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Review article

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Lateral flow test engineering and lessons learned from COVID-19

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Abstract

The acceptability and feasibility of large-scale testing with lateral flow tests (LFTs) for clinical and public health purposes has been demonstrated during the COVID-19 pandemic. LFTs can detect analytes in a variety of samples, providing a rapid read-out, which allows self-testing and decentralized diagnosis. In this Review, we examine the changing LFT landscape with a focus on lessons learned from COVID-19. We discuss the implications of LFTs for decentralized testing of infectious diseases, including diseases of epidemic potential, the 'silent pandemic' of antimicrobial resistance, and other acute and chronic infections. Bioengineering approaches will play a key part in increasing the sensitivity and specificity of LFTs, improving sample preparation, incorporating nucleic acid amplification and detection, and enabling multiplexing, digital connection and green manufacturing, with the aim of creating the next generation of high-accuracy, easy-to-use, affordable and digitally connected LFTs. We conclude with recommendations, including the building of a global network of LFT research and development hubs to facilitate and strengthen future diagnostic resilience.

Sections

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Key points

- Lateral flow tests (LFTs) were adopted at an unprecedented scale during the COVID-19 pandemic, enabling access to testing beyond healthcare settings.
- Only 0.4% of the 3 billion COVID-19 tests performed through to mid-2022 were conducted in low-income regions, raising ethical concerns and constraining our collective ability to respond to a pandemic.
- Key barriers to COVID-19 LFT development and adoption include lack of access to well characterized samples, limited accuracy, lack of connectivity, lack of evidence of cost-effectiveness, regulatory delays and centralized manufacturing capabilities.
- LFTs could also play an important part in the detection of other diseases of epidemic potential and antimicrobial resistance.
- Bioengineering approaches, such as the use of nano- and quantum materials, nucleic-acid-based LFTs, CRISPR and machine learning, will improve the sensitivity, specificity, multiplexing and connectivity features of LFTs.
- We recommend investing in an international LFT research and development hub network to spearhead the development of a pipeline of innovative bioengineering approaches to design next-generation LFTs.

Introduction

Diagnostics have emerged as a crucial countermeasure to the spread of COVID-19, and by late 2022, more than 3 billion tests for SARS-CoV-2 had been conducted worldwide[1]. Reverse transcription-polymerase chain reaction (RT-PCR) remains the gold standard for diagnosing COVID-19, and genomic sequencing has become vital for tracking variants. However, lateral flow tests (LFTs), albeit less sensitive than PCR, have enabled an unprecedented scale of global testing in clinical and public health, owing to their simplicity, low cost, accessibility, rapid results and ability to detect infectiousness[2] (Fig. 1a).

The bioengineering underpinnings of LFTs (also known as rapid diagnostic tests (RDTs), lateral flow assays, lateral flow immunoassays or immunochromatographic tests) date back decades. The first latex agglutination and immunoassays in the 1950s[3] and subsequent refinement of the solid-phase lateral flow assay in the 1980s[4,5] led to the first LFT pregnancy tests, which were revolutionary in empowering women to manage their own health (Fig. 1b). By the 1990s, the first malaria LFTs were being used by trained healthcare providers, although it took two decades before the pre-qualification requirements of the World Health Organization (WHO) were settled. LFTs have since been developed to diagnose infectious diseases in primary healthcare settings worldwide, including for malaria, human immunodeficiency virus (HIV), Strep A (group A *Streptococcus*) and influenza A/B, and selected LFTs are now available for self-testing at clinics and pharmacies worldwide. In 2016, the WHO recommended HIV self-testing with LFTs, based on their effectiveness to reach key populations and increase case detection; nonetheless, adoption remains limited[6]. Compared to other infectious diseases, for which LFT development can take years, SARS-CoV-2 antigen LFTs were developed and deployed within months (Fig. 1b). In 2022, the WHO 'strongly endorsed' COVID-19 self-testing with antigen LFTs[7], putting the public at the heart of the public health response.

The simplicity of LFTs comes with technical limitations and usage trade-offs. Notably, they are less sensitive than PCR and rely on visual readout. LFTs also lack digital connectivity for data collection and linkage to care. However, innovations in ultra-sensitive nanomaterials, clustered regularly interspaced short palindromic repeats (CRISPR)-based detection, mobile app connectivity and deep learning have greatly improved LFT technology, albeit often at an early stage of technological readiness, reflecting a disconnect between bioengineering research priorities and practical use cases.

In this Review, we discuss the design principle of LFTs, and highlight key lessons learned from their use in the COVID-19 pandemic, including access, accuracy, affordability, manufacturing, regulation and funding[8]. We examine the implications of decentralized LFT testing for pandemics, endemic infections and antimicrobial resistance, and discuss bioengineering approaches aimed at meeting the REASSURED criteria (that is, having real-time connectivity, ease of sample preparation, being affordable, sensitive, specific, user-friendly, robust and reliable, equipment-free or environmentally friendly, and deliverable to end-users)[9]. Finally, we summarize research and development (R&D) priorities for researchers, industry, funders and policymakers.

Lateral flow tests

Target analytes and samples

LFTs can be designed to target different analytes, such as antigens (for example, SARS-CoV-2 nucleoproteins) and antibodies (IgG or IgM) (Fig. 2a). LFTs can also detect nucleic acids, although such tests are not commercially available, except in China and from a single US company[10]. LFTs can detect analytes in blood, urine, saliva or vaginal swabs, with sampling protocols (sample collection, buffers, incubation time) varying by disease, sample matrix and analyte[11].

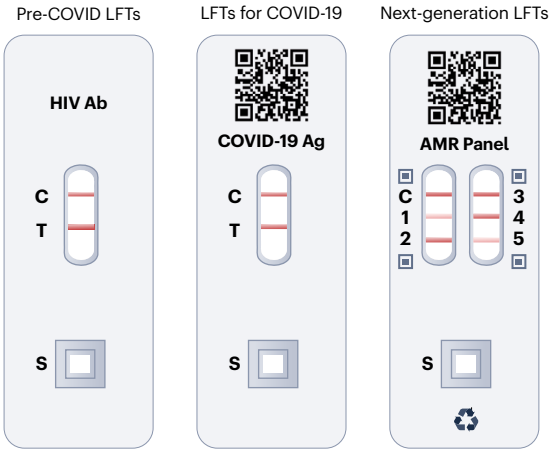
Flow

In LFT-based diagnostics, the sample is first placed onto a cellulose sample pad, and then travels by capillary force to the conjugate pad, where previously dried nanoparticle–receptor complexes are resuspended in the sample buffer (Fig. 2a). Here, gold or latex nanoparticles are most frequently used owing to their ease of manufacture, low cost, wide availability, stability, ease of functionalization with proteins, and in the case of gold, strong plasmonic absorption[12]. In addition, magnetic beads, nanodiamonds[13], quantum dots and other particles have been explored[14]. Mass transport is governed by flow, diffusion and dispersion owing to membrane porosity, but is typically flow-dominated. Flow in LFTs can be described by four flow regimes[15]; alternatively, Washburn and Darcy equations[16] can be applied to model the flow.

Detection

As the sample flows, the target analyte forms complexes with 'detector' receptors on the nanoparticles. Once the complexes reach the test line, which is typically printed with a second 'capture' receptor that is electrostatically bound to the membrane[17], the analyte is bound in a 'sandwich' (Fig. 2b). The accumulation of nanoparticles at the test line generates the signal. Here, binding is limited by target–receptor reaction kinetics rather than by mass transport (Fig. 2c). A control line

a



	Pre-COVID LFTs	LFTs for COVID-19	Next-generation LFTs
Targets	• Antibody • Antigen	• Antibody • Antigen	• Antibody • Antigen • Molecular
Use cases	• Self-testing • Clinical diagnosis • Screening	• Self-testing • Surveillance • Screening	• Self-testing • Clinical diagnosis • Screening • Surveillance • Environmental monitoring
Settings	• Homes • Clinics • Community	• Homes • Mass gatherings • Schools • Borders • Workplaces	• Homes • Mass gatherings • Schools • Borders • Workplaces • Clinics • A&E
Materials	• Gold nanoparticles • Latex beads	• Gold nanoparticles • Latex beads • Quantum dots	Ultra-sensitive materials: • Enzymatic nanoparticles • Nanodiamonds
Result capture	• Manual	• Manual	• Digital • Automatic connection to healthcare pathway

b

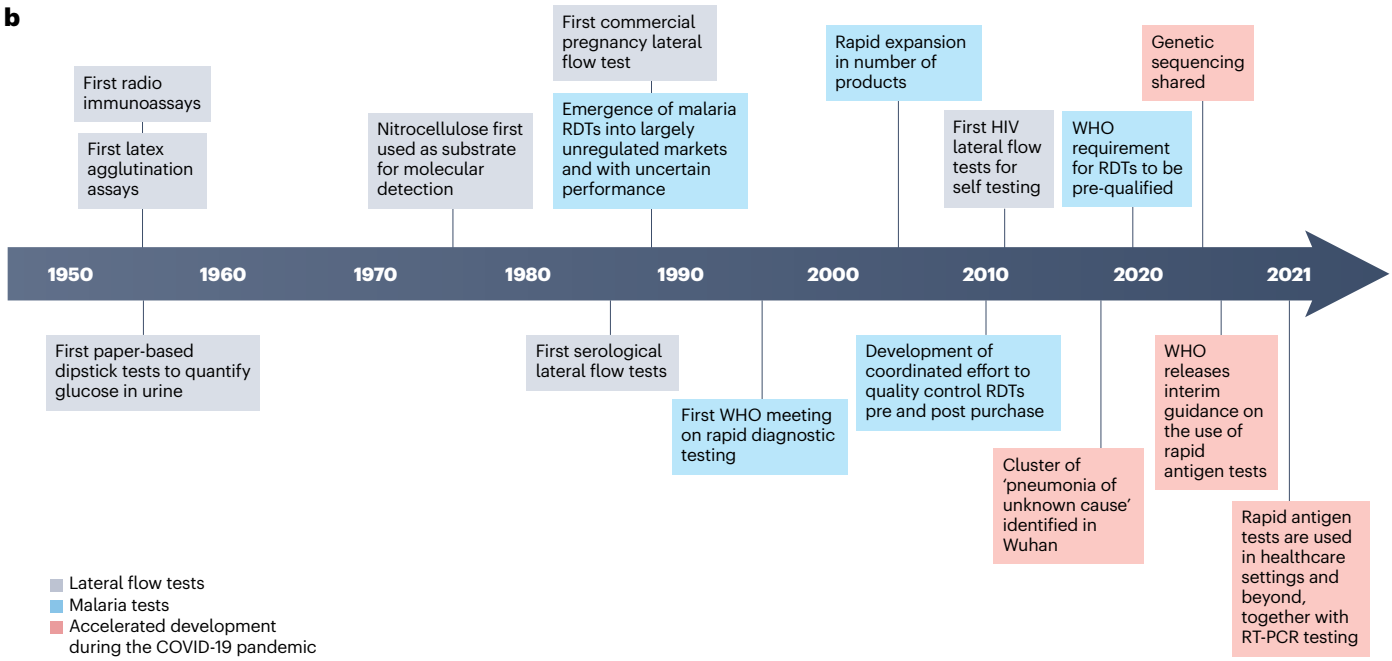


Fig. 1 | Lateral flow tests. a, Characteristics of pre-COVID-19 lateral flow tests (LFTs), LFTs deployed in the COVID-19 pandemic, and next-generation LFTs. **b**, Timeline of key advances in lateral flow testing. WHO, World Health Organization; HIV, human immunodeficiency virus; RDT, rapid diagnostic test; AMR, antimicrobial resistance.

binds the nanoparticles with or without analyte complexation, verifying that the sample has flowed appropriately and that detection complex molecules are functional.

Results

Most LFTs are read qualitatively by visual inspection after 5–30 minutes (Fig. 2d). Alternatively, fluorescent nanoparticles can be used for detection, which may require readers, adding cost, but standardizing results and reducing error owing to subjective interpretation. In addition, quantitative readout data can be captured[18,19].

Commercial kit components and users

A typical LFT kit contains a nitrocellulose membrane strip with dried nanoparticles bearing detector receptors on a glass-fibre conjugate pad, housed in a plastic cassette with a QR code and an identification (ID) number (Fig. 2d). In addition, LFT kits designed for nasopharyngeal samples contain a collection swab, typically a flocked, rayon or Dacron tip on a polypropylene shaft, an extraction tube containing a buffer to extract the target antigens, a plastic waste bag, and written guidance for use, including links to further information or videos. LFTs can be administered by trained health professionals ('professional use' tests), or self-administered ('self-tests').

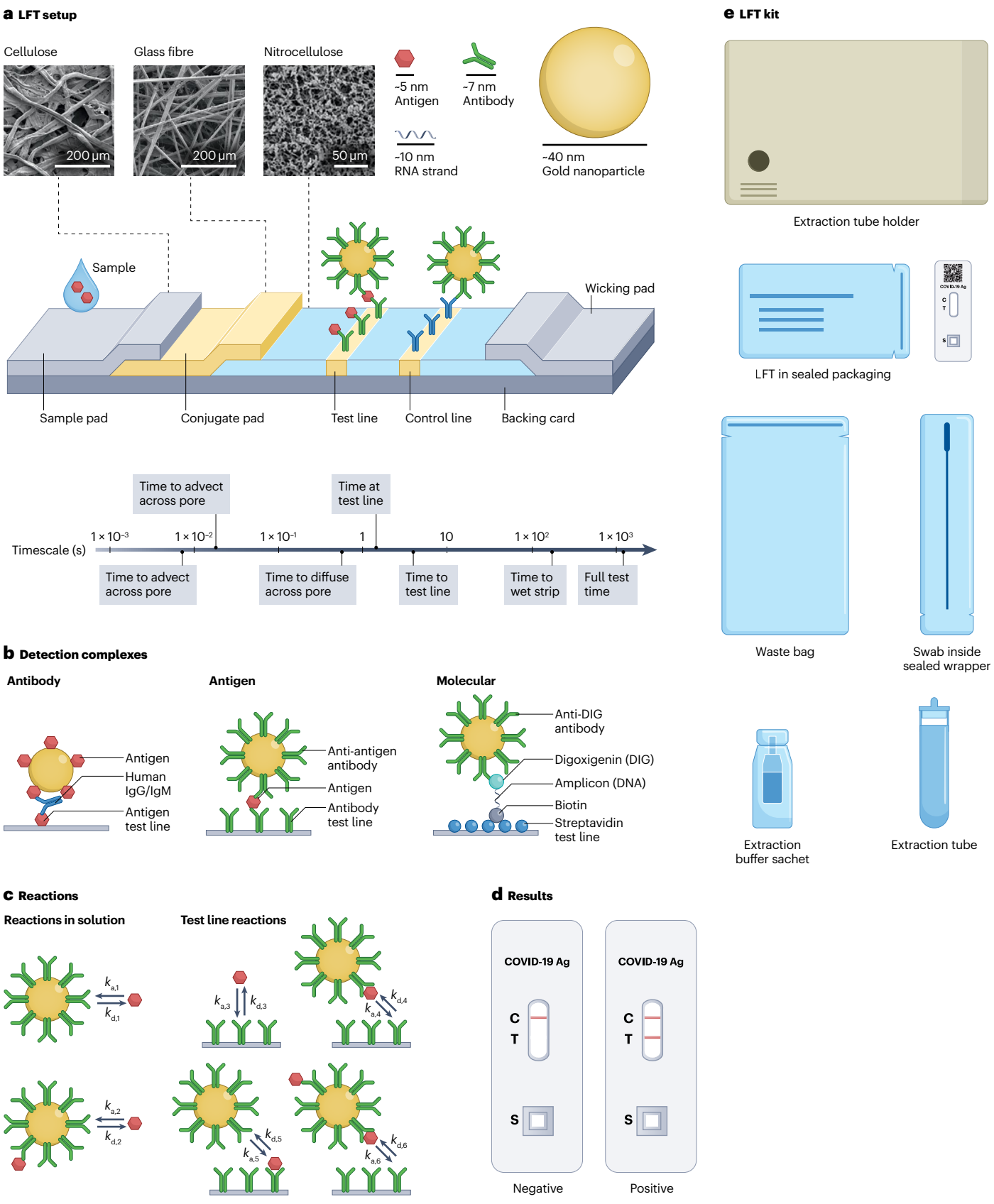

a LFT setup
Cellulose
Glass fibre
Nitrocellulose
200 µm
200 µm
50 µm
~5 nm Antigen
~7 nm Antibody
~10 nm RNA strand
~40 nm Gold nanoparticle
Sample
Wicking pad
Sample pad
Conjugate pad
Test line
Control line
Backing card
Time to advect across pore
Time at test line
Timescale (s)
1 × 10⁻³
1 × 10⁻²
1 × 10⁻¹
1
10
1 × 10²
1 × 10³
Time to advect across pore
Time to diffuse across pore
Time to test line
Time to wet strip
Full test time
b Detection complexes
Antibody
Antigen
Human IgG/IgM
Antigen test line
Antigen
Anti-antigen antibody
Antigen
Antibody test line
Molecular
Anti-DIG antibody
Digoxigenin (DIG)
Amplicon (DNA)
Biotin
Streptavidin test line
c Reactions
Reactions in solution
Test line reactions
d Results
COVID-19 Ag
C
T
S
Negative
COVID-19 Ag
C
T
S
Positive
e LFT kit
Extraction tube holder
LFT in sealed packaging
Waste bag
Swab inside sealed wrapper
Extraction buffer sachet
Extraction tube

Fig. 2 | Lateral flow test components. a, A typical lateral flow test (LFT) is composed of a cellulose sample pad, which absorbs the sample, a glass fibre conjugate pad, which stores dried nanoparticle–receptor conjugates, a nitrocellulose membrane with a test line of immobilized capture receptors, and an absorbent pad to wick the sample. The sample is dropped onto the sample pad, and flows down the strip by capillary action into the conjugate pad, where it resuspends the nanoparticle–antibody complex, which binds to the target analyte. These complexes flow into the nitrocellulose and continue to the test line, which is printed with antibodies that bind to a different paratope of the nucleoprotein. The control line is functionalized with antibodies that bind to the antibodies on the nanoparticles, or an alternative species. **b**, Assay designs for different analyte types. **c**, The different interactions between the analyte, the detection receptors on the nanoparticle and the capture receptors on the membrane are illustrated (not to scale). Association (k_a) and dissociation (k_d) rates are enumerated for different affinity binding reactions in LFTs. **d**, The test is housed in a plastic cassette with a well for sample addition (S), internal contact points to guide flow, and a readout window with test (T) and control (C) line markings. Some tests have a QR code and an identification (ID) number (COVID-19 only). **e**, COVID-19 LFT kits typically contain a nitrocellulose test membrane strip with dried nanoparticles bearing detector receptors (typically antibodies) on a glass-fibre conjugate pad, housed in a plastic cassette with a QR code and an ID number (note that most pre-COVID-19 LFTs lack these); a nasal swab (for anterior nares or mid turbinate), typically a flocked, rayon or Dacron swab with a polypropylene shaft; an extraction tube containing a solution to extract viral antigens; a plastic waste bag; and written guidance for use, including links to further information or instruction videos. Ag, antigen. Part **a** adapted with permission from ref. 184, Royal Society of Chemistry, and adapted from ref. 96, Springer Nature Limited, and adapted from ref. 15, Annual Reviews. Part **c** adapted from ref. 15, Annual Reviews.

Lessons learned from COVID-19

Large-scale testing

LFTs were adopted on an unprecedented scale during the COVID-19 pandemic, demonstrating their feasibility and acceptability on a global basis. LFTs have had multiple clinical and public health use cases[20,21], such as testing to confirm diagnosis in symptomatic individuals, testing to screen asymptomatic individuals with known exposures or in high-risk groups, such as healthcare workers, care home (elder home) workers, or first responders, screening of asymptomatic individuals at schools, workplaces or mass gatherings, air, land or sea border testing to slow the introduction of new variants, testing to determine the effectiveness of anti-viral treatment, testing for surveillance, and infection-control-based testing in healthcare facilities to facilitate flow of patients[22,23].

Professional use and self-tests have enabled LFT-based testing to be expanded beyond healthcare facilities and into community settings and homes (Fig. 3a). COVID-19 testing programmes have been implemented on a city scale (for example, the United Kingdom Liverpool Community testing pilot)[24], and on a national scale (for example, nationwide testing in Slovakia)[25]. In England, 20 million tests were used in less than 12 months, outpacing RT-PCR testing[26] (Fig. 3b).

In many high-income regions, COVID-19 self-tests have been widely available since 2021, often subsidized or free to the public through pharmacies or online ordering. A 2022 WHO survey found that COVID-19 self-testing policies have been in place or under consideration in 101 countries[7] (Fig. 3a). Self-tests have been used in population surveillance studies, such as the Real-time Assessment of Community Transmission (REACT)-2 study in the UK[27], and have been widely accepted and preferred for self-testing in Europe and the USA[28–30], demonstrating safe and error-free use, as well as correct interpretation of results[31–34]. In low- and middle-income regions, COVID-19 self-tests have also shown high acceptability[35,36] and close agreement between results from professional use and self-testing (in Malawi and Zimbabwe)[7], mirroring earlier findings by the Self-Testing Africa (STAR) initiative on HIV self-testing[37]. The WHO survey found that regions implementing COVID-19 LFT self-testing perceived many benefits, including more timely diagnosis and self-isolation, increased access to testing and uptake in the population, increased testing frequency, increased adherence to public health and social distancing measures, decreased transmission, and earlier return to pre-COVID-19 activities[7], as compared to regions that had not implemented LFT self-testing. However, access to self-tests remains inequitable, with substantially lower adoption in low- and middle-income regions (Fig. 3a and Box 1).

Despite wide use and acceptability, COVID-19 LFTs and the care pathways in which they are used have limitations, particularly in terms of false positives and false negatives (Supplementary Table 1). Concerns (in particular, in low- and middle-income regions) include limited educational interventions, inadequate service delivery models for vulnerable populations, inequities in access, unclear regulations alongside inadequate WHO Emergency Use Listing[38], low-quality tests, variability between tests[39] and sampling sites[40], data loss for public health surveillance, coercive testing, contrived results, unclear guidance for managing positive results, and lack of confirmatory testing. Therefore, more rigorous implementation research is needed, including trials evaluating the clinical effectiveness and cost effectiveness of different LFT-based testing strategies and algorithms.

Accuracy

The accuracy and, in particular, the sensitivity of LFTs is lower than that of reference RT-PCR methods, ranging between 34.1% and 88.1% for SARS-CoV-2 antigen LFTs, with an overall specificity of 99.6% (here, data from instructions-for-use-compliant evaluations in symptomatic participants were used), with sensitivity varying between brands[41]. Analytically, rapid antigen tests can detect virus at levels equivalent to approximately 100,000 to 1,000,000 SARS-CoV-2 viral genome copies per millilitre[39], whereas molecular methods, such as RT-PCR, can detect 1–100 copies per millilitre, and thus, the presence of SARS-CoV-2 at 24–48 hours before LFTs turn positive. Such a trade-off between sensitivity and simplicity has long limited the use of LFTs for certain pathogens. The success of COVID-19 antigen LFTs can be in part attributed to the pathophysiology of SARS-CoV-2 (Fig. 3c), that is, its short incubation period and high transmission rates, which are well suited to rapid, frequent testing[2,42]. In addition, pre-symptomatic and asymptomatic people generally shed sufficiently high antigen loads from nasal and throat samples for timely LFT detection. Moreover, the high antigen load of infectious individuals (established by viral culture) correlates well with COVID-19 LFT analytical sensitivity and specificity[2,43]. Owing to the long tail of SARS-CoV-2 infection, viral RNA can remain detectable long after live SARS-CoV-2 can no longer be cultured from patient samples, that is, during the non-infectious recovery phase. In addition to being 'overly sensitive' in establishing infectiousness, molecular test methods are problematic for large-scale, high-frequency testing programmes, given the need to send samples to centralized laboratories, challenges in scaling-up laboratory capacity, and subsequent delays in receiving test results (which can take days).

Thus, LFTs benefit COVID-19 testing in identifying infectiousness or risk of transmission. LFT testing has enabled healthcare workers to return to work, schools and workplaces to reopen, and economic

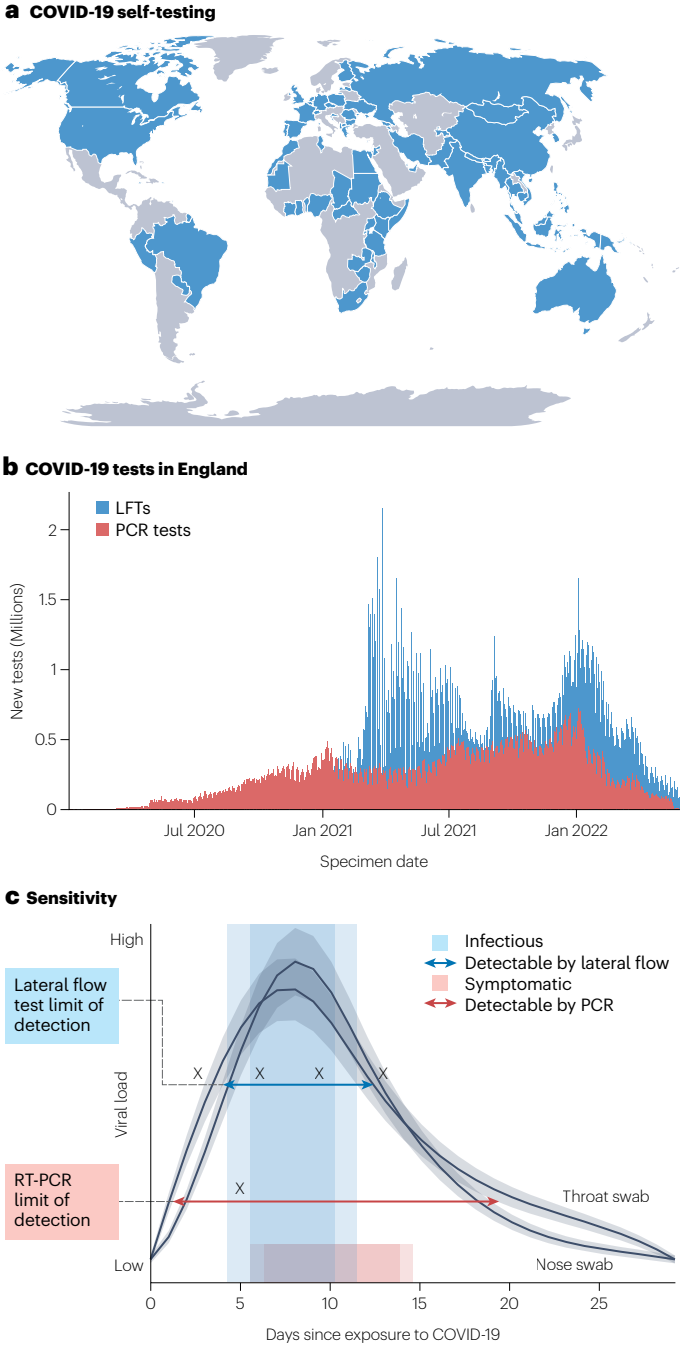


Fig. 3 | Lessons learned from COVID-19. a, The map shows the global distribution of regions in which a COVID-19 self-testing policy was in place, was being considered or was being piloted as of March 2022, adapted from the World Health Organization (WHO)[7]. **b**, Rapid adoption of lateral flow tests (LFTs) in England, following their introduction in 2021, surpassing polymerase chain reaction (PCR) use. Data from the UK Coronavirus Dashboard[26]. **c**, LFT sensitivity in comparison to PCR. LFT sensitivity aligns with the infectious period of COVID-19 and can detect COVID-19 one to two days after PCR can. The low-cost, portable and rapid format of LFTs allows more frequent testing.

recovery, including mass gatherings, border testing and travel testing. The viral load threshold for transmission has been proposed to be about 1,000,000 copies[44], and therefore, rapid antigen tests are considered to be a good public health tool with which to identify infectious people and those at risk of transmitting SARS-CoV-2 to others, thus reducing community transmission[45], with the advantages of ease of use, lower cost, rapid turnaround, and the ability to enable serial daily or weekly testing, which is not currently feasible using RT-PCR testing[2]. In a pandemic, rapid diagnosis of disease can offset loss of sensitivity, allowing the implementation of public health measures, such as self-isolation and contact tracing, without delay in interrupting the chain of transmission.

The WHO has established a target product profile[46] for COVID-19 antigen LFTs for use in suspected COVID-19 cases and close contacts, highlighting the application of LFTs in areas where reference molecular testing is unavailable, or where molecular turnaround times obviate their utility. Specifically, the WHO recommends more than 80% sensitivity (the probability of a positive test, conditioned on truly being positive) and more than 97% specificity (the probability of a negative test, conditioned on truly being negative) for LFTs, using an authorized molecular test (that is, authorized for emergency use by the WHO or the US Food and Drug Administration (FDA)) as reference. Independent evaluations of hundreds of commercial LFTs have been conducted, many supported by the Foundation for Innovative New Diagnostics (FIND)[47–49], the Paul Ehrlich Institute[50], and other public health authorities[43].

Even though LFT sensitivity correlates well with infectiousness, false-negative COVID-19 LFT test results remain an issue, particularly early in an infection, when false-negative tests can lead to inadvertent high-risk contacts and ongoing transmission. Therefore, the timing and frequency of LFT testing are important in early symptomatic infection and in screening individuals (before travel or mass gatherings), because infectious individuals may have tested negative by LFT in the prior 24 hours.

Antigen LFT performance and utility also vary with prevalence[45,51], requiring careful policies and different testing strategies in different epidemiologic settings; for example, self-isolation and repeated testing in high-prevalence, high-vulnerability settings may be warranted for symptomatic individuals even with a negative COVID-19 LFT result, whereas confirmatory RT-PCR testing may be warranted in low-prevalence settings. LFT accuracy also varies slightly for different COVID-19 variants, because of mutations and pathophysiology changes[52]. Despite slight differences in sensitivity, most LFTs remain effective in detecting the major variants of concern, including the Delta[53] and Omicron[54] variants, which contain most mutations in the genes encoding the spike (S) protein, whereas most antigen tests use the nucleocapsid (NP) protein as target.

Fortuitously, COVID-19 antigen LFTs have sufficient accuracy for effective large-scale testing of SARS-CoV-2. However, LFT platforms developed for COVID-19 cannot automatically be transferred to other diseases of epidemic potential, many of which will be more difficult to detect by LFT.

Development and scale-up

Diagnostics have long been underfunded and underused in global health. From the start of the COVID-19 pandemic, a huge amount of funding was directed to SARS-CoV-2 test development and uptake. The US Rapid Acceleration of Diagnostics (RADx) programme invested more

than US$1.5 billion (2020) in diagnostics, including for the development of new diagnostics to boost existing laboratory capacity[55]. The UK government spent an estimated £13.9 billion to make testing freely available between Q2 2020 and Q2 2021 (ref. 56). Member states requested that the WHO prepare a strategy to support access to diagnostics[57], testing and vaccines in low- and middle-income regions, which led to the establishment of the access to COVID-19 tools accelerator (ACT-A)[1]. FIND and the Global Fund, alongside the WHO, co-convened the 'ACT-A Diagnostics Pillar', which supported independent evaluation of SARS-CoV-2 antigen LFTs performance, emergency authorization and multiple programmes to increase access to COVID-19 testing in the Global South, including negotiated ceiling prices for SARS-CoV-2 LFTs and RT-PCR kits (Box 1).

RT-PCR tests can be rapidly developed for a new pathogen based on shared sequence data. By contrast, antigen LFT development requires weeks to months in the best of circumstances, including the design of capture receptors (typically antibodies) against target analytes. In addition, companies typically develop their own proprietary reagents, often based on recombinant antigens. Unsurprisingly, international standardization of diagnostic reagents has been problematic during the pandemic. Reference measurement frameworks and standard development help to ensure accurate diagnostics and their availability during an outbreak of a new pathogen. Moreover, established standards can fast-track our understanding of disease pathogenesis by providing comparability of test results.

Millions of LFTs can be produced per month to meet global demand at affordable prices; however, such scale-up requires investment in manufacturing infrastructure and time. Lack of LFT manufacturing capacity was a major COVID-19 response bottleneck until the end of 2020. Coupled with the higher costs of molecular assays and the required instruments and infrastructure, many places lacked sufficient testing capacity in the pandemic's initial months[57]. That said, a major pandemic achievement was the timeline to develop, scale and deploy new LFTs for a previously unknown virus, which was ultimately compressed from several years to months (Fig. 1b). The first commercial antigen LFT received emergency use authorization in May 2020 (ref. 58), five months after the first COVID-19 case was reported. Many LFT manufacturers claim that the development timeline could have been even shorter, noting that SARS-CoV-2 is relatively straightforward for antigen detection. The main bottlenecks were access to samples for test optimization and validation, and slow regulatory processes[59] (Box 2). In a G7 report tasking policy makers to enable LFT readiness in 100 days[60] for the next outbreak, diagnostics manufacturing capacity and regulation were identified as key areas for improvement.

Differing resources, national regulatory requirements, purchase mechanisms, logistics and policy approaches led some regions to adopt LFTs at large scale sooner than others—especially high-income regions. LFT costs and uptake have varied by country during the pandemic, from free tests through government subsidies to end-user prices as high as US$20 per test[7]. Some low- and middle-income regions experienced difficulties in accessing tests once high-income regions had bought up supply (a problem also seen for COVID-19 vaccines), despite the importance of LFTs in settings with limited molecular testing capacity and rural populations. Regional manufacturing and logistical capabilities for LFT supply became a global concern, given minimal test manufacturing capacity in Africa and elsewhere[61]. Importantly, funding made available for LFT development and manufacturing during the COVID-19 pandemic could be lost[62], but will be required if the world aims to meet the challenge of having LFTs ready in 100 days for the next pandemic, and to address underlying supply chain issues affecting diagnostic access globally[63,64].

Digital data capture

COVID-19 LFT results from self-testing, positive or negative, are often not reported[65], leaving test use data and true case counts unknown, thereby complicating surveillance; for example, only 14% of LFT results up until the end of May 2021 were reported to UK Test and Trace[65]. Digital technologies have been deployed throughout the pandemic response[66], but opportunities for digital LFT data capture, quality assurance, linkage to care, and resource planning were largely missed (Fig. 4). Public health agencies have been slow to adopt digital innovations, with the first WHO guidelines on digital health interventions for health system strengthening published in 2019 (ref. 67).

In the USA, several FDA-authorized LFTs have a companion app, through which the user manually enters test results. LFTs can also contain an integrated reader to detect fluorescent signals and digitize results.

Box 1

Global testing inequities

The COVID-19 pandemic has revealed enormous inequities in access to tests, vaccines and therapeutics. The Access to COVID-19 Tools (ACT)-Accelerator diagnostic pillar (ACT-A Dx), part of the ACT-A mechanism, was established to increase equitable access to COVID-19 testing globally. By mid-2022, ACT-A had helped to secure high-volume supply agreements for antigen lateral flow tests (LFTs) at price ceilings of around US$2.50 per test (2021 value), had secured technology transfer and licensing agreements, and had procured more than 158 million tests through the Global Fund's C19RM mechanism[1]. Despite these efforts, of the 3 billion tests conducted worldwide by 2022, only 0.4% were used in low-income regions, which comprise 7.8% of the global population[1].

These disparities in testing coverage not only affect our collective ability to respond to the pandemic, but raise ethical concerns. The World Health Organization (WHO) Director General has highlighted that "nobody is safe until we are all safe"[186]. ACT-A Dx identified COVID-19 testing levels of at least 1 test per 1,000 people per day as minimal targets for disease mitigation and for the early identification of new variants (ACT). Nonetheless, at the start of 2022, ACT-A faced a collective US$14 billion funding shortfall[187] for vaccines, diagnostics and therapeutics; simultaneously, as the Omicron variant wave peaked in early 2022, testing levels worldwide declined rapidly.

Disparities in LFT testing are found in disadvantaged groups in high-income regions as well as in low-and middle-income regions. The UK Liverpool large-scale voluntary asymptomatic testing observational study reported social, ethnic, digital access and spatial inequalities[188], highlighting that free and voluntary community testing requires adequate support, such as financial aid to enable individuals to isolate or non-digital routes for testing, to minimize inequalities.

In the future, decentralized test manufacturing, bulk purchasing and distribution of tests, cross-border regulatory harmonization, affordable pricing, self-testing, independent clinical evaluations and increased testing capacity could accelerate equitable diagnostics access.

In pilot programmes, digital LFTs were provided for travellers entering the USA at certain airports, with voluntary, app-enabled reporting to the US Centers for Disease Control and Prevention[68]. However, most commercial LFTs provide only a qualitative visual output. Test line intensity depends on multiple factors; in particular, low SARS-CoV-2 antigen concentrations can cause faint test lines, which may be wrongly interpreted as a negative result, risking transmission and a loss of public trust.

Digital approaches to interpreting LFT results have been rare[69,70], and have not yet been widely operationalized. A UK research team at i-sense, in partnership with the Africa Health Research Institute, developed an image library of 11,000 field-acquired HIV LFT photographs and deep learning models to classify results for quality assurance. This approach reduces the number of false positives and negatives, compared to visual audit by nurses and community health workers[19]. The same models were applied to COVID-19 LFTs in partnership with the UK REACT study, and a workflow was developed to analyse more than 500,000 COVID-19 antibody LFT self-tests[71]. Alternatively, machine learning has been applied to analyse LFTs for UK National Health Service (NHS) staff on a smaller dataset[72]. These image datasets are taken in real-world conditions and contain weak positives and invalid tests on a variety of devices, enabling more robust classification.

Re-imagining lateral flow tests

LFTs may make a difference in detecting a range of other infections[73], particularly the WHO's list of priority diseases of epidemic potential, antimicrobial resistance and other acute and chronic infections.

WHO priority diseases of epidemic potential

The development and evaluation of diagnostics for diseases of epidemic potential are often only funded during outbreaks, and are sometimes abandoned once the outbreak abates, leaving regions ill-prepared for the next pandemic[74]. In 2015, in response to the Ebola outbreak in West Africa, the WHO convened experts to develop an R&D blueprint for action to prevent epidemics[75], focusing on emerging diseases with the potential to generate a public health emergency, and for which no or insufficient tools existed, aiming at reducing the time between identification of a nascent outbreak and approval of countermeasures (Supplementary Table 2). Commercial LFTs are currently not available for four of the eight known priority diseases of epidemic potential: Crimean Congo haemorrhagic fever, Middle East respiratory syndrome coronavirus (MERS-CoV), Nipah and other henipaviruses, and Rift Valley fever. For the remaining four, bioengineering challenges remain to be addressed. Low sensitivity limits the use of filovirus LFTs (for example, Ebola); thermal stability is needed for Lassa fever LFTs; and Zika LFTs may need to be multiplexed to detect both antigen and IgM to improve specificity. Moreover, 'disease X', referring to a serious global epidemic caused by an unknown pathogen, will necessitate an even more agile approach to LFT development and preparedness[76].

Industry has historically been reluctant to invest in the development and commercialization of LFTs for pathogens of pandemic potential, owing to an uncertain market size (even during outbreaks), and inconsistent or zero demand in the case of no outbreaks. In addition, well characterized specimens, essential for test development, are often difficult to access. Moreover, performance studies required

Box 2

Regulatory considerations

The COVID-19 pandemic has affected the approach of regulators with regard to approving lateral flow tests (LFTs), including early engagement with and guidance to test developers. Emergency use authorization procedures, longstanding in the UK and USA, but only recently developed by some regulatory agencies for COVID-19, led to more agile review processes in some regions[189]. The World Health Organization (WHO) used its own Emergency Use Listing[38] process to evaluate SARS-CoV-2 LFTs, allowing procurement by global agencies. Under emergency authorization procedures within the legal frameworks of several regulatory agencies, a high number of COVID-19 LFTs were authorized[190]. The first emergency authorization for professional use tests was granted in May 2020[191], and in December 2020[192,193] for self-tests.

However, regulatory bottlenecks delayed uptake of COVID-19 LFTs on a global scale. Access to sufficient clinical samples to meet regulatory requirements was problematic during the troughs between waves of variants. A lack of regulatory harmonization between regions meant tests approved in one jurisdiction were not granted wider authorization. As endorsed by the WHO, regulatory convergence and reliance on approvals by stringent regulatory authorities could avoid unwarranted regulatory roadblocks. Because many non-COVID-19 LFTs are designed for use in settings and diseases not found in the Global North, regional regulatory efforts, such as those proposed by the African Medical Devices Forum[194], merit support.

The rapid development of target product profiles, first by the UK regulator Medicines and Healthcare products Regulatory Agency (MHRA) and later by the WHO[46], provided clear expectations to manufacturers on desired design features, and were welcomed by industry. The US Food and Drug Administration (FDA) and the WHO Emergency Use Listing published minimal acceptable requirements for LFT verification and validation that evolved with the pandemic, and encompassed many of the specifications within the published target product profiles.

The lessons learned from COVID-19 should continue to inform in vitro diagnostics regulations and manufacturing practices. If prioritized, approvals for diagnostics globally, particularly in the Global South, could begin to mirror the speed achieved for COVID-19 test approvals in jurisdictions such as the USA. This will require consistent regulatory guidance to manufacturers and specimen availability. Continued regulatory reforms that balance risk with effective post-market measures, such as digital solutions for rapid test performance feedback, as well as external quality assurance testing data, will assist in timely approvals. Regulations capitalizing on recognition and reliance mechanisms, through adoption of harmonized regulatory requirements, can make this a reality.

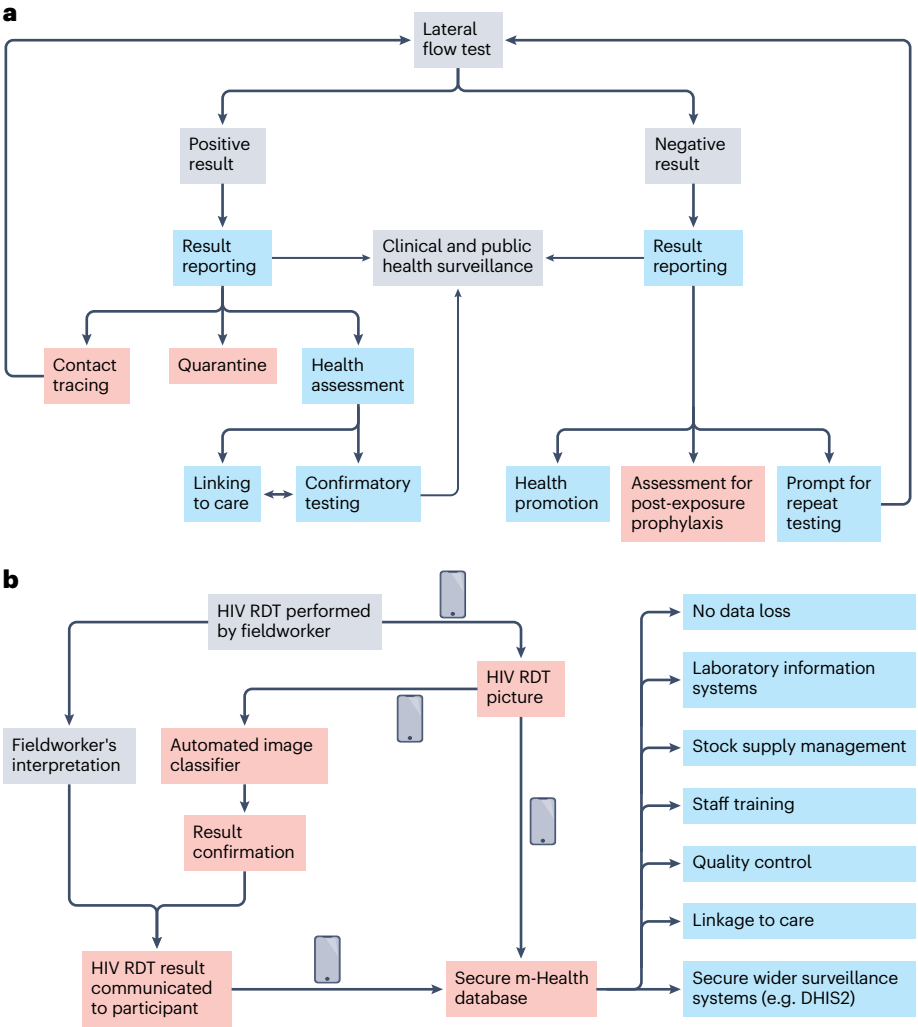


Fig. 4 | Lateral flow test user pathways. **a**, A generalized schema of clinical user journeys, following a lateral flow test (LFT) in a variety of settings. Not all steps will be required for all conditions (such as COVID-19, malaria and human immunodeficiency virus (HIV)). Steps that are specific to certain types of infection and not relevant to all are shown in red. For example, contact tracing and quarantine are required for COVID-19, but not for malaria. Post-exposure prophylaxis is important in some diseases for high-risk groups (for example, post-exposure prophylaxis for HIV, and oseltamivir for influenza). There is good evidence that LFT user pathways effectively link patients to care, particularly following a positive test result for conditions such as malaria and HIV[185]. **b**, The concept of a future m-Health system including an automated LFT classifier and data capture and transmission to a secure m-Health database. Beyond LFT data capture, care and surveillance systems (for example, District Health Information System 2 (DHIS2)) data could be linked to laboratory information systems, stock supply management, staff training and LFT quality control. RDT, rapid diagnostic test. Part **b** reprinted from ref. 19, Springer Nature Limited.

for regulatory approval are costly. Prior to SARS-CoV-2, progress had been made to mitigate these challenges, for example, in the EU-funded ZikaPlan. Moreover, biobank networks have been set up by the Africa Centres for Disease Control and Prevention[77] and FIND. Importantly, diagnostic standards[78] need to be established to save product development time, and public health needs must be addressed by research[76], including the design of multiplex tests to diagnose undifferentiated fevers at the primary care level, tests co-created with end-users, usable and effective self-tests, and data capture systems for result reporting.

Antimicrobial resistance

The 'silent pandemic' of antimicrobial resistance continues to be a substantial global burden, further exacerbated by the COVID-19 pandemic, because screening and surveillance capacity for resistant bacteria gave way to COVID-19 services. Globally, an estimated 4.95 million (3.62–6.57 million) deaths were associated with bacterial antimicrobial resistance in 2019 (ref. 79), and the highest death rates attributable to resistance were in Western sub-Saharan Africa, with 27.3 deaths per 100,000 (20.9–35.3; ref. 79), disproportionately affecting those unable to access expensive second-line antimicrobials[80]. The Review on Antimicrobial Resistance (commonly known as the O'Neill report) highlights that by 2050, ten million lives a year and a cumulative US$100 trillion of economic output are at risk owing to the rise of drug-resistant infections in the absence of action to reduce antimicrobial resistance[81].

The COVID-19 pandemic has reduced public access to care, and antimicrobial prescribing and childhood immunizations have decreased[82,83]. The number of people treated for drug-resistant tuberculosis declined by 15% in the pandemic's first year, and global spending on tuberculosis testing, treatment and prevention services dropped by US$500 million (ref. 84). The pandemic's true impact on global antimicrobial resistance is yet to be confirmed, and new surveillance data must be gathered to update national strategies.

Current methods of determining antimicrobial resistance and susceptibility often rely on bacterial culture, with phenotypic susceptibility testing requiring 36- to 72-hour turnaround times after sample collection, which is too slow for effective antibiotic stewardship in emergency settings or short clinic visits[85]. The time-to-result can be reduced by rapid, low-cost, point-of-need diagnostics, including by

multiplex LFTs with data capture. Priority antimicrobial resistance use cases for LFTs include tests to differentiate bacterial and viral infections, and tests to diagnose sexually transmitted infections (for example, *Neisseria gonorrhoeae* and *Chlamydia trachomatis*). However, the optimal biomarker panels for these diagnostics are often not known; genotypic markers do not always reflect phenotypic behaviour, and the most relevant resistance mutations can change over time and by geography[85], presenting challenges for test development and commercialization. The level of multiplexing in highly accurate antigen detection in LFT formats remains limited to just a few targets (in general, less than five, and often not more than two).

Cost-effective decentralized testing

LFTs need to be integrated within a surveillance system or a care pathway, alongside other preventative, therapeutic and diagnostic tools. LFT research has long focused on early-stage technologies; however, real-world use should be investigated, including individual, setting and system-level design considerations to ensure that end-users are linked to care[86] and that test results inform surveillance and infection-control interventions. Digital care pathways can link LFT self-tests to health systems and electronic patient records (Fig. 4), as was demonstrated by digitally linking self-sampling for chlamydia to care in a proof-of-concept online pathway in the UK[87]. Similarly, digital tools have been integrated with community-based testing using LFTs in South Africa, increasing case detection, reporting and follow-up[88].

Patients should be encouraged to report their results so that they can be linked to care and advice through digital capture; in parallel, digital tools should be designed to ease the burden on patients, and improve provider-to-provider communication. Although control of test results may be advantageous for privacy reasons, care-seeking and behavioural changes also occur without digitally reporting positive test results. Although the importance of reporting varies by pathogen and setting, self-testing and control over the disclosure of results are a key benefit in making diagnostics accessible, as has been shown in demographics hesitant to test for HIV in traditional clinic settings[78]. Importantly, self-testing and digital reporting have shown perceived privacy benefits compared to in-person testing[89].

New LFTs are needed for the diagnosis of various infections, such as urine-based tuberculosis testing, neglected tropical diseases testing[90], LFTs to support the triple elimination of mother-to-child transmission of HIV, syphilis and HBV, and improvements to malaria LFTs to ensure full coverage of pathogenic species and genetic evolution in the parasites.

Implementation research or randomized controlled trials can identify the effectiveness and cost effectiveness of LFT strategies, including test-and-treat programmes linking high-risk people to antivirals (such as nirmatrelvir/ritonavir, Paxlovid)[91] for SARS-CoV-2. Similar approaches can inform the use of therapies for other infections, such as respiratory syncytial virus, and of currently underused therapies, such as oseltamivir for influenza.

LFTs can also be used for monitoring chronic infections and response to treatment. For example, future LFTs capable of viral load monitoring could empower people with HIV to self-monitor, as do glucose tests for people with diabetes. Nucleic-acid-based LFTs may also be amenable to conditions such as human papillomavirus (HPV)-linked cervical cancer. Beyond human health, LFTs could find application in animal health and environmental monitoring[92], for example, in wastewater-based epidemiology.

Next-generation lateral flow tests

Bioengineering approaches can aid in improving the sensitivity, specificity, sample collection and digital data capture of LFTs.

Sensitivity and specificity

Increasing the sensitivity of LFTs could democratize decentralized testing. The sensitivity of LFTs is limited mainly by the nanoparticle properties, read-out methods[93], binding kinetics and mass transport[15]. The type (for example, fluorescent or plasmonic nanoparticle) and properties (for example, size and morphology[94]) of nanoparticles and the corresponding read-out determine the smallest detectable number of bound nanoparticles at the test line. Assuming perfect analyte-to-nanoparticle binding, the number of bound nanoparticles translates to analyte concentration, because at the detection limit, the number of analyte molecules is smaller than the number of nanoparticles, assuming approximately one analyte molecule per bound particle. To optimize performance, the ratio of the signal provided by each nanoparticle to the background signal produced by substrates, samples or the environment needs to be maximized. In reality, however, binding is imperfect and described by receptor–ligand kinetics and mass transport, which determine specific (analyte-mediated binding) and non-specific binding rates. Therefore, the ratio of the signal provided by each specifically bound nanoparticle to the signal produced by non-specifically bound particles and the background arising from substrates, samples or the environment needs to be optimized.

Sensitivity is typically reduced by low-signal positive samples near the detection limit, whereas specificity is decreased by negative samples with high signal. Therefore, sensitivity can be improved by lowering specificity and vice versa, affecting interrelated assay design choices, such as nanoparticle concentration and the surface density of capture ligands; here, higher concentrations of nanoparticles can increase specific binding rates at low analyte concentrations, but can also increase non-specific binding (depending on nanoparticle properties and surface chemistry). Decreasing the flow rate similarly increases specific and non-specific binding. Reducing non-specific binding by optimizing buffers allows higher nanoparticle concentrations without compromising specificity. Optimization, however, is limited by sample type, test time and ease of use, for example, the lack of a centrifuge at the point of care. The choice of materials[95] and architecture[96] of LFTs — membrane, conjugate, sample and absorbent pads, blocking materials and buffers — all determine the playoff between specific and non-specific interactions; for example, smaller pore-size membranes can have a higher sensitivity per volume of sample, at the cost of slower flow rates. In addition, complex samples, such as faeces or whole blood, may require processing and extraction steps.

Theory and modelling approaches can also be applied to study the mechanisms underlying test sensitivity and specificity, for example, by integrating reaction and mass transport theory to generate computational models[15]. Sensitivity may be further improved by bottom-up, target-focused approaches, using high-affinity receptors and amplification strategies, for example, and top-down device engineering approaches to improve the signal-to-noise ratio of transducers and nanoparticle readout.

Sample collection and preparation

The quality of tests and samples affects sensitivity and specificity[97]. Sample collection and preparation are essential steps in any assay, but are often not addressed in the academic literature, and are not well adapted to the setting in which the test is administered. Samples

tested on LFTs — including from whole blood, plasma, serum, saliva, urine, stool, vaginal, sputum, nasal and nasopharyngeal swabs — have diverse properties and compositions[11], which need to be considered in the test design. Integrating blood lancets[98] can reduce the number of components and handling of sharps. LFT sample pads and buffers can even the flow, control sample buffering[11] or act as a filter; however, some specimens (such as serum) require pre-treatment after sampling.

Sample preparation typically includes extraction, purification and concentration, which must be accomplished without sacrificing usability or reproducibility. Protocols for LFT sample preparation depend on sample type, assay, target and setting, and can be optimized for high-throughput, rapid or point-of-care testing[99]. For example, paramagnetic particle systems can be used for purification and concentration to enable automated, high-throughput testing[100], and magnetic-bead-based commercial kits allow rapid, point-of-care sample preparation[101]. Magnetic nanoparticles can also be harnessed for sample enrichment and detection in the same LFT[102].

Molecular testing, in particular RT-PCR, is typically less robust against contaminants and inhibitors[103] than immunoassays. Alternatively, nucleic acid amplification tests, often based on isothermal amplification (for example, recombinase polymerase amplification (RPA) or loop-mediated isothermal amplification (LAMP)), can be integrated with an LFT read-out[14,104], moving nucleic acid amplification tests closer to point-of-care testing.

Sensitive nucleic acid detection

The combination of LFT test formats with nucleic acid amplification and detection heralds a new era of highly sensitive and specific infectious disease diagnostics[105–110]. A number of products are in development, and at least one company has achieved FDA approval, integrating standard RT-PCR amplification and an LFT readout into an easy-to-use format. A single-use disposable molecular test is also commercially available for COVID-19 and sexually transmitted infection testing from self-collected swabs[10]. Isothermal LAMP or RPA amplification can be combined with LFT outputs using functionalized primers to create dual hapten-labelled amplicons that bind to both the test strip and a colorimetric label. These methods are often highly sensitive; however, non-specific amplification can result in decreased specificity, and there can be compatibility issues of the amplification formulation with LFT test line binding. Amplification for LFT nucleic acid detection can also be achieved by displacement amplification or rolling circle amplification[111].

Cited as a 'technology to watch out for' in 2022 (ref. 112), CRISPR-based diagnostic (CRISPR-Dx) systems, such as specific high-sensitivity enzymatic reporter unlocking (SHERLOCK)[113,114] and DNA endonuclease-targeted CRISPR trans reporter (DETECTR)[115], increase the range of molecular targets suitable for LFT-based detection and have been used for diagnosis of SARS-CoV-2[116,117] (Fig. 5a). Typically, isothermal molecular amplification, such as reverse transcription (RT)-LAMP or RT-RPA, is used as an initial step to improve diagnostic sensitivity, followed by a CRISPR-based detection step triggered through highly specific recognition of a target nucleic acid sequence by a guiding RNA (gRNA)–Cas complex. Here, Cas12 or Cas13 are mostly used, which collaterally cleave a reporter when activated by target binding. Once cleaved by the activated Cas, the reporter can bind to the LFT test line as well as to the control line. Alternatively, dead Cas9 (dCas9) binds target sequences without cutting, resulting in co-localization of the dCas9, target DNA, and a nanoparticle-based colorimetric label at the LFT test line.

CRISPR-Dx are versatile platforms, ideal for rapid outbreak response, with the first laboratory CRISPR-Dx for COVID-19 available within months of the beginning of the pandemic[118]. Importantly, CRISPR-Dx can be more sensitive than antigen LFTs for COVID detection[119,120], and integrate multi-step and thus, more streamlined protocols, moving towards translation from the laboratory to the point of care and resource-limited settings. The (SHERLOCK testing in one pot) STOPCovid[121], COVID SHINE (SHERLOCK and HUDSON Integration to Navigate Epidemics)[120,122] and a wearable COVID-19 face mask[123] demonstrate highly sensitive LFT detection with streamlined protocols, involving only one or two user interactions. Several assays[124–126] have focused on developing COVID-19 LFT diagnostic protocols using minimum equipment with the potential to be portable. Finally, driven by the need to identify COVID variants, CRISPR has also been used to detect mutations, including single nucleotide variations[119,120,127].

CRISPR-Dx could extend beyond COVID-19 LFT diagnosis and may allow rapid diagnosis of diverse diseases and variant or resistance monitoring. In particular, CRISPR-Dx benefit from high sensitivity in diverse clinical samples[110,119,128,129], streamlined 'one-pot' protocols and freeze-dried, cell-free assay formats for usability and stability[121–123,127,130,131], as well as smartphone-integrated result interpretation contributing to digital surveillance programmes[122,127,132]. CRISPR-Dx have initially required laboratory equipment for amplification and readout; however, these platforms can also operate with battery power or without power at room temperature[123,125,127]. For antimicrobial resistance monitoring, however, isothermal molecular amplification[131] needs to be avoided, and multiplexing should be implemented to improve accuracy, sensitivity and variant detection[114,119,120,126,127,132,133]. Importantly, CRISPR-Dx could be applied in low-resource settings, which will require integration into commercially viable products and field evaluations.

Materials and sensors

Sensitivity and specificity can be improved by developing receptor ligands with high kinetic on-rates and low non-specific binding, such as nanobodies[134,135]. Owing to their small size (around 15 kDa), they can reach less accessible paratopes of analyte molecules and confer greater chemical and thermal stability than antibodies. Complementary to improving binding at low-analyte concentrations, methods are being developed to multiply the number of targets.

'Top-down' amplification strategies can improve detection limits. In most LFTs, optical absorption-based imaging of plasmonic nanoparticles determines the detection limit, governed by the lowest number of particles required to produce a detectable change in light absorption and the number of binding events required to reach that threshold. Size optimization of gold nanoparticles[93], the design of catalytical nanoparticles that develop a chromogenic substrate[136] and other chemical modifications[137] can further increase the signal per particle. In addition, read-out methods can be improved to reduce the number of required binding events; for example, the plasmonic peak can be used to subtract background in two-wavelength imaging[138], or alternative read-outs can be applied, such as thermal contrast[139].

Dual dynamic range regimes[136], signal amplification strategies[140], sensitive labels[141], and dual-wavelength-based imaging[138] have improved the analytical sensitivity and dynamic range of paper-based biosensors, improving their quantification capabilities. Fluorescent nanoparticles, such as quantum dots[142,143], have also been investigated to improve detection limits. Similarly, sensitivity is limited by the

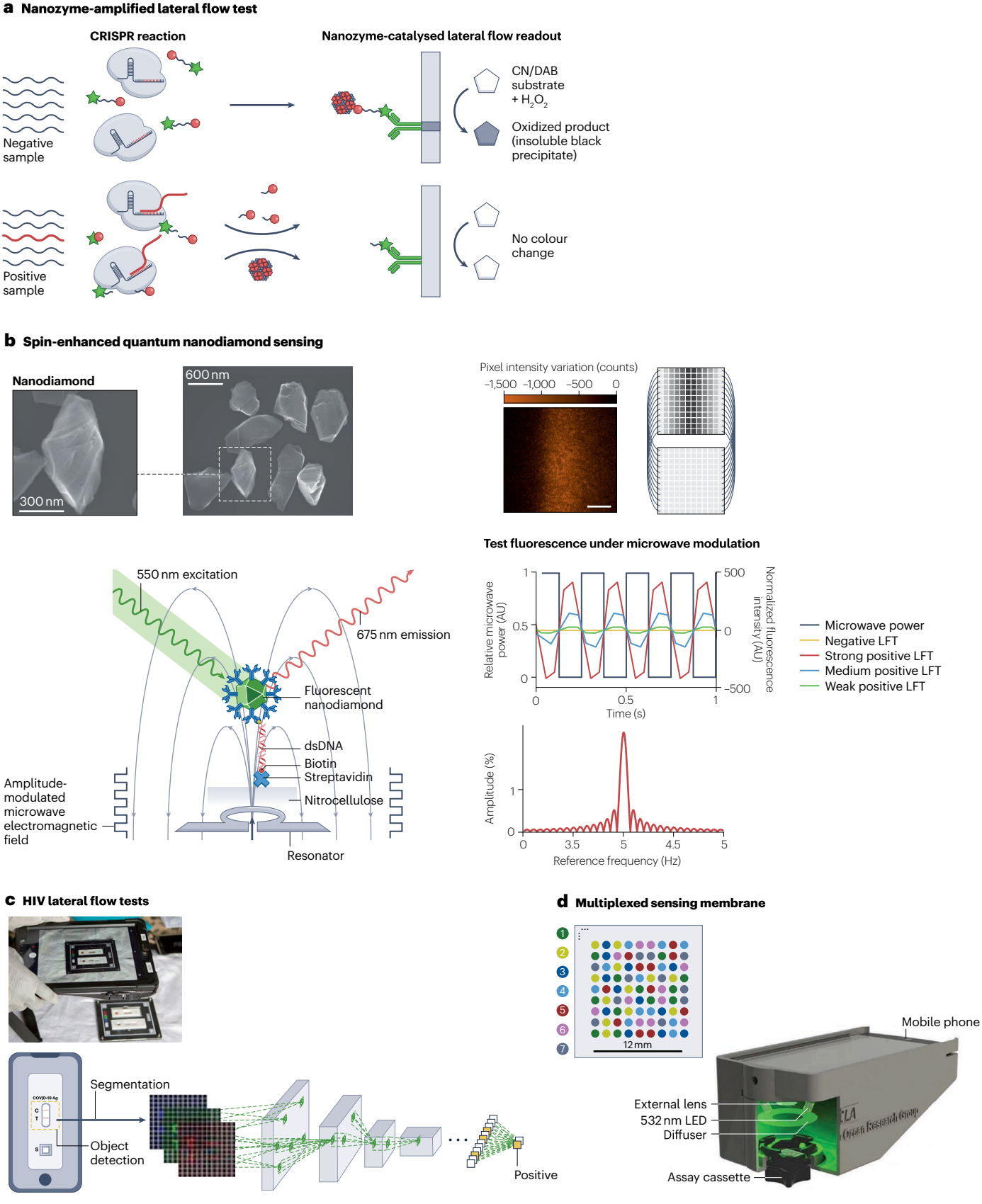

a Nanozyme-amplified lateral flow test
CRISPR reaction
Nanozyme-catalysed lateral flow readout
Negative sample
Positive sample
CN/DAB substrate + H_2O_2
Oxidized product (insoluble black precipitate)
No colour change
b Spin-enhanced quantum nanodiamond sensing
Nanodiamond
600 nm
300 nm
Pixel intensity variation (counts)
−1,500 −1,000 −500 0
550 nm excitation
675 nm emission
Fluorescent nanodiamond
dsDNA
Biotin
Streptavidin
Nitrocellulose
Amplitude-modulated microwave electromagnetic field
Resonator
Test fluorescence under microwave modulation
Relative microwave power (AU)
Normalized fluorescence intensity (AU)
Time (s)
Microwave power
Negative LFT
Strong positive LFT
Medium positive LFT
Weak positive LFT
Amplitude (%)
Reference frequency (Hz)
c HIV lateral flow tests
Segmentation
Object detection
Positive
d Multiplexed sensing membrane
12 mm
Mobile phone
External lens
532 nm LED
Diffuser
Assay cassette

Fig. 5 | Bioengineering next-generation lateral flow tests. a, Cas-based reactions can be combined with a nanozyme-amplified lateral flow test (LFT). Target RNA is mixed with the guiding RNA (gRNA)–Cas13 complex and reporter RNA to trigger the clustered regularly interspaced short palindromic repeats (CRISPR) reaction. Subsequently, streptavidin-functionalized nanozymes are mixed with the CRISPR reaction product that contains the biotinylated reporter RNA to form a complex. The test strip is preprinted with anti-fluorescein amidite to draw up the mixture. The uncleaved reporter RNA–nanozyme complexes are captured at the test line. Finally, the substrate is added for colour development. **b**, Spin-enhanced quantum nanodiamond sensing and background subtraction can be implemented in LFTs to enable ultra-sensitive virus detection. The scanning electron micrographs show nanodiamonds. Pixel intensity variation is shown at the test line on an LFT strip with immobilized nanodiamonds under an amplitude-modulated microwave field. Background subtraction allows ultra-sensitive virus detection. In the amplitude-modulated field, mean fluorescence intensity varies over time. A lock-in algorithm quantifying modulation amplitude over a range of frequencies, gives a sinc function with a peak at the modulation frequency. Nanodiamonds are immobilized at the test line in a sandwich structure in the presence of double-stranded DNA (dsDNA) amplicons. AU, arbitrary units. **c**, Healthcare workers collect images of human immunodeficiency virus (HIV) LFTs in the field, and machine learning allows automatic classification of LFT results. **d**, Deep-learning-enabled point-of-care sensing using multiplex paper-based sensors and a mobile-phone reader with an inserted vertical flow assay cassette. The algorithmically determined immunoreaction spot layout of the multiplexed vertical flow assay membrane contains several distinct spotting conditions, each of which uniquely reacts with the sensed analyte and the signal-forming gold nanoparticles. CN/DAB; 4-chloro-1-naphthol/3,3′-diaminobenzidine, tetrahydrochloride. Part **a** adapted from ref. 131, Springer Nature Limited. Part **b** adapted with permission from ref. 138, Elsevier, adapted from ref. 13, Springer Nature Limited, and reprinted from ref. 13, Springer Nature Limited. Part **c** image courtesy of African Health Research Institute. Part **d** adapted from ref. 165, Springer Nature Limited.

signal-to-noise ratio, that is, the absolute signal can be increased by longer exposure times; however, background and sample auto-fluorescence mask low signals. Nanoparticle signals can be separated from background fluorescence by spin manipulation of fluorescent nanodiamonds[13] (Fig. 5b). However, fluorescence-based imaging approaches require a dedicated reader, adding cost and complexity. Alternatively, mobile-phone-based[144] readers, standalone readers[15] and optics integrated into the test cassette[68,145] have been explored. Surface-enhanced Raman scattering (also requiring a reader) may enable sensitive readout[146–148], and separate detection of specific and non-specific binding[149] (Fig. 5c). In addition, magnetic[150] and electrochemical[151] transduction techniques have been demonstrated for LFTs.

Signal enhancement strategies are limited by non-specific binding, because lowering the detection limits increases the detection of non-specific binding. Designing the transduction mechanism to differentiate between specifically and non-specifically bound labels, by producing a signal only for particles bound to capture ligands[152] could improve sensitivity, allowing high nanoparticle concentrations and thus, rapid binding kinetics without increasing negative signals.

LFTs using fluorescent nanoparticles or other labels that require specific excitation conditions often need additional hardware for automated result capture (a 'reader'). Here, sensitivity and stability of measurement should outperform stand-alone LFTs[153–156]. Such readers can be used to detect approximately 80 fluorophores per diffraction-limited spot size, potentially pushing the limit of sensitivity to the single-molecule level[157,158]. This might not be appropriate in some applications, such as self-testing, for which additional components reduce affordability and usability. However, LFT–reader combinations could be economical in the clinic and for use by healthcare workers, where multiple tests are carried out by a single user. Here, portability, speed and affordability will probably compare positively with laboratory testing. Although requiring specialist hardware, digital connectivity or readers using smartphone cameras could automate result capture. However, readers can limit the volume of tests that can be performed, and some commercially available readers are only guaranteed by manufacturers for a limited number of tests before replacement. More compact readers, zero maintenance and cost-effectiveness[159] could address this bottleneck.

Multiplexing

Decentralized testing using multiplex LFTs (xLFTs) with data capture and reporting may provide early alerts of outbreaks, help to detect infections and antimicrobial resistance, and support effective triaging in health systems. For example, xLFTs can detect multiple targets and differentiate between multiple flaviviruses[160], sexually transmitted infections and drugs. xLFTs have also been developed to distinguish SARS-CoV-2, influenza and their co-infections[161], and HIV/syphilis xLFTs[162] are commercially available. xLFTs combined with symptom and demographic data could pave the way for nuanced decision-making when paired with digital tools; however, systemic and engineering challenges (such as cross-reactivity or interference between multiple test lines in the limited test strip area, reducing specificity) have limited xLFT commercialization thus far. In addition, the need to identify a single set of LFT parameters (for example, buffer and materials) for each target analyte compromises sensitivity.

Cross-reactivity can be mitigated by implementing multiple parallel flow pathways, vertical flow assays[163], or other paper-based configurations[164] with spatial separation of the immunoreaction spots and perpendicular flow of the sample fluid through the membrane. Vertical flow assays can contain about 100 spatially isolated immunoreaction spots in a single test[165] (Fig. 5d), and contain the same assay reagents and inexpensive materials often used in LFTs, enabling manufacturing scale-up. However, vertical assays may result in lower sensitivity owing to the short binding time.

Large-scale manufacturing of xLFTs requires the printing of multiple test spots in a single disposable strip, and thus, multiple dispensing nozzles with different quality-control measures, potentially increasing production costs. Furthermore, additional test lines complicate result interpretation, although this can be mitigated through the use of digital interpretation or test spot array patterns that are recognizable to users[166,167]. The development and commercialization of xLFTs are further limited by the need to validate multiple biomarkers with clinical importance, low market demand and a complicated regulatory approval pathway.

Digital connection and deep learning

The future of public health is increasingly digital. As of 2019, 65% of the global population subscribe to mobile phones, with the fastest growth in sub-Saharan Africa[168]. Accordingly, large-scale, real-world image

datasets could be used to train and validate image classification models[19,71,169] (Fig. 5c) for digital LFT data capture, including in low- and middle-income regions[19]. These datasets can be expensive to produce, and the number of real-world positive cases may vary by disease and setting. However, commercial test providers and public health agencies are already collecting images for test registration and verification, and thus, image collection could be automated in these pipelines to continually improve image classification models. Test providers may be hesitant to introduce algorithms requiring updates when new tests are deployed; however, given the similar visual appearance of qualitative LFT results, algorithms could be updated with smaller datasets once a large dataset is captured for a single test. Test registration and result entry with a single photograph can reduce the data entry burden and encourage users to report results. Probabilistic algorithms providing a measure of uncertainty can reduce confusion arising from false results for users[170].

Smartphone-read results allow more complex LFT configurations, including quantitative and multiplex tests, without increasing complexity for the user. In addition, information can be linked to each measurement, enabling real-time, geo-linked surveillance[154]. Digital solutions should prioritize interoperability and integrate with existing platforms (not exacerbate digital exclusion), should encourage trust with easy-to-use systems and protect personal information, and they should be co-designed with end-users for optimized usability[89]. Moreover, machine learning can be applied to optimize, analyse and quantify paper-based multiplexed tests[165]. However, widespread use of transformative digital solutions will need to be implemented in existing healthcare pathways; these solutions will require acceptability, high data quality and access; and legal, ethical, privacy and data security, and organizational and workforce barriers must be overcome[66].

Green manufacturing

LFTs are typically single use and disposable, producing non-biodegradable plastic waste[171] and sometimes also electronic waste. As an alternative to plastic components, card or biodegradable plastics have been implemented in commercially available tests[172]. However, it is important that these materials retain the advantages of plastic, including robustness and protection of the assay strip, the ability to create the pressure points necessary for controlled flow and low-cost and scalable manufacturing methods. In addition, they should be lightweight, easily transportable and easily printable to include QR codes and lot numbers. In clinical settings, LFTs may often need to be incinerated regardless of material composition, although materials could be included that reduce the environmental toxicity of LFT components and the volume of packaging.

The environmental impact of LFTs that contain new materials and components needs to be considered. The REASSURED guidelines[9] assert the need for environmentally friendly tests that do not require non-existent waste infrastructure or risk the introduction of toxic chemicals into the environment[9]. For example, synthetic biology can be used for animal-free antibody production[173], reducing the overall carbon footprint. If electronic components are necessary, paper-based batteries[174] may aid in reducing toxic waste. Moreover, multiplexed tests can reduce the use of multiple tests.

The sustainability of LFTs can be improved by frequent evaluation of the usage of tests in domestic settings by manufacturers. In addition, redundant components and the size of components could be reduced, or components could be combined. Regulators can improve LFT sustainability by introducing incentives for reduction of materials and toxic chemicals, sustainable design and reusable components, by introducing regulation for the clear labelling of recyclable components, flexible to different disposal requirements for different modes of disease transmission, and by reducing regulatory barriers to changes in packaging and cassette design.

Translation

The LFT and broader in vitro diagnostics market have historically had smaller investments owing to high technological and regulatory barriers, a perceived low health economic value, and smaller financial returns compared to vaccines and therapeutics. Additional barriers, such as unpredictable demand, as well as manufacturing and distribution challenges, further limit the scale-up of new diagnostics.

Target product profiles, technical specifications series[175] and preferred product characteristics[176] are strategic documents that can be used by manufacturers to guide the fast-tracked development of products, and to assist in the identification of regulatory requirements, based on use cases. In autumn 2020, the WHO published four priority target product profiles for COVID-19 diagnostics[46].

Manufacturers should use information gained from the implementation of device risk management and the development of a regulatory strategy driven by implementation and impact requirements. The regulatory strategy should not only consider regulatory requirements in the proposed regions of sale, but also assay quality assurance measures of global buyers, to determine whether further testing or additional quality assurance steps are required.

LFTs and accompanying testing kits and processes need to be designed to be inclusive and easy to use by people with diverse health literacy in various settings[177]. This extends to guidelines for delivery, sample pack design and user instructions[178], minimally invasive biological sampling, and a minimum number of sample preparation steps[9].

Tests are often developed and validated using synthetic samples, which may mask challenges introduced by real-world samples, such as non-specific binding. Access to qualified specimens as well as reference and control materials (ideally by WHO international standards) are key to assay validation (including clinical evaluation). In particular, biobanked, characterized, clinical specimens assist in the development, verification, validation and quality assurance of assays. In addition, availability of reference laboratories, access to reference methods and technology-appropriate written standards (ideally international in nature) are important.

Only a few proof-of-concept diagnostics technologies have been translated into commercially available products. To increase translation, the ultimate product requirements need to be considered early in the research process, not just in terms of target analytical and clinical performance, but also regarding robustness (including stability in temperature and humidity, and over time), time to test results, ease of use, connectivity and affordability. Moreover, manufacturability and scale-up need to be ensured.

Research often focuses on specific elements of diagnostic tests, which may be broadly applicable and disease-agnostic; however, each diagnostic product is intended for a specific clinical application, target user, testing population and use setting. Product design should thus meet the requirements for each specific application and consider end-user needs. Co-creation, community engagement and gold standard frameworks for evaluation are needed for effective test deployment,

including early engagement of end-users, healthcare providers, academic researchers, industry, public health authorities and public health agencies.

Outlook

LFTs have been hailed "the heroes of the pandemic"[179], transforming COVID-19 testing globally. This simple, low-cost platform is gaining the recognition it deserves, but has long been underfunded, overshadowed by investments in laboratory-based and point-of-care molecular and sequencing diagnostics. Moreover, there are major inequities in access to tests, raising ethical concerns and affecting our collective ability to respond to the pandemic.

Bioengineering will play a key part in increasing the sensitivity and specificity of LFTs, enabling multiplexing and data capture, as well as manufacturing in low-resource settings[180]. The combination of LFT test formats with nucleic acid amplification and detection could provide the next generation of LFTs, albeit currently limited to one product on the market. Moreover, emerging technologies could be implemented in LFTs, such as the use of nano- and quantum materials to improve sensitivity, CRISPR to improve specificity and deep learning approaches to allow digital connectivity and quality assurance.

However, a reduction in funding for LFT research post COVID-19 may hamper efforts to capitalize on gains in decentralized testing, especially self-testing, which may be critical to address future pandemic threats. Prior to COVID-19, funding for infectious disease research declined between 2007 and 2018 (ref. 74). In 2021, the UK reduced its commitment to overseas development aid from 0.7% to 0.5% of GDP, with some research funding cut by up to 85% (ref. 181). However, coordinated long-term investment in a global network of R&D LFT hubs is needed to develop and retain people and skillsets, to share knowhow, to standardize reagents and to pilot 'test beds' in which to evaluate effectiveness and cost-effectiveness, and to grow the manufacturing capability developed during the pandemic. These hubs could help to nurture a pipeline of innovative bioengineering approaches across the translational 'valley of death'.

COVID-19 typically presents high viral load (and antigen levels), which can be detected by currently available LFTs; however, other diseases may prove more challenging to detect using LFTs. Neither LFTs nor point-of-care tests currently exist for 50% of the WHO priority diseases of epidemic potential. Importantly, LFTs are also urgently needed to detect antimicrobial resistance, human papillomavirus-associated cervical cancers, and acute and chronic infections, as well as for viral load and animal and environmental monitoring. Globally convergent regulatory pathways are needed, tackling issues of intellectual property, expanding generic (non-branded) diagnostic production capability in low- and middle-income regions to bring down costs[182], and making tests manufacturable, accessible, acceptable and usable by the broadest cross-section of society. For LFTs to be successful in reducing transmission and linking patients to care, investment in communication and education on their use is needed, updated according to continual monitoring of testing behaviours. Linking to care pathways, harnessing digital technologies[66] wherever feasible and acceptable, and co-creation of tests with end-users are essential[168].

In the wake of the COVID-19 pandemic, it is time for governments around the world to embrace some 'lateral thinking' and dare to dream the future of decentralized health and affordable self-testing. Bioengineering and LFTs will play a key part in democratizing health, ensuring we honour the Sustainable Development Goals and "leave no one behind"[183], and strengthening resilience before the next pathogen strikes.

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Competing interests

M.M.S. and M.B. have filed patent application GB2110729.7 relating to nanozyme-catalysed biosensing. M.M.S. has filed patent applications GB2015943.0 and US20200116725 (A1), and is co-inventor on patents ES2365536 (T3) and WO2007063300 (A3) relating to nanomaterials and assays for biosensing. B.S.M. and R.M. have filed patent application WO2020049303A1 (nanodiamond assay). A.O. has pending and issued patents on point-of-care sensors and related technologies. A.M.J. is President of the Academy of Medical Sciences. M.M.S. is a co-founder and director of Signatur Biosciences Ltd. N.E.W. is a cofounder and consults for 52 North Health Ltd. J.J.C. is a co-founder and director of Sherlock Biosciences. E.J.T. is an advisor to Quest Diagnostics. N.F. and R.W.P. are funded by the Wellcome Trust project: The Accelerating Diagnostic Access Project (ADAP). All remaining authors have no competing interests to declare.

Additional information

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