interest

There are no conflicts to declare.

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