Sensors for surveillance of RNA viruses: a One Health perspective

Ye Chen*, Peng Wang*, Fen-ni Zhang*, Hui Dai*, Xin-yi Jiao, Xin-yu Wang, Qi-wen Yu, Mei Kang, Shuo Su, Di Wang

RNA viruses, especially those capable of cross-species transmission, pose a serious threat to human, animal, and environmental health, as exemplified by the 2024 outbreak of the highly pathogenic avian influenza H5N1 virus in cattle, unpasteurised milk, and workers on dairy farms in the USA. This escalating risk of a new RNA virus pandemic highlights the urgent need to implement One Health strategies. However, the centralised virus detection systems currently in use fall short of meeting the required level of virus surveillance and infection diagnosis, particularly in resource-limited regions. In this context, the latest advancements in RNA virus-sensing technologies offer promising solutions. Through interdisciplinary collaboration, these sensors can achieve sensitivity and reliability similar to that of standard laboratory equipment and offer several advantages, such as compact size, affordability, and operational simplicity. In this Review, we highlight the latest advances in sensing technologies for detecting different biomarkers of viral infections (RNA, antigens, and antibodies). We further compare the sensing principles and performances of these technologies and discuss the possibility of deployment of these sensors in the One Health approach and the challenges expected in this pursuit. In conclusion, the widespread use of RNA virus sensors is expected to enhance the effectiveness of surveillance systems for infectious diseases.

Introduction

RNA viruses have increasingly been identified as pathogens responsible for widespread spillover events and pose major threats to human health. For example, the transmission of dengue virus resulted in over 6·5 million cases with more than 7300 deaths in 2023,[1] and as per the COVID-19 dashboard, COVID-19, caused by SARS-CoV-2, has accounted for over 7 million fatalities between December, 2019 and July, 2024. Since March, 2024, cattle in multiple dairy farms across 13 US states have tested positive for the highly pathogenic avian influenza H5N1 virus, with four dairy farm workers involved in spillover events due to close contact with infected cattle, contaminated milking equipment, exposure to unpasteurised milk, or a combination of these factors.[2] Thus, monitoring viral biomarkers in animals, humans, and the environment is crucial for an early indication of emerging (newly identified or previously unknown) and re-emerging (resurfaced after a period of decline or low incidence) viruses, enabling timely and effective control measures.

Particularly, the spread of emerging zoonotic infectious diseases is exacerbated by changes in ecological factors, and studies have revealed the increasing diversity of high-risk RNA pathogens in both domestic and wild animals.[3,4] Furthermore, the high mutation rate and short replication cycles of RNA viruses make them prone to generating new subtypes and variants, posing substantial challenges to their surveillance.[5] These challenges are compounded by the fact that current efforts in screening for viruses and identifying and controlling infections are more advanced in the context of humans, with less attention paid to the contexts of animals and the environment.

To promote the implementation of the One Health strategy, an integrated and unifying approach that recognises the interconnections between humans, animals, and their shared environment, detecting and identifying pathogenic RNA viruses at the human–animal–environment interface, is a priority for comprehensive zoonotic disease control.

RNA virus detection mainly relies on established laboratory techniques, such as ELISA, reverse transcription quantitative PCR (RT-qPCR), serological antibody determination, immunochromatographic methods (such as rapid tests), and reverse transcription loop-mediated isothermal amplification (RT-LAMP).[6] These methods are typically expensive, time-consuming, and are performed by professionals in specialised laboratories. Additionally, sample contamination and degradation can occur during the transportation of fresh (frozen) biological samples, such as swabs, faeces, and blood, making centralised detection systems inefficient for collating comprehensive information on RNA viruses. Development and implementation of more efficient methods of RNA virus detection are imperative to address these challenges.

RNA virus-sensing technologies, which incorporate biosensing materials with signal transducers, can detect various biomarkers of RNA viruses, such as viral RNA, antigens, and antibodies, and convert these biological signals into measurable responses for the identification of pathogens and diagnosis of diseases.[7] These sensors, characterised by their compact size, affordability, and operational simplicity, hold great promise for the acquisition of information on RNA viruses.

The COVID-19 pandemic has notably accelerated advancements in RNA virus-sensing technologies, bridging the performance gap between them and conventional laboratory-grade equipment (table). The latest breakthroughs in optical technologies and nanotechnologies have improved the sensitivity of RNA virus sensors; in addition, interdisciplinary research studies spanning synthetic biology and materials chemistry have collectively driven advances in the development of biosensing materials,

Lancet Microbe 2025; 6: 101029

Published Online December 13, 2024
https://doi.org/10.1016/j.lanmic.2024.101029

*Contributed equally

Key Laboratory of Fujian-Taiwan Animal Pathogen Biology, College of Animal Sciences, Fujian Agriculture and Forestry University, Fuzhou, China (Y Chen PhD); **Department of Atmospheric and Oceanic Sciences, Fudan University, Shanghai, China** (P Wang PhD); **Department of Biomedical Engineering, Zhejiang University, Hangzhou, China** (Prof F Zhang PhD, Prof D Wang PhD); **Department of Immunology, School of Basic Medical Sciences, Peking University Health Science Center, Beijing, China** (H Dai PhD); **Department of Epidemiology, School of Public Health, Shanghai Institute of Infectious Disease and Biosecurity, Fudan University, Shanghai, China** (X Jiao BS, X Wang BS, M Kang MPH, Prof S Su PhD); **Clinical Research Center, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China** (M Kang); **Research Center for Frontier Fundamental Studies, Zhejiang Lab, Hangzhou, China** (Q Yu PhD, Prof D Wang)

Correspondence to:
Prof Di Wang, Department of Biomedical Engineering, Zhejiang University, Hangzhou, 310007, China; Research Center for Frontier Fundamental Studies, Zhejiang Lab, Hangzhou 311121, China
diwang@zhejianglab.com

or

Prof Shuo Su, Department of Epidemiology, School of Public Health, Shanghai Institute of Infectious Disease and Biosecurity, Fudan University, Shanghai 200032, China
shuosu@fudan.edu.cn, **ssh5658485@163.com**

or

Dr Ye Chen, Key Laboratory of Fujian-Taiwan Animal Pathogen Biology, College of Animal Sciences, Fujian Agriculture and Forestry University, Fuzhou 350002, China
fafuchenye@163.com

For more on the **COVID-19 dashboard** see https://data.who.int/dashboards/covid19/deaths/

For more on the **One Health strategy** see https://www.who.int/health-topics/one-health/

further enhancing their performance.[38] Moreover, the high-throughput and fast-iteration capabilities of these sensors offer a promising approach to counter the increasing diversity and rapid evolution of variants in RNA viruses.[18] Expanding the application of novel RNA virus sensors could considerably enhance the ability of surveillance systems to detect circulating RNA viruses and diagnose diseases caused by these pathogens.

Here, we summarise and discuss the latest advancements in RNA virus sensors and their performance and applicability in practice. Furthermore, we outline the gaps that need to be addressed for large-scale deployment of these sensors. We believe that these insights could help to guide future advancements in RNA virus-sensing technologies, which are expected to boost the One Health approach.

Sensors for viral RNA detection

Detection of specific fragments of viral RNA genomes is one of the most accurate methods for identifying RNA viruses and confirming clinical diagnoses.[39] This process typically involves several steps, such as sample pretreatment, virus or cell lysis, RNA extraction, nucleic acid amplification, and target fragment detection.[40] Although conventional RNA detection methods based on RT-qPCR are sensitive and can detect pathogens even in samples with low viral load, they generally rely on lengthy protocols, expensive reagents, complex equipment, and highly trained staff.[41]

Various sensing approaches have been developed to accelerate and simplify nucleic acid amplification procedures (figure 1A). For example, accelerated thermal cycling can be achieved by utilising the plasmonic heating effect of nanoparticles (figure 1A), for instance, enabling the amplification and fluorescent detection of SARS-CoV-2 RNA within 17 min.[8] In contrast to RT-qPCR, methods based on RT-LAMP, nucleic acid sequence-based amplification, and rolling circle amplification can achieve single-step isothermal amplification of RNA or DNA (figure 1A).[9,11,42] These simplified approaches can be further accelerated by the catalytic effect of plasmonic hotspots. By integrating plasmonic nanosurfaces into the confined space of a microfluidic chamber, plasmonic "hot" electrons can be injected into a nanolitre volume, achieving a nine-fold acceleration in the kinetics of RNA or DNA amplification.[9]

To simplify the procedure for obtaining quantitative sensor readouts, amplification approaches are usually coupled with optical detection (figure 1A). For example, by combining RT-LAMP and rolling circle amplification with colourimetric detection of the hydrogen ions released during amplification, a sample-to-answer turnaround time of 13 min was achieved for saliva samples of individuals with COVID-19, with an accuracy similar to that of RT-qPCR.[9]

CRISPR-Cas-based fluorescence detection is another powerful optical method (figure 1A). This approach is highly specific owing to the sequence-specific recognition of the target and offers multiplexed, rapid, and quantitative detection. A large-scale multiplex nucleic acid detection method developed using the Cas13 enzyme is capable of performing over 4500 detections simultaneously within a single microwell array and showed the ability to differentiate between 169 human-associated viruses, including dengue virus, Zika virus, and different subtypes of influenza A virus.[18]

As their sensitivity continues to improve, some RNA sensors can even detect low-copy samples directly, eliminating the need for nucleic acid amplification and considerably enhancing the efficiency of viral RNA detection.[43,44] For example, an amplification-free nucleic acid immunoassay implemented on a lateral flow strip[10] uses DNA probes designed to selectively bind to the SARS-CoV-2 genome and a fluorescent nanoparticle-labelled monoclonal antibody that binds to DNA–RNA hybrids (figure 1A). This inclusion helps achieve a limit of detection (LOD) of 0·5 copies per µL. In a multicentre, randomised, double-blind trial involving 734 samples (593 throat swabs and 141 sputum) obtained from 670 individuals, the assay achieved a sensitivity of 100% and specificity of 99% for both types of samples.

Electrochemical sensing technology can also enable the detection of unamplified RNAs (figure 1A).[45,46] By using DNA nanopyramid scaffolds to immobilise complexes of Argonaute proteins and guide DNA on the surface of electrochemical electrodes, nucleic acid (cell-free DNA and RNA) enrichment and signal transduction within the Debye length can be achieved on the sensor surface. Continuous detection of interstitial fluid indicates the ability of such a system to monitor cell-free RNA in real time for up to 14 days, with an LOD of 0·3 fM.[46] This high level of sensitivity, combined with the simplicity and low power consumption of electrochemical sensing systems, highlights the great promise of sensors for continuous monitoring of ultra-trace quantities of unamplified viral RNA in crucial scenarios, such as analysing the body fluids of medical workers in high-risk circumstances to provide early warnings of infection.

The rapid development of viral RNA sensors provides a versatile toolbox for strengthening key components of the One Health approach, particularly by enhancing surveillance across various host species and environments for known pathogens. Detection of target RNA viruses in insect vectors and wild animals could provide both time-specific and location-specific early warnings of viral activity before human infections occur.[47,48] The example of the 2024 H5N1 virus spillover in US dairy farms also highlights the need for continuous viral RNA monitoring in animals, the environment, and products on farms.[2]

Viral RNA sensors can effectively support these large-scale or distributed surveillance tasks by expanding testing capability within the same budget. Upon the onset of an outbreak, an adequate supply of viral RNA sensors with short turnaround times could help to maintain pace with the increasing number of affected individuals, thus reducing the burden on the health-care system (figure 2). In addition, the deployment of viral RNA sensors to monitor sewage could reveal the scale of the outbreak and community dynamics of emerging human viruses,[50] thereby facilitating timely and tailored governmental interventions.

	Sensing principle	Pathogen	Detection time	Temperature	Sensitivity or limit of detection	Specificity	Year	Reference
RNA sensor	Plasma-enhanced PCR	SARS-CoV-2	17 min	Rapid thermal cycling using plasma heating: 58–90°C	3·2 copies/μL	NA	2020	Cheong et al (2020)[8]
	Plasma enhanced RT-LAMP	SARS-CoV-2, H1N1	13 min	Temperature control: 65–95°C	SARS-CoV-2: 5 copies/μL H1N1: 4 copies/μL	NA	2023	AbdElFatah et al (2023)[9]
	(HC-FIA) DNA probe	SARS-CoV-2	45 min	Incubation at 56°C	500 copies/mL	99·2%	2020	Wang et al (2020)[10]
	Paper-based biomolecular sensor	ZIKV	180* min	Temperature control: 37–95°C	1 fM	NA	2016	Pardee et al (2016)[11]
	Cysteine-based, zinc oxide nanoparticle electrochemical biosensor	DENV2, ZIKV, CHIKV, YFV	NA	RT	ZIKV: 0·0421 PFU /mL YFV: 0·0437 PFU /mL CHIKV: 0·062 PFU/mL DENV2: 0·0382 PFU/mL	NA	2020	Simão et al (2020)[12]
	Microfluidic device using Cas13	SARS-CoV-2	48 min	Temperature control: 25–65°C	40 copies/μL	100%	2022	Chandrasekaran et al (2022)[13]
	CRISPR/Cas12a plus LAMP	SARS-CoV-2	120 min	Temperature control: 37–95°C	0·8 copies/μL	100%	2022	Najjar et al (2022)[14]
	(CRISPR/Cas12a plus LAMP) Ferrobots	SARS-CoV-2	36·75 min	95°C for thermal lysis; 65°C for RT-LAMP reaction	25 copies/μL	100%	2022	Lin et al (2022)[15]
	CRISPR-based sensor	SARS-CoV-2	90 min	RT	17 aM	NA	2021	Nguyen et al (2021)[16]
	Linear molecular beacon probes	CSFV, SARS-CoV-2	30* min	RT	SARS-CoV-2: 0·24 nM CSFV: 0·28 nM	NA	2022	Du et al (2022)[17]
	Microfluidic device using Cas13	DENV, ZIKV, HCV	180* min	Temperature control: 41–95°C	NA	NA	2020	Ackerman et al (2020)[18]
	CRISPR/Cas13, Cas12a, and Csm6	DENV, HIV, ZIKV	90 min	Temperature control: 37–65°C	DENV, HIV, ZIKV: 2 aM	NA	2018	Gootenberg et al (2018)[19]
	AuNPs with antisense oligonucleotides (ASO-LAMP)	SARS-CoV-2	70 min	Incubation at 65°C	10 copies/μL	100%	2021	Alafeef et al (2021)[20]
	LAMP	SARS-CoV-2	35 min	Temperature control required; RNA extraction at 95°C and LAMP reaction at 60°C	300 copies/reaction	100%	2021	Deng et al (2021)[21]
	CRISPR-Cas13a/C2c2 system, combining RPA	ZIKV, DENV	150 min	Temperature control: 37–95°C	2* aM	NA	2017	Gootenberg et al (2017)[22]
Antigen sensor	Single molecule nanopore sensor	SARS-CoV-2	210 min	Temperature control: 25–95°C	0·2 pM	NA	2023	Ren et al (2023)[23]
	LSPR-enhanced LFIA	SARS-CoV-2	20 min	RT	212 pg/mL	100%	2023	Gupta et al (2023)[24]
	MolEMS-based FET sensor	SARS-CoV-2	10 min	RT	6 aM	NA	2024	Ma et al (2024)[25]
	D4 assay chip	EBOV	>60 min	RT	0·01 ng/mL	NA	2021	Fontes et al (2021)[26]
	Rapid immunochromatography	DENV, ZIKV	NA	RT	DENV: 4 ng/mL ZIKV: 18 ng/mL	DENV: 100% ZIKV: 86%	2017	Bosch et al (2017)[27]
	OECT	SARS-CoV-2	10 min	RT	23 fM	NA	2021	Guo et al (2021)[28]
	Immune-resonance sensor	SARS-CoV-2, H1N1	10 min	EBT	SARS-CoV-2: 19·7 fg/L; H1N1: 0·11 fg/L	100%	2024	Li et al (2024)[29]
	Bionic microchannel impedance sensor	SARS-CoV-2	5 min	EBT	1 fg/mL	NA	2023	Li et al (2023)[30]
	LOC	Norovirus	20 min	RT	81·8%	NA	2021	Chung et al (2021)[31]
	SES	SARS-CoV-2	10 min	RT	10 pg/mL	NA	2024	Li et al (2024)[32]

(Table continues on next page)

	Sensing principle	Pathogen	Detection time	Temperature	Sensitivity or limit of detection	Specificity	Year	Reference
(Continued from previous page)								
Antibody sensor	PRNT	SARS-CoV-2, EBOV, DENV, ZIKV	3-5 days	Incubated at 37°C	100%	100%	2021	Bewley et al (2021)[33]
	ELISA	SARS-CoV-2, EBOV, DENV, ZIKV	>120 min	Incubated at 37°C or RT	90%	100%	2020	Lassaunière et al (2020)[34]
	CLIA	SARS-CoV-2	NA	RT	81·8%	99·3%	2020	Nicol et al (2020)[35]
	LFIA	SARS-CoV-2	15 min	RT	81·8%	95·3%	2020	Nicol et al (2020)[35]
	Simoa	SARS-CoV-2	>30 min	RT	100%	94%	2020	Norman et al (2020)[36]
	OECT	SARS-CoV-2	5 min	RT	1 fM	NA	2021	Liu et al (2021)[37]
	LSPR-enhanced LFIA	SARS-CoV-2	20 min	RT	185·3 pg/mL	100%	2023	Gupta et al (2023)[24]
	LOC	SARS-CoV-2	120 min	Temperature control: 37–95°C	100%	100%	2022	Najjar et al (2022)[14]

Detection time is the latency period from the introduction of the sample to the sensor's definitive output. ASO-LAMP=antisense oligonucleotide loop-mediated isothermal amplification. AuNP=gold nanoparticle. CHIKV=chikungunya virus. CSFV=classical swine fever virus. CLIA=chemiluminescence immunoassay. DENV=dengue virus. DENV2=dengue virus 2. EBT=exhaled breath temperature. EBOV=Ebola virus. HC-FIA=hybrid capture fluoroimmunoassay. HCV=hepatitis C virus. LAMP= loop-mediated isothermal amplification. LFIA=lateral flow immunoassay. LOC=lab on a chip. LSPR=localised surface plasmon resonance. MoIEMS=molecular electromechanical system. NA=not applicable. OECT=organic electrochemical transistor. PFU=plaque forming unit. PRNT=plaque reduction neutralisation test. RPA=recombinant polymerase amplification. RT=room temperature. RT-LAMP=reverse transcription loop-mediated isothermal amplification. SES=miniaturised soft electromagnetic swimmer. Simoa=single molecule array. YFV=yellow fever virus. ZIKV=Zika virus. *Approximate values.

Table: **Summary of state-of-the-art RNA virus sensors**

Furthermore, low-income countries and resource-limited regions often have insufficient specialised equipment and trained staff to effectively detect RNA viruses. In this context, the ease of use and cost-efficiency of viral RNA sensors offer a promising solution for fostering regional collaboration.

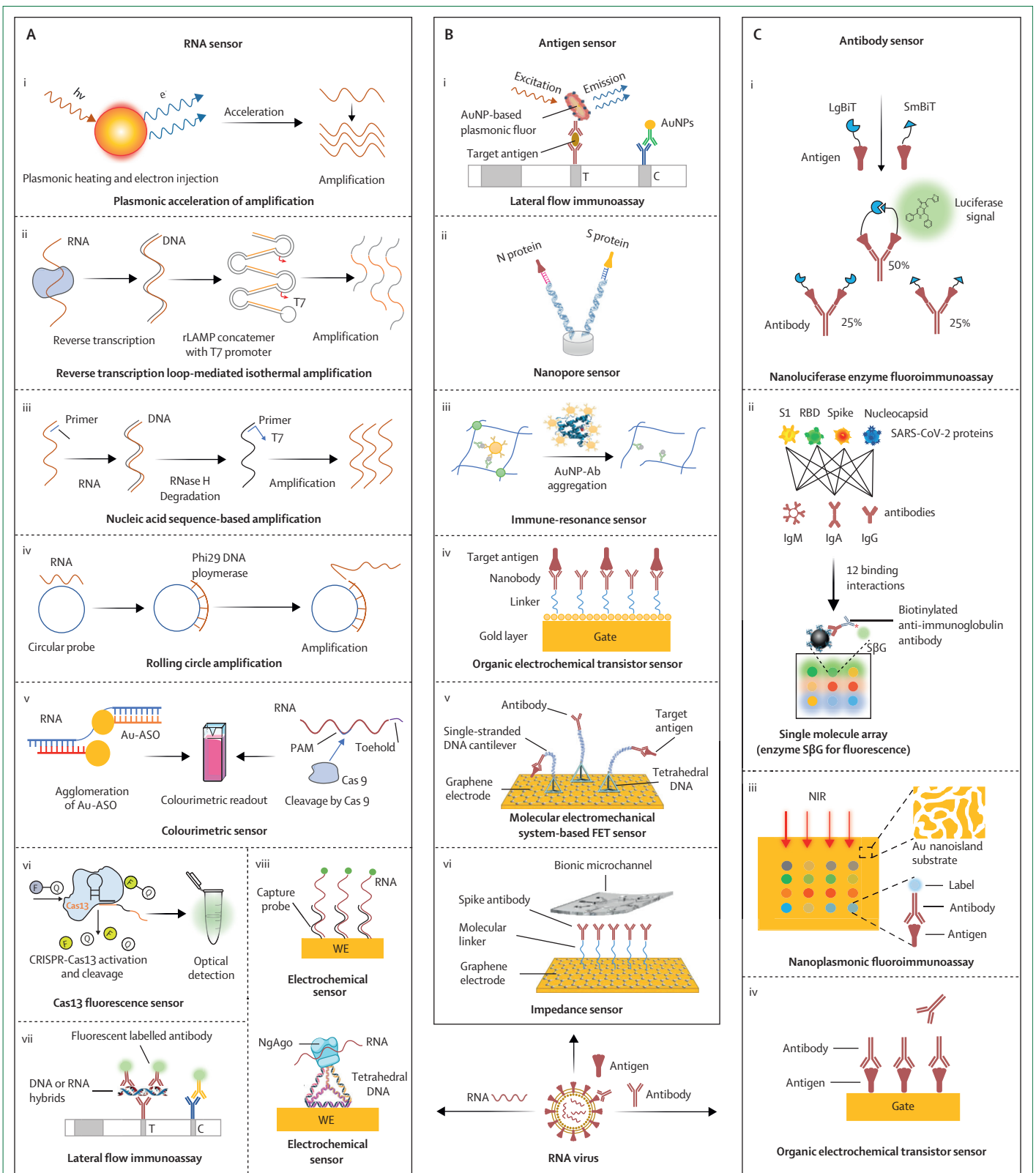
Sensors for viral antigen detection

Antigen sensors identify pathogens by detecting their proteins or other molecules that can induce an immune response. Compared with nucleic acid testing, the major advantages of antigen detection include immediate availability of results and no need for nucleic acid extraction, rendering antigen detection suitable for on-site screening and large-scale monitoring of epidemics. However, conventional colloidal gold immunochromatography has low sensitivity, hampering its use during the early or late stages of infection when viral load is low, which can lead to false-negative results.[51] In addition, this method does not offer the level of specificity required for accurately identifying specific subtypes of viruses and confirming diagnoses.[52] A series of novel RNA virus antigen-sensing technologies are actively addressing these limitations, aiming to enhance sensitivity and specificity while maintaining the speed and convenience of analysis.

In antigen sensors, specific antibodies and aptamers are typically used to capture target viral antigens, and elaborate designs of nanomaterials and nanostructures are commonly used to increase their sensitivity (figure 1B). For example, plasmonically active antibody-conjugated fluorescent gold nanorods can make conventional lateral flow assays (LFAs) ultrasensitive (figure 1B). With sample-to-answer times of less than 20 min, plasmonically-enhanced LFAs achieved approximately 30-fold improvements in their dynamic range (10^{-1}–10^{3} pg/mL) and LOD (93 fg/mL) compared with those of the 4-h-long gold-standard ELISA method, achieving 95% clinical sensitivity and 100% specificity for antigens in nasopharyngeal swabs from individuals with COVID-19.[24]

Fluorescent LFA offers the advantages of low cost and ease of operation. The fluorescent LFA signal can be easily read using a handheld fluorescence reader, enabling quick results with minimal labour. The high sensitivity could also reduce false negatives, thereby enabling broader application of antigen detection in the early stages of infection (figure 2). Nanopore sensing has been proven to be highly sensitive to molecules passing through and is increasingly being used for nucleic acid sequencing.[53] This approach can also be applied to antigen detection by combining nanopore sensing with position-encoded DNA molecular probes (figure 1B); the method enabled direct detection of SARS-CoV-2 antigens in unprocessed human saliva, with an LOD of 0·09 pM.[23]

Field-effect amplification is widely applied in electrical sensors to enhance their sensitivity in antigen detection. Organic electrochemical transistors have emerged as a promising platform for biosensing owing to their robust



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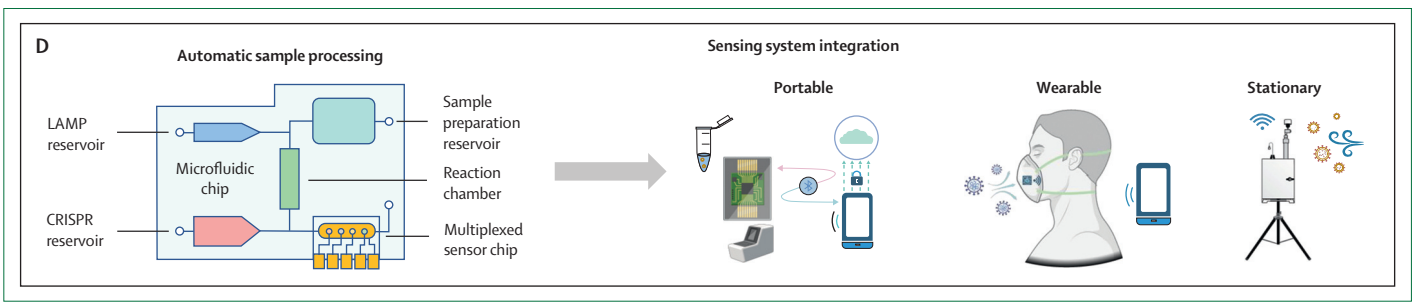


Figure 1: **RNA virus sensors with potential applicability in surveillance systems**
(A) Nucleic acid amplification methods and RNA sensors, including (i) plasmonic acceleration of nucleic acid amplification, (ii) RT-LAMP, (iii) nucleic acid sequence-based amplification, (iv) rolling circle nucleic acid amplification, (v) colourimetric sensor, (vi) Cas13 fluorescence sensor, (vii) lateral flow immunoassay, and (viii) electrochemical sensor. (B) Antigen sensors, including (i) lateral flow immunoassay, (ii) nanopore sensor, (iii) immune-resonance sensor, (iv) organic electrochemical transistor sensor, (v) MolEMS-based FET sensor, and (vi) impedance sensor. (C) Antibody sensors, including (i) nanoluciferase enzyme fluoroimmunoassay, (ii) single molecule array, (iii) nanoplasmonic fluoroimmunoassay, and (iv) organic electrochemical transistor sensor. (D) Integration of RNA virus sensors with microfluidics could develop various sensing systems for convenient and automatic virus detection and monitoring. Ab=antibody. Au=gold. Au-ASO=gold nanoparticle–antisense oligonucleotide. AuNPs=gold nanoparticles. FET=field-effect transistor. LgBiT=large BiT. MolEMS=molecular electromechanical system. NgAgo=Natronobacterium gregoryi Argonaute. NIR=near-infrared. PAM=protospacer adjacent motif. RBD=receptor-binding domain. rLAMP=conversion of loop-mediated isothermal amplification to RNA. RT-LAMP=reverse transcription loop-mediated isothermal amplification. SmBiT=small BiT. WE=working electrode.

ability to amplify electrical signals (figure 1B).[54] Even a few binding events at the gate electrode can cause large modulations in the channel current. By modifying the gate electrode with biosensing materials, an organic electrochemical transistor sensor can rapidly quantify viral antigens at levels ranging from a single molecule to nanomolar concentrations in unprocessed human saliva and serum.[28,55]

The field-effect transistor is another commonly used transducer for antigen detection. Using graphene as the gate material and modifying the graphene with DNA-probe assemblies, field-effect transistor sensors can directly detect protein concentrations as low as 6×10^{-18} M in biofluids (figure 1B).[25]

Electrical sensors can also facilitate wireless detection by modifying their electrical components with biosensing materials. A wireless immunoassay leverages the immunoresponsive hydrogel-modulated radio frequency resonant sensor to capture and amplify the recognition of viral antigens and a flexible readout network to transduce the immunological bindings into electrical signals (figure 1B). This sensor can simultaneously detect antigens of SARS-CoV-2, H1N1 Influenza A Virus, and respiratory syncytial virus in aerosols with sensitivity at the fg/L level and an analysis time of less than 10 min without the need for wires or batteries.[29] This approach is particularly useful for multiplexed detection of RNA viruses in locations with insufficient power resources.

In another example, anti-spike antibodies on activated graphene specifically bind with spike proteins of SARS-CoV-2 (figure 1B). This binding lowers the surface resistance of graphene, converting the biochemical signal into an electrical signal via a backend circuit. The signal can then be read wirelessly using a smartphone through near-field communication (NFC).[30]

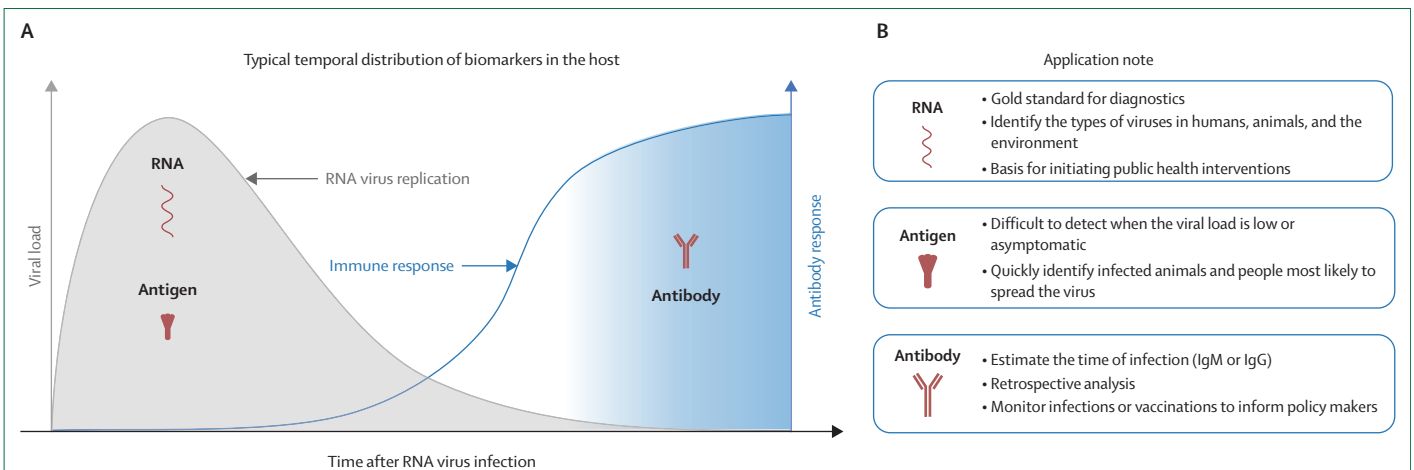
Non-specific binding is another key challenge in the development of antigen sensors. Cross-reactivity resulting from recognition of molecules other than the analyte of interest can produce false-positive responses. Screening for high-affinity antibodies against viruses is an effective means to improve the accuracy of antigen detection. This strategy is well exemplified in an assay developed for the detection of Ebola virus.[26] First, secreted glycoprotein was chosen as a diagnostic target because of its abundance in the serum during the initial stages of Ebola virus infection. Second, single-chain antibody phage display was used to generate high-affinity antibodies specific for the Ebola virus-secreted glycoprotein, followed by a high-throughput antibody-pairing strategy to identify the most sensitive combination of capture and detection antibodies. Lastly, a single-step sandwich immunoassay with sensitivity up to the fM range was inkjet-printed; the system was capable of detecting Ebola virus infection in a standard non-human primate model in 60 min, much earlier than an RT-qPCR (3–16 days).

Monoclonal antibodies also serve as effective tools to develop selective antigen sensors. By immunising mice with recombinant proteins that closely resemble the true conformations of viral proteins, highly specific monoclonal antibodies can be obtained against target antigens. An immunochromatographic sensor developed thus helped to identify all four dengue virus serotypes and Zika virus in human serum samples without cross-reactivity.[27] However, additional experiments with a larger sample size should be conducted to further validate the performance of this sensor.

These examples suggest that advanced antigen sensors, with their greatly improved sensitivity and specificity, could complement viral RNA detection technologies by enabling rapid and accurate detection of RNA viruses in humans, animals, and the environment. This advancement can facilitate early warnings of potential cross-species transmission and help to reduce the risk of viral spillover and outbreaks.

Sensors for viral antibody detection

Antibody detection is also crucial for retrospective analysis of infections and for evaluating immune status after



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Biomarker	Sensing principle	Sensitivity	LOD	Specificity	Detection time
RNA	HC-FIA[10]	100%	0·5 copies/μL	>99%	45 min
	CRISPR/Cas 13[13,19]	>99%	40 copies/μL	>93%	48–90 min
	ECS[45]	100%	0·025 copies/μL	100%	30 min
Antigen	Nanopore[23]	NA	0·2 pM	100%	210 min
	LFIA[24]	>95%	212 pg/mL	100%	15–20 min
	ImmHR[29]	>85%	0·11–19·7 fg/L	100%	10 min
Antibody	Simoa[36]	100%	NA	94%	>30 min
	OECT[37]	100%	1 fM	100%	5–10 min
	NP-FIA[49]	100%	1·6 ng/mL	>99%	>150 min

Figure 2: **Application and comparison of various types of RNA virus sensors**
(A) Typical temporal distribution of different biomarkers in the host after RNA virus infection. In the early stage of infection, the concentrations of viral RNA and antigens rise with the viral load, triggering an immune response that leads to antibody production. As the viral load decreases, the immune system gradually clears the viral RNA and antigens, and antibodies persist to maintain ongoing protection. (B) Application of different biomarkers detection methods in combating viral outbreaks and epidemics. (C) Comparison of representative RNA virus-sensing technologies. The deeper the shade of blue, the higher the level of performance. ECS=electrochemical sensor. HC-FIA=hybrid capture fluoroimmunoassay. ImmHR=immunoresponsive hydrogel-modulated resonant. LFIA=lateral flow immunoassay. LOD=limit of detection. NA=not applicable. NP-FIA=nanoplasmonic fluoroimmunoassay. OECT=organic electrochemical transistor. Simoa=single molecule array.

vaccination (figure 2).[39] Conventional methods for antibody detection are limited by their poor sensitivity and accuracy. These methods are often incapable of detecting antibodies in the early stages of infection and do not possess the resolution to quantify changes in immune response over time. Additionally, high concentrations of non-specific antibodies in the blood can lead to false-positive results.[39] Therefore, the development of novel antibody-sensing technologies is urgently required to address these limitations.

Various optical sensors have been developed for the sensitive detection of antibodies against RNA viruses (figure 1C). The split-luciferase antibody sensor provides a new strategy for luminescent biosensing (figure 1C).[56,57] In this strategy, one antibody fragment is bound to an antigen fused to large BiT (LgBiT; nanoluciferase fragment I) and the other to small BiT (SmBiT; nanoluciferase fragment II). Their interaction reconstitutes the enzyme and facilitates the production of an active luciferase signal. Serological studies of individuals with COVID-19 have shown that the split-luciferase antibody sensor has a sensitivity of 89% for detecting anti-spike protein antibodies and 98% for anti-N protein antibodies, with a specificity of over 99% for both.[57]

Single molecule array (Simoa) technology is another enzyme-based fluorescence sensor for highly sensitive antibody detection. Simoa is a multiplexed assay that uses dye-encoded antigen-coated beads to quantify levels of IgG, IgM, and IgA antibodies against different viral antigens (figure 1C). The ultra-sensitivity of Simoa allows for a 4000-fold plasma dilution, thus requiring only a minute (<1 μL) sample volume.[36] Similar to the cases of RNA

sensors and antigen sensors, plasmonic effects are commonly used to enhance optical signals in antibody-sensing systems. For example, a substrate composed of nanoscale gold islands with abundant nanogaps can provide near-infrared fluorescence enhancement of up to approximately 100-fold due to plasmonic resonance and local electric field enhancements (figure 1C), providing an LOD of approximately 1·6 ng/mL for SARS-CoV-2 IgG antibody.[49] The plasmonic effect can also improve the sensitivity of LFA in antibody detection,[24] thereby allowing for detection in the early stages of infection and providing rapid feedback on immune response. Many other LFA-based methods have also shown great promise in providing low-cost and accurate detection.[58,59]

Besides optical sensors, advanced electrical sensors can also achieve highly sensitive antibody detection. For example, using an organic electrochemical transistor (figure 1C), an IgG detection limit of 10 fM can be achieved in serum and saliva; the detection range in serum was from 10 fM to 100 nM.[37] The entire testing process can be completed within 5 min. Thus, the advanced sensing material design and transducing technologies developed for antigen sensors are also mostly applicable to antibody sensors. The highly improved sensitivity, specificity, and throughput of antibody sensors allow for the interrogation of interactions between antibody isotypes and viral proteins and could help to test and understand the heterogeneous clinical presentations of infections in individuals.

Given its straightforward, quick, and cost-effective nature, antibody detection is an effective method for reflecting past infection history in large-scale surveillance aimed at monitoring individual exposure and assessing population immunity following immunisation. However, conducting large-scale antibody testing across multiple animal species remains a challenge.

First, to efficiently monitor large animal populations, strategies such as random sampling, pooled testing, or both, are often used to manage throughput and cost constraints.[60]

Second, detecting antibodies across different animal species places higher demands on sensing technologies, such as multiplexed detection and more specific sensing materials to differentiate between different antibodies. Here, setting sentinel animals is a promising solution: rather than capturing a wide range of wild species, some animals that are more susceptible to specific viruses (eg, aquatic birds for avian influenza) can be used as sentinels.[61,62] Monitoring these species can provide early warnings of viral threats without the need to screen the entire wildlife.

Third, distinguishing between antibodies generated from vaccination and those resulting from natural infection might require assays targeting both structural and non-structural proteins of viruses.[63]

Integration of sensing systems for RNA virus detection

RNA virus detection often requires multiple steps, including pretreatment of raw samples, RNA extraction, nucleic acid amplification, and optical detection. In established laboratory methods, these steps are usually processed separately, which increases the complexity of operations and introduces the potential for operational errors. In contrast, RNA virus sensors are compatible with microfluidic technology, and their integration enables on-chip execution of essential liquid handling functions, such as distributing, mixing, separation, and enrichment.[64,65] This approach could effectively streamline RNA virus detection procedures and support multiplexed detection.

Furthermore, the microscale and functional integration of microfluidics with RNA virus sensors reduce reagent and sample consumption, in addition to improving the speed and sensitivity of detection. For example, a programmable microfluidic-sensing platform uses a swarm of millimetre-sized magnets as mobile robotic agents for handling magnetised sample droplets.[15] This platform can automatically perform RT-LAMP and colourimetric detection of SARS-CoV-2 in clinical samples, with a ten-fold to 300-fold reduction in reagent costs and three orders of magnitude reduction in instrumentation cost. Various low-cost microfluidic-sensing devices have also been developed for the efficient and simultaneous detection of viral antigens, antibodies, and multiple types of biomarkers.[13,14,16]

The integration of RNA virus sensors with microfluidic chips might help to develop automated RNA virus detection devices for various applications, including portable devices for field detection, wearable devices for exposure and infection analysis, and stationary devices for air and water monitoring (figure 1D). These devices could effectively support the acquisition of extensive information on RNA viruses in humans, animals, and the environment.

Challenges and prospects of RNA virus sensors

Despite the rapid advancements in RNA virus-sensing technologies, several challenges persist.

First, to be fully functional, many emerging sensing technologies still require specialised equipment, which compromises their cost-efficiency and restricts their widespread adoption. For example, plasmonic heating-accelerated nucleic acid amplification relies on specialised equipment containing an expensive laser array.[8] Similarly, a large-scale multiplex nucleic acid detection system using Cas13 requires droplet generators and fluorescence microscopy for sample preparation and signal detection.[18] Thus, more simplified and integrated designs in various sensing technologies are needed to improve accessibility, particularly in low-income and middle-income countries.

Second, the biosensing materials used in these sensors might become inactivated upon contact with complex samples (eg, faeces and whole blood). Additionally, developing highly specific biosensing materials for antigen and antibody detection remains challenging. Although monoclonal antibodies have shown good specificity in antigen detection, their production remains limited in resource-constrained settings.[66] Therefore, developing

Search strategy and selection criteria

We searched PubMed for articles published in English between January, 2016 and October, 2024, and the websites of WHO, using the following terms: ("RNA viruses" OR "Epidemiology" OR "Coronaviruses" OR "Dengue virus" OR "Zika virus" OR "Influenza viruses" OR "Measles Virus") AND ("nucleic acid" OR "molecular" OR "antigens" OR "spike proteins" OR "spike S" OR "spike N" OR "antibodies" OR "IgG" OR "IgM" OR "IgA") AND ("sensors" OR "tests" OR "detection" OR "diagnostics" OR "assay" OR "amplification"). Full-text articles were included whenever they involved state-of-the-art RNA virus sensors for detecting various biomarkers in emerging or re-emerging RNA virus outbreaks and the role of RNA virus detection in One Health strategies. Relevant international and national guidelines and policies were also included.

sensors that require less stringent production protocols and can use more readily available materials is now crucial.

Third, current multiplexed detection primarily relies on the repetition of transducers and the use of various biosensing materials. Although this approach increases throughput, a targeted design for detecting specific viruses is often not viable. Finetuning the sensor structure, detection range, and offset might be necessary to detect different viruses accurately, as mismatches between targets and transducers can considerably affect sensitivity.

Fourth, correct interpretation of sensing results is crucial and requires a comprehensive understanding of the characteristics and limitations of biomarkers and their sensors (figure 2). Notably, the sensitivities and specificities of the diagnostic methods discussed can vary with the actual prevalence or infection rate of the screened population,[67] which emphasises the need for confirmatory diagnosis after screening in large-scale epidemiological investigations, regardless of the high sensitivity reported during the development phase.

Lastly, the data obtained from RNA virus detection contain valuable information worth exploring, such as virulence, transmissibility, and resistance to antivirals. At the same time, leveraging this potential requires prior knowledge and further research.[68,69]

Addressing these challenges requires multidisciplinary collaboration, including the development of high-affinity antigens or antibodies through synthetic biology, the design and manufacture of sensor chips (including optical, nano, and electrochemical types) based on materials chemistry and microfabrication or nanofabrication, and the proper use of these sensors.[7,38] Furthermore, the suitability of sensors and devices for large-scale production, which can accelerate prototyping and manufacturing processes, should be considered. As the cost of RNA virus sensors decreases, their shelf life extends, and the sensing devices become more user-friendly, providing medical, veterinary, and environmental professionals (and even households) with ready-to-use detection tools will become increasingly feasible.

To enhance virus surveillance and outbreak preparedness, WHO and the Foundation for Innovative New Diagnostics have established a strategic collaboration aimed at strengthening diagnostic capacities in resource-limited countries.[70] Cooperation among international organisations, such as WHO, the Food and Agriculture Organization of the UN, the UN Environment Programme, and the World Organisation for Animal Health, has also been strengthened to improve the surveillance and control of zoonotic disease outbreaks.[71] RNA virus-sensing technologies are expected to support these efforts by scaling up diagnostic capabilities, thereby addressing crucial health security threats at the human–animal–environment interface.

Contributors

SS, DW, YC, PW, FNZ, and HD conceptualised the study and contributed to the writing and revision of the manuscript. QWY and FNZ prepared the display items. YC, QWY, XYJ, and XYW performed the literature search. MK contributed to the section on the challenges and recommendations. All authors comprehensively reviewed and edited the manuscript.

Declaration of interests

We declare no competing interests.

Acknowledgments

This study was funded by the National Key Research and Development Program of China (grant number 2023YFD1802000), National Natural Science Foundation of China (grant number T2450074), the Program of Shanghai Academic Research Leader (grant number 23XD1420700), and Medical Science Data Center of Fudan University, National Natural Science Foundation of China (grant number 82102180), the Zhejiang Provincial Natural Science Foundation of China (grant number Z24H180008), and Shanghai Hospital Development Center "Research physician innovation and translational ability training program" (grant number SHDC2022CRS028). The Rising-Star Program of Shanghai 2024 Science and Technology Innovation Action Plan (Yangfan Special Project, 24YF2734300). We thank Professor Shuang Hao of Northeastern University for her support and guidance, and thank Professor Andres Merits of University of Tartu for his advices on manuscript preparation.

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