Innovations in one-step point-of-care testing within microfluidics and lateral flow assays for shaping the future of healthcare

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A R T I C L E   I N F O

A B S T R A C T

Point-of-care testing (POCT) technology, using lateral flow assays and microfluidic systems, facilitates cost-effective diagnosis, timely treatment, ongoing monitoring, and prevention of life-threatening outcomes. Aside from significant advancements demonstrated in academic research, implementation in real-world applications remains frustratingly limited. The divergence between academic developments and practical utility is often due to factors such as operational complexity, low sensitivity and the need for trained personnel. Taking this into consideration, our objective is to present a critical and objective overview of the latest advancements in fully integrated one-step POCT assays for home-testing which would be commercially viable. In particular, aspects of signal amplification, assay design modification, and sample preparation are critically evaluated and their features and medical applications along with future perspective and challenges with respect to minimal user intervention are summarized. Associated with and very important for the one-step POCT realization are also readout devices and fabrication processes. Critical analysis of available and useful technologies are presented in the SI section.

1. Introduction

Lateral flow assays (LFA) and microfluidic assays are popular diagnostic systems (Koczula and Gallotta, 2016; Yang et al., 2022) and should meet the seven World Health Organization (WHO) guidelines for a point-of-care test (POCT) summarized in "ASSURED" (affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, and deliverable to end users) (Land et al., 2019) (Fig. 1). In general, the field of application of POCTs has significantly broadened to various operational areas detecting a broad range of analytes. These can be mainly grouped into biological analytes, such as nucleic acids, enzymes, antibodies, small molecules, cells and tissues. With the continuing discovery of new biomarkers, biorecognition elements used for the specific capture of the target marker have been continuously evolving (Morales and Halpern, 2018). Natural (antibodies (Dkhar et al., 2022), enzymes (Gupta et al., 2022), nucleic acids (Yoo et al., 2020)) and synthetic biorecognition elements (molecularly imprinted polymers (MIPs) (Denmark et al., 2020)) are frequently used in biosensors. Typically, an optical or electrochemical label, that is connected with the biorecognition element, provides a semi-quantitative or quantitative signal that can be easily interpreted by the naked eye, a spectrometer, or

electrochemically (Nguyen et al., 2020). The projected revenue for global POCT is anticipated to increase from $45.4 billion in 2022 to $75.5 billion by 2027, exhibiting a compound annual growth rate (CAGR) of 10.7 % (Markets and Markets, 2023). This growth can be attributed to various factors, including advanced technology, heightened health awareness, and an increasing demand from developing countries (Luppa, 2018). Despite their popularity, most commercial POCT systems often do not meet the ASSURED criteria (Nilghaz et al., 2016) and fail to fulfill requirements (1) in low-resource settings, (2) in settings with shortage of skilled personnel and (3) in automation of operation steps to eliminate user-associated inconstancy in the test results. Mostly, those tests are typically operated in fully automated fashion within centralized health centers for diagnosis. This presents a major challenge, especially in underdeveloped nations where there is often a shortage of both the necessary instrumentation and skilled physicians to conduct these tests, leading to limited diagnostic capabilities. To overcome this burden miniaturization of the detection systems into portable and cost-effective devices or the utilization of even a smartphone offer fast results on-site without the need for trained personnel or complex sample preparation, making them a more convenient option compared to larger and more complex instruments (Zhang

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J. et al., 2020). Most POCT platforms typically necessitate additional hands-on steps, such as sample preparation, washing, and the introduction of additional solutions to provide sensitive testing. This can lead to intricate operations for the user and increased susceptibility to errors in the detection process (Fox et al., 2006). Furthermore, it was noted that the test's performance saw a significant decline attributed to the absence of user expertise, ultimately undermining the test's accuracy (Fox et al., 2006). Especially, with the current shortage of healthcare professionals and the additional training and workload for complex POCT procedures may compromise the care provision (Ortiz and Loeffelholz, 2023). In order to enable the test procedure to be conducted by non-specialized personnel or even patients themselves, the integration of sample processing steps and detection into a fully automated one-step POCT platform is an essential prerequisite. Following the sample introduction to the POCT device, amplification and washing steps do not require any sample handling, allowing automated testing with minimal manual interaction. This ensures the elimination of the risk of contamination or user error, which could otherwise lead to inaccurate results (Sharma et al., 2015). Additionally, to enhance the commercial adoption of one-step POCT systems, it is crucial to hermetically store the immunoassay reagents, either in lyophilized or dried form on the test. This safeguards against biodegradation, ensures the reliability of testing, and facilitates shipping and storage at ambient temperatures (Yager et al., 2006). Hence, the optimal POCT system allows specific and sensitive analyte detection, yet eliminating the need for cumbersome and complex equipment and ensuring easy operation for lay patients (Kimura and Asano, 2022).

1.1. Complementary recent reviews and focus of this article

In recent years, several notable reviews have emerged in the field of LFA and microfluidics for POCT (Liu et al., 2019; Nguyen et al., 2020; Roy et al., 2022; Sachdeva et al., 2021). While most of them, focus on specific disease diagnostic such as COVID-19 (Pérez-López and Mir, 2021; Shaffaf et al., 2021), hepatitis (Duchesne and Lacombe, 2018), reviewed specific detection techniques such as nucleic acid detection (Li

et al., 2021; Liu and Tsutsui, 2023) or primarily emphasize the development and incorporation of new technologies (Sena-Torralba et al., 2022) and signal amplification strategies (Nguyen et al., 2020) they have limited in-depth discussions on their translation into practical applications and barely consider the operation experience by the end-user. A recent review closely aligns with the content of our review. However, their focus is primarily directed towards simplifying the incubation and washing steps in POCT (Kimura and Asano, 2022). Our review instead concentrates on the advancement made in one essential area that is vital to achieving the goal of producing high-performing immunological devices for on-site use: the development of sensing strategies that allow one-step POCT systems with a focus on minimizing user involvement by allowing only the sample to be applied to the assay. At the same time, our review consistently focuses on the aspect of mass production and the associated commercialization of the POCT product, aiming to bridge the gap between research and industry. Moreover, most reviews address paper-based LFAs and microfluidic assays individually (Liu et al., 2022; Omidfar et al., 2023; Torul et al., 2023; Zhang and Liu, 2022). Instead, we consolidate the latest state-of-the-art one-step methodologies of both topics devised by the scientific community over the past five years. It highlights strategies that can jointly facilitate the development of one-step POCT platforms designed for home-testing, while addressing the key challenges that lie between prototype creation and mass production. First, we provide examples of one-step POCT devices that have made it to the market. Secondly, our focus shifts to signal amplification advancements that can further enhance the sensor performance and have been successfully integrated into one-step POCT devices. Finally, we explore the effect of test design on the one-step assay and the integration of sample preparation steps into POCT platforms. In an accompanying SI section we also provide a critical analysis of available readout devices and especially fabrication processes which are crucial for any POCT to successfully reach the market. In our conclusion, we analyze and explore prospects for the future.
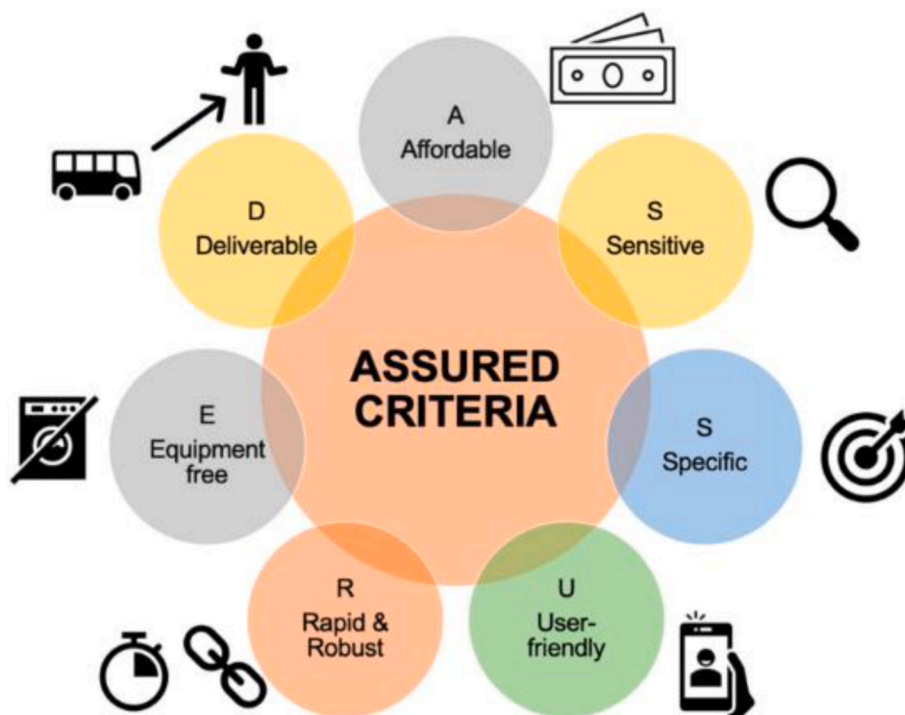

Fig. 1. An illustration of the ASSURED criteria for diagnostic platforms. Reproduced/adapted from (Bhardwaj et al., 2022) (https://creativecommons.org/licenses/by/4.0/).

2. Commercial one-step POCT platforms

Although lab-on-chip systems have been in existence for several decades, most of them have not yet been commercialized or regularly utilized as research-grade instruments outside of specialized laboratories. For immunological devices to successfully enter and thrive in the market, they must meet several crucial criteria that ensure their practicality, accessibility, and efficiency. Firstly, they need to be simple to operate, allowing users to perform tests with minimal effort and without specialized training (Zhang et al., 2021). This leads to the second requirement: the devices must function effectively without the involvement of trained professionals, making them ideal for widespread use in various settings. Additionally, these devices must be cost-effective, ensuring they are affordable for a broad audience, which is critical for large-scale adoption. The ease of fabrication in bulk is another essential factor, as it guarantees that the devices can be mass-produced efficiently, maintaining quality and availability (Cong and Zhang, 2022). Lastly, a rapid turnaround time is vital, providing quick results that are necessary for timely decision-making in clinical and on-site applications (Sachdeva et al., 2021). By fulfilling these requirements, immunological devices beyond the LFA concept can overcome barriers to market entry and achieve widespread adoption, ultimately advancing the field of Point-of-Care Testing (POCT). To address the mentioned requirements in POCT and in response to the growing trend of health-conscious behavior, commercially available fully-integrated one-step POCT devices have been developed (Table 1) (Torul et al., 2023). All devices have in common, that signal generation and analysis are automatically initiated upon sample application to the test and therefore, enables user-independent highly sensitive platforms. In addition, a portable and handheld reader enables on-field analysis and the proper material and format decision of the sensor format allows profitable industrial scale up production.

3. Signal amplification for one-step POCT platforms

The WHO reports that nearly three-quarters of all deaths globally are caused by non-communicable diseases (NCDs), with cardiovascular diseases being the leading cause of NCD deaths annually, along with cancer, chronic respiratory illness, and diabetes (World Health Organization, 2023). Therefore, early detection of disease biomarkers through highly sensitive analytical devices is crucial in reducing mortality rates. POCT is especially beneficial in low-to middle-income countries where NCD deaths are more prevalent, as POCT is more accessible and convenient compared to conventional laboratory analytical devices, which are often expensive, labor-intensive, and cumbersome. However, the lack of sensitivity and specificity has made POCT unreliable and susceptible to false negatives and false positives (Ortiz and Loeffelholz, 2023). Therefore, developmental focus in the scientific community has been toward improving the assay performance and the pursuit of ever-lower detection limits. While there have been numerous reported record-breaking limits of detection (LODs) for specific analytes, there

has unfortunately been an over-emphasis on publications with limited consideration of practical applications, especially in resource or personnel-limited settings. To enhance sensitivity, users often must add an additional signal amplification solution or perform an extra washing step, both of which introduce additional operational complexities and may elevate the risk of errors (Kimura and Asano, 2022) rendering the gained LOD performance often times useless. In the following discussion, we will concentrate on the most recent promising developments that demonstrate enhanced sensitivity for one-step POCT platforms. This advancement aims to eliminate complex operational procedures and, consequently, mitigate the potential for errors.

3.1. Optical POCT

Presently, most POCT utilize colorimetric signals that are created through colored labels. Antibody-conjugated colloidal gold nanoparticles (AuNPs) are often the preferred choice for quantification, as they have biocompatible properties, are easily modified and functionalized, and exhibit surface plasmon resonance in the visible range. This has made gold nanoparticles the benchmark in the field (Amendola et al., 2017; Zhou et al., 2015). Despite its advantageous properties, AuNPs in LFA tests are not without drawbacks. These drawbacks include low AuNP capture efficiency (<5 %) by the recognition elements, speed of flow (Moghadam et al., 2015) and insufficient visual contrast detection, leading to subpar sensitivity and detection limits (Qin et al., 2012). To increase the color contrast, several novel metallic (Beck et al., 2023) and non-metallic NPs (Wu et al., 2023) as well as well-known signal amplification strategies through conjugation, aggregation and growth of nanoparticles have been employed (Zhou et al., 2019). However, the latter typically require the manual addition of extra amplification solutions (Tng et al., 2022; Panferov et al., 2021), introducing complexity and the potential for errors into the assay, as the signal-enhancing reagent must be added at precise timing and temperature conditions (Ren et al., 2019; Zhou et al., 2021b). To preserve the user-friendly nature typically associated with LFAs, the integration of the signal amplification step within single-step POCT systems has been crucial (Panferov et al., 2023). The growth of nanoparticles is accomplished by storing the amplification solution in a dried form on the test and subsequently delivering the reagent to the test zone through manipulation of the test structure or the incorporation of solution-delivering polymers with a sensitivity enhancement of 5-fold up to 543-fold (Fig. 2a) (Han et al., 2020b; Panraksa et al., 2021). Another amplification approach is the accumulation of multiple nanoparticles that are different in size, on the test line. By storing the NPs on the test, the one-step mechanism is retained and demonstrated a 10-fold up to 30-fold signal enhancement (Fig. 2b) (Chotithammakul et al., 2021; Shen and Shen, 2019; Taranova et al., 2021), compared to the traditional LFA. Recently, loading of liposomes with sulforhodamine B (SRB) showed an improvement of the detection limit of interleukin-6 (IL-6) by an order of magnitude (7 pg mL^{-1}) when compared to the traditional AuNP based LFA (81 pg mL^{-1}) (Fig. 2c) (Rink et al., 2022). Key to this significant lowering of the LOD

Table 1
Examples for commercially available one-step POCT devices.

| Platform	Reader	Sensing strategy	Sample	Volume (μL)	Biomarkers	Time (min)	Reference
iSTAT	Handheld/portable	electrochemical	Whole blood	17–40	lactate, cardiac, hormones	2–15	(Abbott, n.d.)
Triage	Small benchtop/portable	fluorescence	Whole blood or plasma	175–250	cardiac	15–20	(Hitado GmBH)
Atellica VTLi	Handheld/portable	frustrated total internal reflection imaging	Whole blood or plasma	30	cardiac	8	(Siemens Healthineers, n.d.)
LumiraDx	Handheld/portable	fluorescence	Whole blood or plasma	20	cardiac	12	(LumiraDx, n.d.)
FREND	Small benchtop/portable	fluorescence	Serum or plasma, nasopharyngeal swab	35–70	cardiac, respiratory, urology	3	(NanoEntek, n.d.)
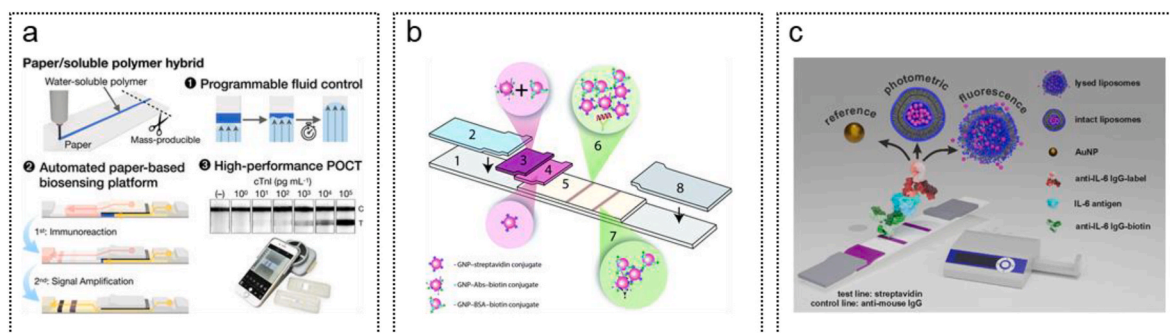
Fig. 2. AuNP-based amplification strategies for one-step POCT. An illustration of the workflow of a programmable fluid control for the signal amplification on a lateral flow assay with a dissolvable PVA barrier. Reproduced/adapted from (Han et al., 2020b) (a). Network of gold nanoparticles. Reproduced from (Taranova et al., 2021) (https://creativecommons.org/licenses/by-nc/3.0/) (b). Loaded liposomes for signal improvement. Reproduced/adapted from (Rink et al., 2022) (https://creativecommons.org/licenses/by/4.0/) (c).

was the optimization of SRB loading and liposome size. Additionally, enzymatic processes have been shown to increase the sensitivity of immunoassays (Bollella, 2022; Calabria et al., 2021). However, the addition of the enzyme substrate requires an additional operation step, making also this test procedure more complicated and prone to manual errors (Joung et al., 2014). To eliminate the extra addition of the enzyme's substrate, one-step strategies have been realized into POCT platforms (Lee et al., 2022; Liu et al., 2020; Parandakh et al., 2023; Rocca et al., 2021; Uddin et al., 2021), by implementing wax structures (Ishii et al., 2018; Preechakasedkit et al., 2018) and optimization of the channel geometry to achieve a simple one-step enzyme amplified immunoassay (Panraksa et al., 2022). Another group have demonstrated a fully integrated POCT device for the health monitoring of astronauts through the chemiluminescence process of horseradish peroxidase (HRP) and luminol. The device consists of a disposable LFA cartridge, where the active reagents were stored in reservoirs and a chemiluminescence reader. The amplification solutions were manually activated through pressure-actuated pouches and controlled through similar valves, thus the assay only requires the addition of the sample (Zangheri et al., 2019). A fully integrated and automated platform based on magnetic digital microfluidic (MDM) technology was demonstrated by the group of Hu et al. (2023) (Fig. 3a). The platform performs microbead-based ELISA in droplets using highly sensitive biotinylated magnetic nanoparticles, as the mobile substrate. These nanoparticles were controlled by an external magnet, allowing for automated immunoreaction and enzymatic amplification with HRP. By adding sucrose-fixed substrate loaded fibers (SCF) onto the LFA sample pad, automated substrate delivery for the signal amplification with Au@Pt nanozyme at the test line was achieved. The LFA demonstrated similar analytical performance for the detection of acetamiprid with a simplified operation procedure. However it should be noted that the addition of an extra fiber, with high absorbance capacity, may lead to an increase of required sample volume (Fig. 3b) (Mao et al., 2023). Han et al.

(2020a) introduced a water-swellable polymer for the automated substrate delivery and enabled a 595-fold higher detection limit for cTnI than the gold standard (Fig. 3c). When the immunoreaction is saturated, the polymer releases the reagent solution for the signal amplification. Even though the release of the substrate is automated and the error susceptibility is reduced by the use of the polymer, the reagent solution still needs to be applied manually to the test.

3.2. Photoluminescence

Fluorescence detection has gained widespread prominence in the field of biomedicine and has been integrated into numerous one-step clinical diagnostic instruments, prominently including fluorescent immunoassays. The gain in sensitivity and LOD makes it highly appealing also for the POCT. A variety of fluorescent reporters, including fluorescent dyes, quantum dots (QDs), carbon dots, and upconverting nanoparticles (UCNPs), are employed as amplification signals, due to their enhanced fluorescence output and stability (Gong et al., 2017; Supianto and Lee, 2022). However, costly optical detection instruments are required to achieve the desired dynamic range and detection limits, preventing its application in low-resource settings and in the at home testing market (Semeniak et al., 2023). In addition to improving readers (see supplementary information) an abundance of amplification strategies have been proposed recently. Similar to colorimetric approaches, the sheer number of fluorescent labels per binding site at the detection area can be increased through nanocarriers. Nanocarriers pose flexible structures housing a multitude of cavities, holes, or pores, making them suitable for the encapsulation of a diverse range of materials (Chen et al., 2021). Currently, the existing nanocarrier options primarily include liposomes (Xing et al., 2016), mesoporous silica (Gubala et al., 2020), as well as nanomaterials based on polymers (Chen et al., 2020; Zhou et al., 2021a), metal oxides (Zou et al., 2021), and carbon (Arshad et al., 2022). By exploiting the electrostatic multilayer deposition of multiple
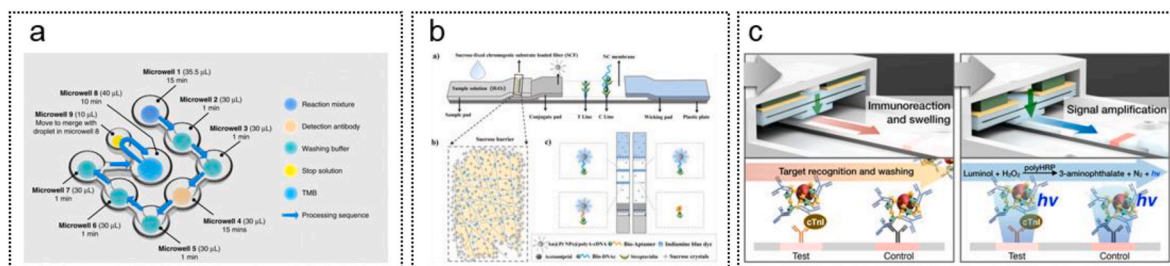

Fig. 3. Enzymatic amplification strategies in one-step POCT. Fully automated magnetic microfluidic platform for the controlled reaction workflow. Reproduced/adapted from (Hu et al., 2023) (https://creativecommons.org/licenses/by/4.0/) (a). Delayed substrate delivery on a paper-based LFA through a dissolvable sucrose barrier. Reproduced/adapted from (Mao et al., 2023) with permission from Elsevier (b). Time-programmable amplification part based on a water-swellable polymer. Reproduced/adapted from (Han et al., 2020a) (c).

QDs onto a graphene oxide nanosheet, a multilayered fluorescent nanofilm improved the sensitivity by 2–3 orders of magnitude for the detection of *Salmonella typhimurium*, compared to the conventional LFA (Yu et al., 2023). Yang et al. (2023) demonstrated a rapid, on-site, and highly sensitive detection of the MPXV antigen with multilayered SiO2-Au core dual-quantum dot shell nanocomposite. The fluorescence can achieve a 238-fold improvement in sensitivity compared with the AuNP-based LFA (Fig. 4a). Additionally, the sensitivity of fluorescence signals in POCT devices has been improved with the integration of metal-enhanced fluorescence (MEF) technology (Campu et al., 2023; Guo et al., 2018; Semeniak et al., 2023; Zhao et al., 2020). MEF works by dramatically increasing the fluorescent signal when fluorophores and metallic nanostructures are placed in close specific proximity, enabling the detection of lower concentrations of biomarkers and earlier disease diagnosis through localized surface plasmon resonance of the metal structure and the nanomaterial (Jeong et al., 2018). For example, a MEF-based microfluidic chip for the detection of cardiac troponin I (cTnI) in spiked human serum was developed. Dense arrays of Au nanorods deposited on the detection area of the microfluidic channel onto which the antibodies were immobilized (Fig. 4b) (Wang Ye et al., 2021). While MEF has gained much attention, especially for the POCT, a significant challenge lies in attaining uniformity in the size, shape, structure, and morphology of the MEF substrates at the nanoscale level (Deng et al., 2013). Another major limitation of fluorescence detection is the autofluorescence of the samples that hinder ultra-sensitive detection (Loiseau et al., 2019). Thus, an extra washing step is required which needs an additional handling process. To circumvent this, a one-step fluorescence immunoassay has been developed where the washing step was eliminated through the installation of switchable peptides that enable fluorescence signal detection in the presence of the analyte (Fig. 4c). Thus, the total analysis time could be halved to less than 30 min (Kim et al., 2022; Liu Qi et al., 2020). To further reduce autofluorescence, lanthanide doped-NPs have been employed into POCT systems due to their large stokes-shift (Jin et al., 2022; Liu Qingyun et al., 2020).

3.3. Surface-enhanced Raman spectroscopy (SERS)

Recently, there has been growing interest in combining POCT devices with surface-enhanced Raman spectroscopy (SERS) (Gunawardhana et al., 2021; Khlebtsov and Khlebtsov, 2020; Khlebtsov et al., 2019; Panneerselvam et al., 2022; Sun et al., 2020), which allows for highly sensitive detection in laboratory settings. Additionally, the high spatial resolution and narrow Raman scattering bands of SERS make it a promising tool for multiplexing (Langer et al., 2020). A few examples have been published, such as the a SERS-based LFA for the detection SARS-CoV-2 IgM/IgG (H. Liu et al., 2021). With that set up, the detection limit was improved by a factor of 800 compared to the traditional LFA. Quantitative analysis was performed with a portable

Raman reader that houses a 785 nm laser for the excitation. To reduce the sample volume, Su et al. (2019) have integrated SERS detection within a microfluidic platform. A microfluidic SERS platform with integrated blood-plasma separation was able to detect creatinine with only 3 μL whole blood within 2 min. However, SERS detection has several limitations that hinder its in-field application. Reproducibility is challenging due to the size variation of metal colloids, uncontrollable aggregation of particles and the uneven distribution of signaling molecules on particle surfaces. Additionally, the sensitivity of SERS in POCT is relatively bad compared to conventional SERS, due to the reduced resolution of portable readers. Further, the analyte is often affected by the POCT's material properties and the design of the platform that is known as "memory effect" (C. Wang et al., 2024). To realistically use SERS in real-world scenarios, further developments are needed to address the mentioned limitations. If achieved, SERS could become a valuable addition to the POCT portfolio.

Fluorescence, MEF, and SERS are innovative concepts with potential, but their requirement for a reader limits their applicability in certain types of POCT, such as home testing and resource-limited settings. However, they remain intriguing and pertinent for POCT conducted in doctor's offices, analytical laboratories, and similar settings.

3.4. Electrochemical sensors

Electrochemical methods hold significant potential for affordable, compact, user-friendly portable devices suitable for various applications (Singh et al., 2021). Their main advantages over optical methods are the simplicity of the hardware needed and the ability to detect in optically scattering and colored samples. Hence, electrochemical measurements can be conducted on colored or turbid samples like whole blood, without being disrupted by fat globules, red blood cells, hemoglobin, or bilirubin (Yao et al., 1993). Wang et al. (2021) demonstrated this nicely with a budget-friendly, and portable microfluidic paper-based electrochemical apparatus, allowing for the immediate isolation of proteins and the direct electrochemical identification of Pb(II) in protein-rich urine samples (Fig. 5a). Beck et al. (2022a, 2022b) proposed superior signal amplification with silver nanoparticles when compared to conventional gold nanoparticles in a one-step electrochemical flow channel device (Fig. 5b). Here, all reagents were dried on the sensor, preserving the one-step LFA methodology. Yakoh et al. (2019) created a device capable of sequentially storing and transporting reagents to the detection channel autonomously, without requiring external power (Fig. 5c). This device consists of two main parts: an origami folding paper (oPAD) and a mobile reagent-stored pad (rPAD). This three-dimensional capillary-driven device streamlines the cumbersome process of multi-step reagent manipulation in complex assays. Even though only buffers were utilized in this scenario, and not actual human samples, the straightforward yet adaptable design allowed for achieving good sensitivity while maintaining the one-step approach.
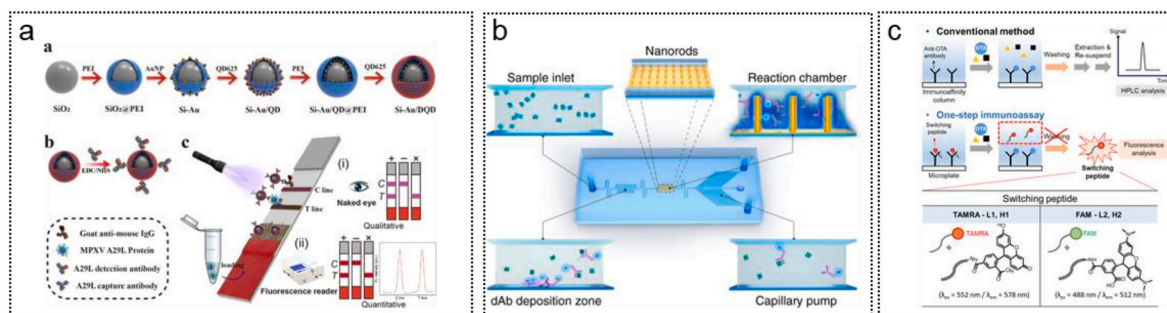

Fig. 4. Fluorescence one-step POCT. Signal amplification by embedding multiple QDs into a nanocarrier made out of SiO$_2$ nanoparticles. Reproduced/adapted from (Yang et al., 2023) (https://creativecommons.org/licenses/by/4.0/) (a). MEF-based microfluidic platform for highly sensitive detection. Reproduced/adapted from (Wang Ye et al., 2021) (https://creativecommons.org/licenses/by/4.0/) (b). One-step immunoassay with switching peptides to avoid an additional washing step (Kim et al., 2022) (https://creativecommons.org/licenses/by/4.0/) (c).
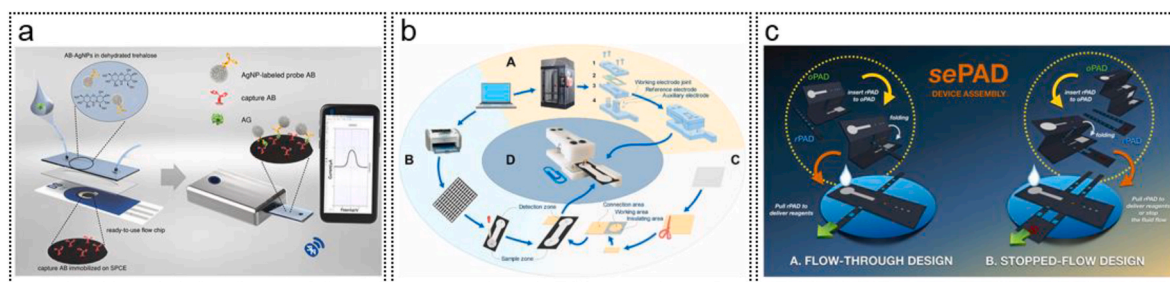
Fig. 5. Electrochemical one-step POCT. Microfluidic biosensor for the detection of NT-proBNP via Ag- nanoparticles. Reproduced/adapted from (Beck et al., 2022b) (a). Paper-based electrochemical sensor with on-line isolation of proteins in urine for the detection of Pb. Reproduced/adapted from (Wang et al., 2021) (b). An origami folding paper (oPAD) and a movable reagent-stored pad (rPAD) for the sequentially transport of reagents. Reproduced/adapted from (Yakoh et al., 2019) (c).

3.5. Nucleic acid amplification

Complementary to the widespread nanomaterial-based amplification methods, nucleic acid amplification techniques (NAATs) have been proofed as reliable and strong detection tool. Currently, the real-time polymerase chain reaction (RT-PCR), the gold standard for virus infection detection, stands out due to its extraordinary sensitivity and specificity. However, RT-PCR suffers from cumbersome machines and skilled professionals and is therefore inapplicable in a POCT setting that does not rely on laboratory-based devices (Esbin et al., 2020). Recently, isothermal nucleic acid amplification strategies such as loop-mediated isothermal amplification (LAMP), strand-displacement amplification (SDA), recombinase polymerase amplification (RPA), helicase dependent amplification (HDA), and CRISPR/CAS have gained massive attention in diagnostic testing as their isothermal character facilitates the integration in POCT devices and ensures a cost- and time-effective approach (Liu et al., 2019; Zheng et al., 2021). Especially, in the COVID-19 pandemic nucleic acid based detection methods ensured accurate and fast results (Esbin et al., 2020). The key advantage of nucleic acid amplification lies in the generation of a large number of copies of the target and therefore, improving the sensor sensitivity (J. Asiello and J. Baeumner, 2011). The main challenge for NAATs is the integration of the multiple step process ((I) nucleic acid purification, (II) amplification, (III) detection) of nucleic acid amplification methods in the one-step POCT device to reduce the hands-on interference and potential contamination (Dong et al., 2019; Wu et al., 2014). Unfortunately, these steps still are often performed separately off-device and manual pipetting is required, but there is great potential for integrating the entire handling into a POCT device, eliminating the need for human intervention (Cunha et al., 2022). Recently, a range of fully integrated one-step platforms based on NAATs have emerged, offering streamlined and user-friendly solutions (Li et al., 2021; N. Wang et al., 2024). For example, Seok et al. (2020) designed a reverse transcription LAMP (RT-LAMP)-based lab-on-paper for molecular diagnostics of Zika, Dengue, and Chikungunya viruses from human serum. The entire nucleic acid testing process, encompassing sampling, extraction, amplification, and detection, is seamlessly executed on a single paper chip within 60 min at 65 °C. Utilizing the engineered structure of paper materials and dried chemicals on the all-in-one chip, serum samples containing the target virus RNA are effortlessly introduced via automatic flow from distilled water injection. An autonomous and fully integrated sample-to-answer device, for the specific detection of HIV-1 virus from human whole blood was developed to streamline the whole detection process (Phillips et al., 2019). Following sample addition, the result can be observed within 90 min. Additionally, users need to execute only four steps to commence testing: loading the sample and rehydrating mixture, adding wash buffer, sealing the inlets with adhesive, and activating the temperature control circuit by connecting to a power source like a computer, cellphone, or portable battery. Focusing on multiplexing, Xie et al. (2024) developed a magnetic-based all-in-one platform for the detection of foodborn pathogens. Using this platform,

the time required from sample collection to obtaining results was approximately 60 min, and the entire process on the chip was completely automated.

3.5.1. Commercial one-step NAAT devices

These advancements have resulted in the emergence of several scalable production devices that have now entered the market (Fig. 6) (Li et al., 2020, 2021). USTAR has developed an EC-certified COVID-19 RNA paper-based test that allows for PCR-quality detection at home using a portable kit without the need for additional instruments. Results can be observed within 35 min, and the product offers a sensitivity of 95.4 % and a specificity of 99.8 %. However, it's worth noting that the user is required to add two additional buffers to the assay, resulting in two additional steps for the user to complete (USTAR Biotechnologies LTD., n.d.). Visby Medical™ has launched a portable and fully integrated PCR-platform for the simultaneously detection of three respiratory health biomarkers, such as COVID-19, influenza A and influenza B. The device enables accurate testing with the results being available under 30 min (Visby Medical, n.d.). Similarly, Cue Health has released a handheld, portable PCR platform capable of detecting COVID-19 in just 20 min. The test kit includes a reader and an assay that can be inserted into the reader after sample addition (Cue Health, n.d.). Detect® has introduced a fully-integrated one-step POCT device, eliminating any sample handling. The device consist of a heater and a lateral flow assay. After collection of the sample trough the nasal swab the tube is closed with the provided detect cap that contains the reagents for the NAT. The tube is then placed into the heater for 55 min for the reaction to take place. After that, the tube and an additional reagent are transferred to the lateral flow assay where, the PCR-quality result can be observed after 10 min (Detect, n.d.). A similar but much more convenient method to test for COVID-19 is introduced by the Lucira POCT device by Pfizer. This test is based on RT-LAMP and is also able to detect 3 viruses with only one test within 30 min. The test is initiated with the addition of the sample and no training is required, thus enabling home-testing (Lucira by Pfizer, n.d.). BIOPIX-T introduced the PEBBLE qcLAMP, a compact and lightweight diagnostic system designed for rapid molecular detection of infectious diseases like COVID-19 and Influenza A at the point of care or demand. The platform performs a real-time quantitative colorimetric loop-mediated isothermal amplification (qcLAMP) within 30 min and the reader enables an innovative approach for heating the test vials and interpreting the results ((Bioopix-T, n.d.)).

3.6. Design modification and fluid control

Aside from tag-related amplification methods, recent research have focused on test structure modifications for enhancing the sensitivity of conventional LFAs (Díaz-González and de la Escosura-Muñiz, 2021). The improvement is mainly attributed to the increase in kinetics of the immunoreaction. For example, to slow down the flow in paper-based LFAs physical barriers such as wax barriers (Fig. 7a) (Sena-Torralba et al., 2020; Strong et al., 2019; Tran et al., 2022), cotton thread (Chen
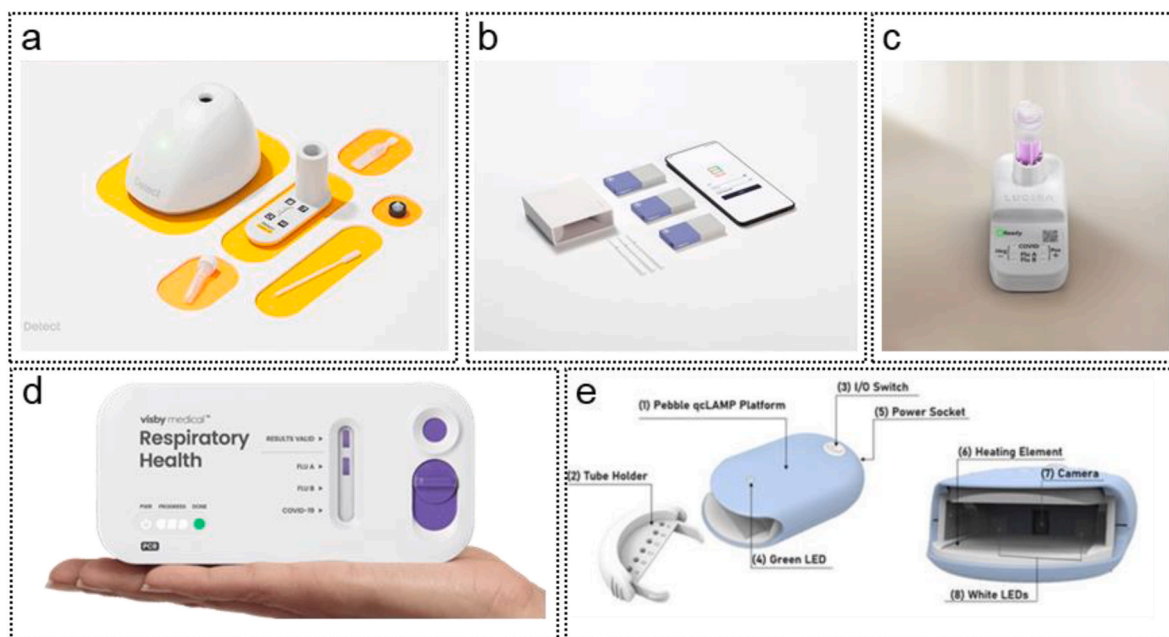
Fig. 6. Commercial one-step NAAT devices. Molecular PCR platform (Detect, n.d.) (a), Reader and tests (Cue Health, n.d.) (b), Reader and assay (LUCIRA, n.d.) (c), Reader and assay (Visby Medical, n.d.) (d), Molecular diagnostic device Pebble (Biopix-T, n.d.) (e).
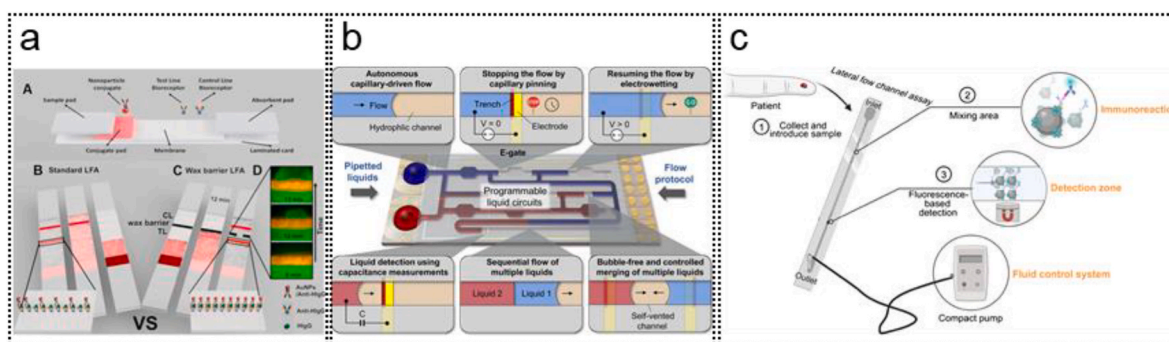

Fig. 7. Sensitivity enhancement through design modification and fluid flow control. LFA with wax barriers. Reproduced/adapted from (Sena-Torralba et al., 2020) (a). Programmable microfluidic with electro-actuated valves and self-vented channels. Reproduced/adapted from (Arango et al., 2020) (https://creativecommons.org/licenses/by-nc/2.0/) (b). Magnetic bead-based platform with active fluid control. Reproduced/adapted from (Strohmaier-Nguyen et al., 2024a) (https://creativecommons.org/licenses/by/4.0/) (c).

et al., 2022; Tan et al., 2021; Zhang et al., 2019), laser patterning (Bikkarolla et al., 2020; Sumantakul et al., 2023), pressed NC membrane (Park and Shin, 2022) have been reported. Modification of the size ratio of the test line and stacking the pads vertically have also shown positive impact on the sensor performance (Afriat et al., 2020; Zadehkafi et al., 2019). While nitrocellulose remains the predominant material for capturing bioreceptors like antibodies in LFAs (Tang et al., 2022), it also bears some limitations such as fluctuations in flow rate and analysis time due to differences in pore structure and sample viscosity. Pore blockage caused by matrix components, the membrane's innate absorbent properties leading to a relatively high sample volume, and inconsistency in dispersing the labeled sample across the membrane due to batch-to-batch variations and buffer reagents (Castro et al., 2022). Thus, there has been a notable trend in exploring alternative membrane materials recently. For example, electrospun polymer nanofibers (Brito-Pereira et al., 2021; Perju et al., 2022; Pimentel et al., 2020), *bombyx mori* cocoons and electrospun silk fibroin (Brito-Pereira et al., 2022), cotton thread (Mao et al., 2015). Yet, an extensive assessment of these alternative materials regarding the sensitivity, reliability and long-term stability is necessary before considering them as dependable

substrate for immunoassays (Díaz-González and de la Escosura-Muñiz, 2021). To overcome these limitations, other researchers have focused their work on channel-based assays, where any membrane- and fleece material was omitted. The most popular immunoassays are built in so-called microfluidics, where microchannels simultaneously houses sample processing and detection. This technology's strengths lie in its cost-effective and scalable manufacturing, simple adaptability for functionalization, faster reaction kinetics, and its flexibility in adjusting flow rates by changing the geometry of the channel or external fluid flow control system (Fig. 7b/c) (Arango et al., 2020; Azizian et al., 2023; Bhuiyan et al., 2022; Jang et al., 2021; Shen et al., 2023; Strohmaier-Nguyen et al., 2024a). In a study by Strohmaier-Nguyen et al. (2024b) sample-absorbing fleece and membrane materials were eliminated for the detection of NT-proBNP in a lateral flow channel assay. By drying antibodies on the test and a multilayered polymer film as the test line, the one-step characteristic has been preserved. Unlike traditional lateral flow assays, the assay developed in this study requires only 20 μL of sample volume (as opposed to 150 μL), easily obtained through a simple finger prick, thereby making it suitable for home testing. Similarly, Hemmig et al. (2020) transformed the conventional LFA into a

fluorescence-based capillary-driven microfluidic without any fleece- and membrane materials. Through a self-coalescence mechanism involving rehydration of reagents to form a homogenous concentration profile and capturing beads in the detection zone, a detection limit of 4 ng mL^{-1} cTnI was achieved using only 1 μL sample volume. The group of Kim et al. (2020) developed a microfluidic immunoassay with a fluid vent control to amplify the signal of the immunoassay by pausing the sample flow. All reagents were dried within the sensor, allowing the immunoassay to be initiated upon sample introduction. In the developed platform, meticulous fluid flow control ensured continuous delivery of reagents to the detection area. This approach led to an improved immunoreaction and a 10 times higher LOD than the commercial LFA kit.

3.7. Sample preparation

Sample preparation, a crucial aspect of precise biomedical analysis, provides the recovery and concentration of the desired biomarker from the sample through purification of the sample and elimination of potential interferences (e.g., non-target contaminants) (Cui et al., 2015). Incorporating this step into one-step platforms is a must and provides the opportunity for accurate and highly sensitive testing, however, is an immensely challenging task. The complexity of preparing the sample varies typically mainly based on the source of sample (predominantly here whole blood, urine, or saliva).

3.7.1. Blood

Whole blood stands as one of the most intricate body fluids utilized in clinical analyses (Anderson and Anderson, 2002) and usually demands plasma separation from blood cells to prevent impediments in the detection process and signal read-out such as high non-specific auto-fluorescence signals (Shrirao et al., 2021) and colorimetric background signals. In traditional paper-based POCT platforms, plasma separation is often accomplished using a porous filtration membrane. This straightforward strategy is hampered by membrane clogging, increased blood volume requirements due to dead volume in the membrane, and analyte loss caused by non-specific adsorption. In recent years, there has been a rise in literature focused on miniaturized methods for plasma extraction (Maria et al., 2017; Maurya et al., 2022; Tripathi et al., 2015; Wang Yudong et al., 2021). However, most of these methods necessitate pre-dilution of the blood sample. While this reduces the concentration of blood cells, it also decreases the concentration of the target analyte, and it presents an extra sample processing step. It thus ultimately impairs the sensor performance and adds inconsistencies to the POCT platform. Thus recently researchers focuses on integrated plasma separation techniques without the need for a blood sample pre-dilution (Bakhtiaridoost et al., 2023; Lee et al., 2019; Qiu et al., 2020; Shi et al., 2023; Su et al., 2019). The integration of an acoustic micro-streaming into a microfluidic device for the detection of HIV-1 p24 protein enabled the isolation of plasma from undiluted whole blood and achieved approximately 31.8 % plasma yield with 99.9 % plasma purity in a span of 5 min. They showcased a detection threshold of 17 pg mL^{-1}, with the acoustic microstreaming also serving as both a micropump and micromixer, facilitating controlled sample flow and enhanced kinetics of the immunoreaction (S. C. Liu S.C. et al., 2021). Lenz et al. (2021) have integrated centrifugal cross-flow filtration to separate serum form whole blood with a purity of 99.99 % and showed higher biomarker retention when compared to the serum that was separated with the benchtop centrifugation. A magnetic separation technique was devised to extract plasma from whole blood. By coating magnetic nanoparticles with antibodies targeting red blood cells, plasma with 99.9 % purity was obtained. In a ferritin test conducted on the lateral flow assay, it exhibited a superior detection limit compared to the filtration membrane (Vemulapati and Erickson, 2018). A significant drawback of the setup is that the biomarker's bioanalysis was conducted off-chip, rather than being integrated into the separation module. By combining the commercially available filter membrane with a microfluidic device, the researcher successfully extracted 20 μL plasma from 100 μL undiluted whole blood in 16 min (Kim et al., 2021). To actively pull the extracted plasma from the membrane, nano-sized gaps were incorporated on both ends of the microchannel. Thus, the whole platform enables powerless application. To overcome the inherent sample absorbing characteristics of the filter membrane, a sedimentation-based plasma separation enables efficient separation and sensitive detection with minimal sample volume was developed for the detection of NT-proBNP in whole blood (Fig. 8a) (Strohmaier-Nguyen et al., 2024c).

3.7.2. Saliva

The conventional collection of saliva is done off-chip and requires additional saliva collector and running buffer in order to initiate the immunoassay on the POCT platform (Jung and Kim, 2022). This step is more cumbersome for the user as the saliva needs to be added to the test separately. In order to achieve more effective regulation and ensure consistent performance in saliva-based testing, it is essential to standardize collection methods, facilitated by the use of standardized collection devices (Cuevas-Córdoba and Santiago-García, 2014). To overcome this burden, the company inne.io has developed a fully integrated minilab for home-testing to track fertility hormones in saliva. The saliva collector houses a sample absorbing pad that is integrated into the LFA (Fig. 8b) (Inne, n.d.). After sampling, the collector is folded onto the LFA, thereby initiating the immunoassay. In combination with the reader, the results are automatically send to the smartphone. Vinoth et al. (2023) created a portable electrochemical microfluidic device, featuring an integrated filtration membrane for the electrochemical monitoring of various salivary biomarkers. The samples are gathered using a plastic dropper, then introduced into the device inlet and allowed to traverse through the microfluidic channels. Another all-in-one electrochemical-based platform for the detection of cotinine was developed by integrating the commercial available cotton-swab-type collector with a 3D printed housing for seamlessness and straightforward introduction of the sample to the assay (Lee et al., 2020).

3.7.3. Urine

The preparation of urine for POCT analysis is simpler and does not require pre-processing. However, there may be cases where the target analyte is present only in very low concentration making a preconcentration step necessary (Cai et al., 2020; Drexelius et al., 2020; Jajack et al., 2019; Zhang et al., 2020). By using gradients as the driving force for the preconcentration proteins have been successfully demonstrated. Specifically proteins were enriched 100-fold using both electric field and pH gradient or by pressure gradients water and interferences were filtered through membrane and the protein of interest was preconcentrated up to 100-fold (Fig. 8c) (Jajack et al., 2019). Most of the on-chip preconcentration steps are a good alternative for the cumbersome bench-top instruments, however, they are still complex as they require additional running or washing buffers, complex geometries or specific parts of the platform need to be manually assembled afterwards. Thus, many further improvements need to be done in order to fulfill the requirements of the one-step POCT.

3.7.3.1. Perspective.
Before a commercial POCT system can be produced through mass fabrication, manufacturers must overcome several challenges, including material selection, meeting sensor performance criteria, ensuring ease of production, simplifying test operation, and developing integration solutions. They are accountable for maintaining product quality, especially for medical devices, while also focusing on minimizing costs and boosting profit margins (Cong and Zhang, 2022). It cannot be emphasized enough that changes in material, process, production techniques all influence the performance of a test system, in part dramatically. Finely tuned optimized assay may result in bad performing
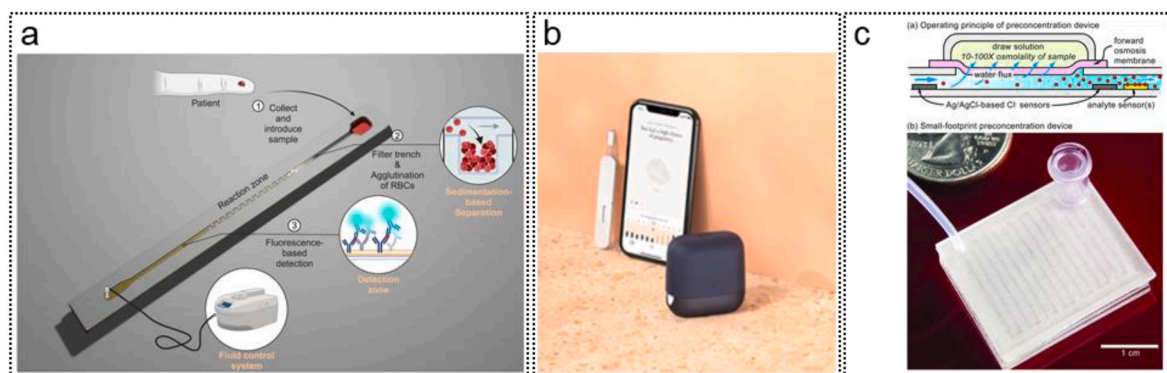
Fig. 8. Integrated sample preparation. Sedimentation-based plasma separation in a microfluidic platform. Reproduced/adapted from (Strohmaier-Nguyen et al., 2024c) (https://creativecommons.org/licenses/by/4.0/) (a). Portable reader and integrated saliva collector and preconcentration step in a LFA. Reproduced/adapted from (inne.io) (b). Integrated preconcentration for a urine based assay. Reproduced/adapted from (Jajack et al., 2019) (https://creativecommons.org/licenses/by/4.0/) (c).

assays when production techniques are altered. It is therefore of utmost importance for novel POCT concepts to consider mass producibility already while inventing new assays, without which these may never be of help to the society at large.

(I) Material and geometric structure for ease of production

The development of POCT devices involves a complex chain of processes, requiring various manufacturing techniques like ablation tools, hot embossing, and roll-to-roll integration. An effective design can significantly reduce effort. Rectangular channels are preferable due to the challenges of achieving rounded structures when using methods like laser ablation or micromilling. Larger channels can simplify tooling, replication, and integration.

A thorough understanding of the assays being performed on a chip and their material requirements is also important in selecting the appropriate material for the production. It is crucial to fully understand the surface properties (e.g., hydrophobicity), light absorbance, mechanical characteristics, and manufacturability (e.g., replication fidelity, surface treatment, bonding methods) of a material used to minimize the potential costs associated with redevelopment. For example, while PDMS is commonly used in lab settings, thermoplastics are generally preferred for industrial scale-up. By addressing these material and design considerations during the design and prototyping stages, while keeping potential scale-up challenges in mind, setbacks can be mitigated, and costs saved.

Manufacturing feasibility should be considered from the start of product development, with expert advice to streamline the process and reduce scale-up failures, especially in academic projects that have application in mind and are not only seeking basic understanding or the innovation of new principles. In fact early involvement of professionals experienced in manufacturing and scale-up would be desirable, as this can save time and effort in converting prototypes into commercial products and assess early on, whether a new assay system has the possibility to be more than a proof-of-principle.

(II) Performance and complexity of POCT device and its operation

For successful commercialization, factors such as performance, system complexity and ease of use of the test must be considered. POCTs with low system requirements, allowing for the miniaturization of necessary hardware, offer broader application possibilities and can be used in low-resource settings (Dincer et al., 2017). High accuracy, sensitivity and specificity in combination with easy test handling, where the sample is simply applied to the test—ideally non- or minimally invasive samples—does not require trained personnel for execution or

sampling (Mohammed et al., 2015). This is particularly advantageous given the current shortage of skilled personnel in medical facilities.

4. Conclusion

Currently, most of the POCT devices remain bulky bench-top instruments or require skilled personnel to operate multiple handling steps. This is because innovations in POCT primarily prioritize cost reduction and sensitivity improvement, which often necessitate multiple operational steps and complex manufacturing. However, there is a growing post-pandemic trend toward developing compact, portable and user-friendly devices, signifying a shift away from complex platforms to on-site, and home-based testing settings. This transformation suggests a positive outlook for accessibility and ease of use, minimizing the need for highly skilled personnel to operate multiple handling steps. Recent research has narrowed the gap between the complexity of devices demonstrated in one-step assay systems and that of bench-top assays. The advancements attained in academic research are now ready to be seamlessly integrated with commercially viable options and gain more and more importance. New sensing technologies, including new labels, test structure and miniaturized read-out systems have overcome the limitations of conventional assays and enable high sensitive one-step POCT platforms. Furthermore, advancements in materials and technical equipment facilitates commercialization in the market. Another crucial factor for the successful marketing and sustainability of one-step POCT devices is to mitigate the risk of sample contamination by untrained personnel. The scale down of all analytical steps such as sample preparation, reagent mixing, separation, chemical reaction and signal detection into a POCT system, eliminate any manual operation and enable reliable testing in rural areas or in home settings.

CRediT authorship contribution statement

Dan Strohmaier-Nguyen: Writing – original draft, Conceptualization. **Carina Horn:** Conceptualization, Supervision. **Antje J. Baeumner:** Writing – review & editing, Supervision, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used ChatGPT in order to improve the language and correct grammar. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.:

Data availability

No data was used for the research described in the article.

Acknowledgment

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bios.2024.116795.

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