REVIEW

Highly sensitive immunodiagnostics at the point of care employing alternative recognition elements and smartphones: hype, trend, or revolution?

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Abstract

Immunodiagnostic tests performed at the point of care (POC) today usually employ antibodies for biorecognition and are read out either visually or with specialized equipment. Availability of alternative biorecognition elements with promising features as well as smartphone-based approaches for signal readout, however, challenge the described established configuration in terms of analytical performance and practicability. Assessing these developments' clinical relevance and their impact on POC immunodiagnostics is demanding. The first part of this review will therefore give an overview on suitable diagnostic biosensors based on alternative recognition elements (such as nucleic acid-based aptamers or engineered binding proteins) and exemplify advantages and drawbacks of these biomolecules on the base of selected assays. The second part of the review then focuses on smartphone-connected diagnostics and discusses the indispensable considerations required for successful future clinical POCT implementation. Together, the joint depiction of two of the most innovative and exciting developments in the field will enable the reader to cast a glance into the distant future of POC immunodiagnostics.

Keywords Biosensor techniques · In vitro diagnostics · Near-patient testing · Nanomaterials · Point-of-care testing · POCT · Smartphone · Recognition elements

Abbreviations

Ab	Antibody
CBP	Calcium-binding protein
CLIP	Combinatorial library of improved peptide
DARPins	Designed ankyrin repeat proteins
IVD	In vitro diagnostics
LFA	Lateral flow immunoassays
LFD	Lateral flow device
MW	Molecular weight
MIP	Molecular imprinted polymer
SAW	Surface acoustic wave
SELEX	Systematic evolution of ligands by exponential enrichment
SPR	Surface plasmon resonance

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Introduction

To date, point-of-care testing (POCT) immunodiagnostics in clinical routine commonly rely on antibodies as biorecognition elements with the biomolecular interaction being read out either visually or via sophisticated equipment provided by the assay's manufacturer. This quasi-default set-up, however, is recently challenged by two major developments: first, several alternative recognition elements with promising features have entered the scene; and second, the ubiquitous availability of smartphones has created a powerful add-on or even alternative for the readout of biomolecular interactions.

High-sensitive diagnostics for POCT usually use molecular recognition protein structures to detect the measurand in complex matrices, such as whole blood or serum. Most widely applied recognition elements

are mono- and polyclonal antibodies (Abs). Due to their extraordinary specificity against antigenic structures, there is an enormous number of different qualitative or quantitative Ab-based methods to assess the concentrations of measurands in single and multiplexed mode. Details are described elsewhere in this volume by Poschenrieder et al.

Alternate biorecognition elements were extensively investigated in the last years [1]. Relevant for immunodiagnostic applications are biotechnologically engineered affibodies, monobodies, DARPins, and anticalins, as well as nucleic acid-based aptamers. Here, the recent advantages of systematically evolving ligands by exponential enrichment techniques help for a suitable selection of affinity binders.

Reasons for the increasing interest in alternative binding partners are not only some obvious disadvantages of Abs but also their often questionable stability and batch-to-batch reliabilities [2]. There are also situations where Abs cannot be produced or lack the required specificity or affinity in the assay. Abs also need to be checked for the respective binding specificities when immobilized onto a sensor surface. This is due to a gradual loss of performance when immobilizing Abs, regardless of their spatial orientation.

Despite the fact that the mainly applied recognition elements in biochemical diagnostics are still Abs, the future will see a significant increase in POCT methods applying alternate biorecognition element candidates. Extended shelf lives and room temperature stored reagents: this is what the in vitro diagnostics (IVD) market demands. Novel binding partners will contribute to these issues.

In this review, we will portray novel recognition elements as viable and robust alternatives to Abs and focus on recent developments in high-sensitive POCT diagnostics.

But also the increasingly complex range of clinical applications in healthcare will be addressed in particular. Smartphones became one of the fastest growing sectors in the technology industry and have a significant impact in medicine. Therefore, we will discuss also smartphone-based immunodiagnostics, since they offer novel possibilities for prevention, diagnosis, and monitoring of chronically diseased subjects [3, 4]. The availability of smartphones offers also the potential of near-patient testing where they are most needed, i.e., in resource-limited settings in developing countries.

Is the introduction of alternative biorecognition elements and smartphones into POCT immunodiagnostics merely a hype, a general trend, or an actual revolution? This review gives an overview on recent advances in the field and tries to answer these questions by assessing the developments with respect to future clinical applications.

Sensors based on alternative recognition elements

Abs are not the only class of biorecognition elements that can be employed for the specific binding of analytes. Alternative compounds, such as synthetic oligonucleotide sequences, ankyrin repeat proteins, or protein scaffolds can be designed to display novel binding sites for antigenic structures. A general feature of these non-antibody binders is that they exhibit a stable core region and surface (peptide) loops of high structural plasticity [5]. The binding proteins are synthesized either by iterative grafting of binding-active peptide loops or by site-directed random mutagenesis in combination with molecular selection techniques (e.g., phage display, SELEX) [5].

This section describes various biorecognition elements beyond classical Abs which are currently available for biosensor-based POCT applications. In Fig. 1, the molecular structures of various binders are illustrated [6]. In fact, the term immunodiagnostics applied for these diagnostic systems is somewhat misleading. The more precise expression is ligand assay-based diagnostics. This term, however, has not found acceptance in the scientific community.

Nucleic acid-based aptamers

Aptamers are single strands of either DNA or RNA oligonucleotides (10–80 bases in length) that can be used to bind different analyte structures with high specificity and affinity and are selected from large combinatorial libraries [7]. On the basis of their three-dimensional structure, aptamers can bind the analyte molecule by adaptive binding, electrostatic interactions, hydrogen bonds, or base-stacking. In general, the affinity constants are in the same pico- or nanomolar ranges as the ones of Abs.

Their use as binding element and possible replacement of Abs in immunoassays is already widely recognized [8–10]. The major advantages of aptamers are in principal their stability and synthetic production rather than the establishment from animal sources.

Aptamers are known to be chemically stable against extreme pH and temperature conditions and can be conjugated to functional groups easier than Abs. Aptamers are created by the systematic evolution of ligands by exponential enrichment, a process called SELEX, which has been investigated extensively since the first description in 1990 [11, 12].

However, despite the tremendous interest by assay developers, the number of different aptamers that bind different antigens is still limited [13, 14]. Single aptamer-based assays may suffer from relatively weak sensitivities. Thus, wide commercialization as IVD is still hampered. To overcome this analytical problem, sandwich-type systems using a pair of aptamer and Ab in a complex have recently been developed as an alternative and are reviewed by Ho Bin Seo et al. [15].
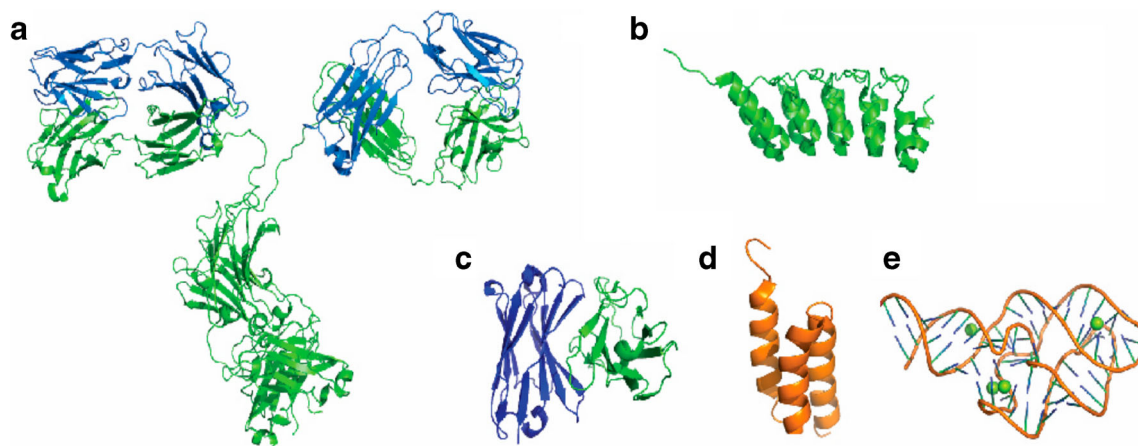
Fig. 1 Molecular structures of Abs and alternative recombinant binder scaffolds used in biosensors. Figure slightly modified, according to Li and Chen [6]. **a** IgG2a mAb with two heavy chains colored in green and two light chains colored in blue; **b** DARPin against tubulin beta chain; **c** anti-fluorescein scFv; **d** HER2-binding affibody; E: 5-hydroxytryptophan aptamer

Abidin et al. reported on developments of cortisol aptasensing towards POCT application [16]. Monitoring the diurnal variations in cortisol serum or saliva concentrations is of medical interest, due to the role of cortisol in stress symptoms. Chronic stress causes major health problems; among them, the most prominent is the risk of acute coronary syndrome. Using a recognition system of aptamer functionalized gold nanoparticles pre-bound with an electroactive compound, the cortisol level could be detected by Sanghavi et al. [17]. The authors realized the competitive binding of the analyte to the aptamer by following the signal generation from the displaced electroactive compound using voltammetry at patterned graphene-modified electrodes.

Concerning lateral flow immunoassays (LFA), Chen and Yang speculated that aptamer-based LFA may be one of the first platforms for commercial use of aptamer-based diagnostics [5].

Bruno evaluated the use of high-affinity DNA aptamers for capture and reporting, coupled to light-emitting quantum dots, and showed first data supporting the thesis that aptamers help to improve the sensitivity of foodborne pathogen (*E. coli*, *Listeria monocytogenes*, and *Salmonella enterica*) detection via LFA test strips [18].

The combination of aptamers with graphene or graphene oxide in order to construct biofunctional sensor surfaces improves the sensitivity and selectivity of the assay system and is called graphene-based aptasensing [19]. In general, graphene offers several advantages for immunodiagnostics such as its flat two-dimensional structure, which exhibits a large surface area and shows extraordinary electrical conductivity and optical properties. Furthermore, graphene is highly reactive for conjugating biorecognition elements with high densities and has low toxicity.

Finally, an approach to avoid the known analytical problems of multiplexed techniques was recently reported by Sze et al. [20]. The authors combine nanopore sensing and nucleic acid aptamer recognition elements. They demonstrate a flexible and scalable platform to sense multiple protein analytes simultaneously by grafting specific sequences along the backbone of a dsDNA carrier. The aptamer-bound analytes then produce unique ionic current signatures and allow for the differentiation of individual protein sizes. The method is suitable for single molecule screening in human serum at ultra-low protein concentrations.

Further engineered binding proteins

Molecular recognition for analytical purposes can also be performed by further non-immunoglobulin protein species. In the early development phase of the classical radioimmunoassays even isolated hormone receptors were applied in ligand assays for quantification of hormone concentrations in blood. Obviously, such attempts were not feasible due to the low stability of the binders. Therefore, it was not surprising that the search for stable binding proteins resulted in new protein technologies. As already mentioned above, a common characteristic is the engineering of a constant backbone protein ("scaffold") as a base for variable recognition/binding surfaces, mimicking the complementarity-determining region of Abs [1, 21, 22]. The monomeric proteins typically contain less than 200 amino acid residues and are very small in comparison to Abs.

This group includes many artificial binding proteins, a selection of which is given below. The progressive nature of these compounds can be seen by the chemical stability of the scaffold protein and by the small molecular size, providing increased surface densities and more intimate contact to the sensor. Implementation of these binding proteins into

biosensor-based POCT applications, however, is still in its infancy.

Reverdatto discriminated two groups of non-antibody binding proteins, originally explored to investigate protein-protein interactions [23].

The so-called "Loop on a Frame" scaffolds imitate Ab paratopes by constructing scaffolds that contain multiple variable loops and incorporate complex combinatorial proteins [24]. As a result, such complex structures are suitable for generating high-affinity surfaces [25].

- **Peptide aptamers:** The group of Reverdatto used a thioredoxin scaffold to develop an efficient design for constructing a Combinatorial Library of Improved Peptide (CLIP) aptamers [25].
- **Monobodies**, also called adnectins, are based on a scaffold structure of the 10th extracellular type III domain of human fibronectin (10Fn3, molecular weight (MW) 10 kDa). This domain consists of 7 barrel-forming β-sheets and 3 solvent-accessible loops on each side, resembling the ligand-binding sites of Abs [26].
- **Anticalins** are synthetic proteins, being capable to bind to small antigens and even small hapten-like molecules with < 1 kD [27]. This type of Ab-mimetic is biotechnologically created from lipocalins which are a family of naturally binding proteins with high structural plasticity. They are found in bacteria, (in)vertebrate cells, and in plants. Lipocalins are involved in immune response, humoral transport, and other biological processes. They are composed of four peptide loops mounted on a stable β-barrel scaffold. Anticalins with a size of about 20 kDa are much smaller than Abs; nevertheless, they possess high target affinity and specificity for various antigenic structures. The anticalin production technology uses site-directed random mutagenesis and selection via phage display against selected molecular target compounds [27]. The number of applications in IVD, however, is still limited. Anticalins may be advancing across new horizons with regard to novel therapy modalities and in vivo imaging techniques [28].

Scaffolds with rigid combinatorial motifs These scaffold-based binders utilize permutations of residues embedded into rigid structural elements at positions that can tolerate side-chain replacements without significantly destabilizing the molecular structure [23].

- **DARPins:** Designed ankyrin repeat proteins (DARPins) are highly stable compounds, derived from the ankyrin repeat proteins, and found as the most abundant binding proteins in the human proteome [29–31]. They are obtained by ribosome or phage display and can be expressed as recombinant proteins in *E. coli*. Each of the repeat motifs is composed of 33 amino acids folded into a β-turn, followed by two antiparallel α-helices and a loop connecting to the next repeat. DARPins form a stable protein framework with a large target interaction surface. Therefore, they offer multiple binding to different epitopes of a single antigen or against several antigens.
- **Affibodies** [29, 32]: The affibody is based on the IgG-binding domain of protein A. This small (MW 6 kDa), three α-helix bundle protein consists of a single polypeptide chain and can be synthesized chemically. Modifications introduced into IgG-binding domains lead to directional head-to-tail polymerization and subsequently, comparable to DARPins, to multiple domains for increased antigen avidity. Affibodies represent useful binding proteins that have been investigated for use in therapy, diagnostic imaging, and biotechnology over the last 15 years [33]. Applications as POCT elements are still to be investigated.

For the sake of completeness, molecularly imprinted polymers (MIPs) have to be mentioned as further alternative recognition elements in this review. MIPs are generated by a polymerization process occurring around a specific template molecule. The quantification of some clinically relevant analytes via MIP-based sensors has been described in detail [34]. Due to their insufficient lower limits of detection and their—as compared to Abs—lower analytic specificity, MIPs, however, do not and supposedly never will play a relevant role in high-sensitive POCT immunodiagnostics. Molecular imprinting will therefore not be discussed in further detail.

In conclusion, all described alternative recognition elements have in common that they are structurally and chemically vigorous and yet can be tailored exactly to the desired binding site. The mostly unproblematic and efficient immobilization onto sensor surfaces compared to Abs together with the small molecular size may lend them to flexible adaptation in many highly sensitive future POCT diagnostics. However, their broad application is hindered by the fact that antibodies as biorecognition elements constitute a mighty and, to date, nearly impregnable competitor: over decades, antibodies have shown impressive flexibility in constructing high-affinity binding partners for a plethora of analytes at reasonable costs.

In Table 1, a summary of pros and cons regarding the application of alternative binders is given. In general, it should be mentioned that these compounds are expensive in identification and design, whereas the production is inexpensive. Contrarily, mAbs are costly in production and maintenance of the respective eukaryotic cell lines [33, 35–37].

Table 1 Advantages and disadvantages of selected alternative biorecognition elements for POCT immunodiagnostic applications [33, 35–37]

Biorecognition element	Advantages	Disadvantages
Nucleic acid aptamers	Small size	Non-specific binding
	High sensitivity	Epitope limitations (not suited for hydrophobic or acidic protein targets)
	High reproducibility	Cost
	Long shelf lives	
Monobodies	Small size	No significant experience with POCT applications
	Stability	
	Ease of engineering	
Anticalins	Bind well to small proteins	Cost
	Stability	No significant experience with POCT applications
	High reproducibility	
	Ease of development	
DARPins	Small size	Epitope limitations (concave shape of binding surface)
	Ideal binders for large conformational epitopes	No significant experience with POCT applications
	Simple post-isolation modification	
	High affinity	
	High solubility	
	High specificity	
	Stability	
	High reproducibility	
	Ease of engineering	
	Rapid production	
	Low cost	
Affibodies	Small size	Not described yet
	High affinity	No significant experience with POCT applications
	High specificity	
	Stability	
	Ease of engineering	
	Quick in vitro production	
	Low cost	
MIPs	Stability	Poor selectivity
	High reproducibility	Epitope size limitations
	Ease of engineering	
	Low cost	
	Long shelf lives	

Smartphone-based immunodiagnostics

In 2018, 7.9 billion mobile phone subscriptions outreached the number of the human population on earth [38]. The availability of smartphones offers the potential of near-patient testing where they are most needed, i.e., in resource-limited settings in developing countries. The growth and acceptance of smartphones among clinicians has been remarkable over the last decade. Over 87% of physicians use a smartphone or tablet [39]. They utilize this emerging technology in order to make rapid and evidence-based decisions aiming towards cost-effective mobile health care and personalized medicine

Table 2 Overview of smartphone-connected POCT immunodiagnostics for conventional clinical chemical analyses. *Corr*: correlation with established system (in parentheses); *nd*, not determined; *LOD*, limit of detection; *LR*, linear range; *Se*, sensitivity; *Sp*, specificity

Analyte	Matrix	Principle	POCT method	Smartphone readout	Analytical performance	Diagnostic performance	Clinical relevance	Reference
Ca^{2+}	Serum (mouse)	Ab detects CBP conformation change	Lateral flow assay, 2D-chromatography	Attachment, image, analyzed with software on desktop computer	LR: 0.25–2.5 mM corr: high (i-STAT)	nd	high	[50]
Vitamin B_{12}	Whole blood (human)	Sample B_{12} and bound BSA-B_{12} compete for gold-nanoparticle-anti-B_{12}	Lateral flow assay	Attachment and app	Corr: high (IMMULITE)	Cutoff 332 pmol/l: Se 100%; Sp 100% Cutoff 258 pmol/l: Se 87%; Sp 100%	Medium	[51]
Ferritin	Whole blood (human)	Gold-nanoparticle-anti-ferritin and anti-ferritin on solid phase build sandwich with sample ferritin	Lateral flow assay	Attachment and app	Corr: high (IMMULITE)	Cut-off 18.0 µg/l: Se 90%; Sp 100%	Medium	[52]
hCG	Buffer	Anti-hCG on silica-encapsulated strontium aluminate nanophosphors	Lateral flow assay	Attachment and app	LOD: 45 pg/ml	nd	High	[53]
NSE	Serum (human)	Anti-NSE on working electrode	Electrochemical	Results of electrochemical detector transmitted to smartphone via Bluetooth and app	LOD: 10 pg/ml LR: 1–500 ng/ml	nd	Low	[54]

[40]. Smartphones have already been used for patient monitoring and diagnostics as well as for more efficient medical education and communication [41, 42]. A detailed review on smartphone-based immunosensors has been presented by Vashist and Luong [42].

State-of-the-art in smartphone-connected diagnostics

In recent years, several advances in smartphone-connected diagnostics have been described [43–45], e.g., both POC diagnostics with built-in sensors (mostly camera and microphone) and external sensors. Five types of smartphone-based POCT-adaptable microfluidic biosensor systems, i.e., imaging, biochemical, immune, hybrid (multiple sensing modalities), and molecular, have been reviewed by Xu et al. [46]. Advantageously, smartphones can be integrated with many other attachments, such as miniaturized cameras, optical sensors, dongles, and electrical circuits, and as such receive growing attention [47–49]. The main challenges stated by Xu et al. are the bulkiness of excessive accessories, the sacrifice of sensitivity when using miniaturized sensors, and the costs and complexity of fabrication of integrated chips [46].

The determination of several common analytes of the clinical routine laboratory via smartphone-based immunodiagnostics has been described (see Table 2). An immunosensor for serum Ca^{2+} using a smartphone-based detector for color readout was for example presented by Park et al. [50]. The authors apply the calcium-binding protein (CBP), which changes conformation upon Ca^{2+} binding. Subsequently, a mAb, recognizing the altered structure of the CBP, forms the immune complex. For the differential diagnosis of anemic conditions, smartphone-based immunodiagnostic systems for the quantification of vitamin B_{12} and ferritin have been described. The vitamin B_{12} assay is based on the competition of sample vitamin B_{12} with a vitamin B_{12}-bovine serum albumin conjugate, immobilized on the nitrocellulose membrane of a lateral flow assay. Readout is then accomplished with a smartphone-accessory and an associated app [51]. Quantification of ferritin was performed as follows: serum ferritin as the analyte is bound by a gold-nanoparticle-anti-ferritin conjugate and by a second anti-ferritin on the surface in a sandwich complex. Color development on test and control lines of the lateral flow assay are then readout via a smartphone attachment and the respective app [52]. Common lateral flow assays for the detection of human chorionic gonadotropin (hCG) ("pregnancy test") have been refined by Paterson et al.: Silica-encapsulated strontium aluminate nanophosphors functionalized with anti-hCG were used as reporters in a lateral flow assay. The flash of the smartphone is employed to excite the phosphors and luminescence is read out by use of a smartphone attachment and a respective app [53]. Moving on to less common analytes, a POCT system for neuron-specific enolase was presented [54]. Employing a microfluidic paper-based analytical device, the

surface of the working electrode was modified with nanocomposites synthesized by amino functional graphene, thionine, and gold nanoparticles and anti-NSE immobilized for immunosensing. The signals of the electrochemical detector were then transmitted to the smartphone via Bluetooth and the measurement results presented via an app. Vashist et al. reported on assays for fetuin-A (alpha$_2$-HS-glycoprotein) [55] and states a current trend towards smartphone-based assays regarding fetuin-A detection [56]. Liu et al. described a surface plasmon resonance (SPR) imaging biosensor where lightweight optical components and sensing element were connected by optical fibers on a phone case [57] for general applications in medicine and environmental monitoring. The fiber-optic SPR structure has been used to detect a broad range of analytes, e.g., cytokines involved in wound healing [58] or cardiac markers in undiluted serum [59]. In order to improve accessibility for consumers to analyze their foods for allergen presence, the development of simple, safe, and rapid assays can be linked with smartphones as detectors. Ross et al. reviewed consumer-friendly, smartphone-based immunochemical food-allergen assays [60], such as milk, egg, crustacean, fish, peanuts, and tree nuts.

Beyond conventional clinical chemical analyses, a lot of effort was put into the application of smartphones in conjunction with POCT for the diagnosis of infectious diseases. Works are mainly focused on the antigenic and serological detection of infections with arboviruses and on sexually transmitted diseases (see Table 3). Smartphone-based diagnostics for simultaneous detection of Zika, Chikungunya, and Dengue viruses at the POC have been reported by Ganguli et al. [71]. However, this system constitutes a nucleic acid testing approach. Using immunodiagnostic principles, Bedin et al. presented a paper-based lateral flow assay for the detection of the non-structural NS1 viral protein of Dengue and Zika viruses [61]. An image of the color developments in the various test zones is taken with the smartphone and analyzed with the respective app. Additionally, an electrochemical immunosensor for the label-free detection of Zika virus protein has been developed to enable the identification of an early-stage infection [62]. The electrochemical sensing system integrates nano/microelectronics for the development of a miniaturized potentiostat, and nano/microelectrodes to develop sensing chips as well as nanostructures for a higher biomolecule load and smaller volumes. The rapid detection of biomarkers of HIV infection in patient samples using a smartphone-connected piezoelectric surface acoustic wave (SAW) microsensor, which requires no microfluidics, optics, analyte labeling, amplification, or washing steps, was reported by Turbé et al. [63]. Another smartphone-based system has also been reported for the genotyping of HBV [64]. For the analysis of the HBV genotypes A–D, the authors applied genotype-specific mAbs. A smartphone has also been used to image the LFA; thereby, LEDs are used for fluorescence

Table 3 Overview of smartphone-connected POCT immunodiagnostics for diagnostics of infectious diseases. *Corr*: correlation with established system (in parentheses); *nd*, not determined; *LOD*, limit of detection; *LR*, linear range; *Se*, sensitivity; *Sp*, specificity

Analyte	Matrix	Principle	POCT method	Smartphone readout	Analytical performance	Diagnostic performance	Clinical relevance	Reference
Dengue Zika	Whole blood, plasma (human)	Anti-NS1 Ab on test strip	Lateral flow assay	Image and app	LOD Dengue: Plasma: 10 ng/ml Whole blood: 20 ng/ml LOD Zika: Plasma: 10 ng/ml Whole blood: 20 ng/ml	nd	High	[61]
Zika	Buffer	Anti-Zika specific envelope protein Ab on surface	Electrochemical impedance spectroscopy	Graphical user interface	LOD: 10 pM LR: 10 pM–1 nM	nd	High	[62]
HIV	Whole blood (human)	p24 and anti-p24 llama antibodies on surface	Surface acoustic wave	Attachment and app	LOD anti-p24: 1.1 nM LOD p24 antigen: 2 nM	nd	High	[63]
HBV	Serum (human)	Genotype-specific monoclonal Ab	Lateral flow assay	Image and app	LOD HBsAg: 2.5, 5, 5, and 10 IU/ml for genotypes A, B, C, and D	nd	Medium	[64]
Mump Measles HSV-1 HSV-2	Serum (human)	Not given	ELISA	Attachment and app	nd	Mumps: Se 100.0%; Sp 97.4% Measles: Se 99.5%; Sp 94.6% HSV-1: Se 100%; Sp 100% HSV-2: Se 100%; Sp 100%	Medium	[65]
HIV T. pallidum Anti-cardiolipin	Whole blood (human)	Chimeric antigens of HIV 1/2, outer membrane protein TpN17 from T. pallidum, and cardiolipin in reagent cassette	ELISA	Dongle	nd	HIV: Se 100%/100%; Sp 87%/91% Treponemal Ab: Se 92%/77%; Sp 92%/89% Cardiolipin Ab: Se 100%/80%; Sp 79%/82% (fingerprick/venous)	High	[66]
HIV T. pallidum	Serum (human)	Not given	Lateral flow assay	Attachment and app	nd	HIV: Se 94.1–97.0%; Sp 100% T. pallidum: Se 86.5–92.4%; Sp 99.0–99.1%	High	[67]
Influenza H5N1	Throat swab (human)	Anti-influenza A nucleo-capsid on nitrocellulose or latex (conjugated with coumarin-derived dendrimer)	Lateral flow assay	Attachment and app	nd	Se 96.6%; Sp 98.6%	High	[68]
E. coli N. gonorrhoeae	Urine (human)	anti-E. coli and anti-N. gonorrhoeae on latex	Immunoagglutination	Image, analyzed with software on a desktop computer	LOD E. coli: 10 CFU/ml LOD N. gonorrhoeae: 10 CFU/ml LR: up to 10^7 CFU/ml	nd	High	[69]
Streptomycin	Chicken me at Milk (bovine)	Streptomycin-specific aptamer	SYBR Green I fluorescence	Image and app	LOD: 94 nM LR: 0.1–100 μM	nd	Low	[70]

excitation and smartphones for signal detection. Compared to existing methods, the authors conclude significantly lower costs using the simpler smartphone HBV genotyping method [64]. Berg et al. established a smartphone-based, mobile microplate reader. Evaluation of this microplate reader as compared to a conventional reader platform for mumps IgG, measles IgG, and herpes simplex virus 1 and 2 IgG yielded a high degree of agreement between both devices [65]. A multiplexed approach for the detection of infections with HIV and syphilis was presented by Laksanasopin et al. [66]. The authors employed a smartphone dongle to replicate all steps involved in ELISAs for the detection of Abs against HIV, of a treponemal Ab for syphilis, and of anti-cardiolipin Abs as a marker for active syphilis. Evaluation of two other commercially available smartphone-based diagnostic systems for the detection of Abs against HIV and *Treponema pallidum* yielded promising sensitivities and specificities as well [67]. Furthermore, the identification of the pathogenic H5N1 Influenza A virus [68] was described recently. Finally, urinary tract infection and gonorrhea were monitored by measuring the extent of immunoagglutination, using the cell phone as detector [69]. Another application combines the use of an aptamer in a POCT method for the determination of streptomycin in food with the fluorescence readout by the use of a smartphone [70]. In principle, the streptomycin-specific aptamer binds the measurand, whereas unliganded excess aptamers hybridize with DNA oligonucleotides. This double-stranded DNA can subsequently be detected by the fluorescence dye SYBR Green. This interesting example shows how alternative binding elements and modern smartphone technologies allow the establishment of an on-site and visual POCT for the detection of antibiotics in daily life.

Critical assessment of smartphone-connected POCT immunodiagnostics

Enthusiasm about the opportunities of smartphone-connected immunodiagnostics, however, should not deter from critically assessing the presented systems. Only a critical view on the recent developments allows identifying the possibilities and limitations of current techniques and defining the directions for future research.

The first point often missed is the choice of the proper analyte. As such, POCT systems for detection of arbovirus infections or of sexually transmitted diseases make sense. This is especially true as they are often relevant in countries with limited resources and lack of clinical laboratory infrastructure. Yet it remains obscure, why analytes such as ferritin, vitamin B_{12}, or NSE should be determined at the POC. These analytes do not constitute emergency parameters. The benefit of measuring them in a POCT system, i.e., immediate results, by far does not outweigh the associated disadvantages, e.g.,

cost and lower analytical quality. Another point regularly ignored already during assay design is the tested sample material. Ideally, the tested sample material is similar to the sample material investigated in clinical routine. One should refrain from extrapolating results collected with analyte in buffer to actual specimens. The complex matrix of sample materials such as serum or urine tends to create higher background signals as compared to analyte-spiked buffer solutions, thereby impacting the analytical performance.

From a technological point of view, most of the presented smartphone-connected POCT immunodiagnostic systems are based on lateral flow assays and as such employ a very traditional IVD technique. One may speculate why more innovative detection approaches like electrochemical sensors did not find broad acceptance: Do they not reach comparable analytical performance? Are they financially prohibitive? Remarkably, the majority of the presented systems in addition to the smartphone also require a smartphone attachment/accessory. This attachment increases costs, may be breakable, and impairs ease of use. Consequently, systems without attachments are preferable.

With respect to the performance of the presented systems, two aspects have to be distinguished: analytical and diagnostic performance. With the data on limits of detection, linear ranges, and correlation to established systems given in the respective publications, most of the systems seem suitable for use in a clinical context—provided the data have been collected with authentic sample material (see above). It has to be criticized, however, that oftentimes diagnostic performance in terms of diagnostic sensitivity and diagnostic specificity have not been evaluated. Integration of smartphone-connected POCT immunodiagnostics in clinical workflows without sound data on their diagnostic performance would be flat out irresponsible. Yet, it is promising, that the few works actually investigating the diagnostic performance found diagnostic sensitivities and specificities sufficient for clinical use.

Another issue should also be taken into account: The World Health Organization's ASSURED criteria of ideal characteristics for POCT in resource-limited settings (**A**ffordable by those at risk of infection, **S**ensitive, **S**pecific, **U**ser-friendly (simple to perform and requiring minimal training), **R**apid and robust, **E**quipment-free, and **D**elivered to those who need it) are not fulfilled by smartphone-connected POCT solutions for the points "U" and "E."

Further considerations for clinical implementation

For a successful future clinical implementation of smartphone-based POCT, several key challenges must be addressed. These include considerations laboratories are well familiar with such as reliability of the testing, analytical performance, miniaturization of microfluidics, material safety

and disposal, and economic feasibility. For smartphone-based POCT, however, requirements, trends, and risks of information and communication technologies should be taken into account additionally. Prompt synchronization between the various mobile devices and central servers, where the data are eventually stored, has to be ensured. Technical specifications of smartphones change persistently. Implementation of ubiquitous smartphone-based POCT not compromised by changing cellphone models as well as fulfilling regulatory requirements is therefore challenging. The sensitive nature of personalized health data furthermore raises concerns about ownership, security, and access rules of electronic data records—especially in scenarios where data are transmitted between devices or servers. Smartphone-based POCT should—as all medical devices—be protected against known vulnerabilities. In order to fend off possible hacking attempts, advanced encryption algorithms will play a major role. They are, however, currently not sufficiently utilized within the healthcare field. Widespread acceptance of smartphone-based POCT as a diagnostic modality will finally critically depend on the adequate and timely identification of and successful defense against tomorrow's threats [4, 72].

Smartphone-based POCT methods are nowadays applied mainly by patients themselves. Possible medical sectors are the diagnosis of infectious diseases and inflammation, monitoring of chronic disease states, sports medicine, and perhaps in the future also therapeutic drug monitoring.

Summary

Non-antibody biorecognition elements are getting more and more relevant for immunodiagnostic applications. Examples are affibodies, monobodies, DARPins, and anticalins, as well as oligonucleotide aptamers. Apart from the latter, the binding proteins/peptides are produced by biotechnological techniques either by rational refinement of peptide loops with prescribed analyte binding or by site-directed random mutagenesis selection in combination with targeted selection display techniques [73].

Due to the obvious advantages of these biorecognition elements, the future will see more viable applications in the medical field of point-of-care testing. Specifically, the coating of sensor surfaces offers benefits in terms of chemical stability. Thus, various sample matrices (whole blood, serum, urine) are applicable and temperature-robust reagents can be used with a longer shelf life. The small size of the binding proteins combined with the rigid scaffolds offers two advantages: first, increased surface densities can be achieved; and second, with the individual assay steps occurring closer to the surface, analyte or label is located closer to the sensing surface as well. Both effects complement each other in improving analytical sensitivity of the vast majority of biosensors. Furthermore multiplexed arrays can be established most efficiently [1].

It should not go unmentioned in this context that given the fact of a high quantity of clinically relevant parameters, the numbers of alternative binders to various analytes are still very low. So it is too early to draw any conclusive appraisals of analytical performance data for clinical applications in comparison to Abs.

The situation is especially surprising for aptamers. Aptamers entered the scientific scene as alternate binders nearly three decades ago and exhibit numerous advantages as compared to antibodies. They were, however, not able to even challenge antibodies' position as the main biorecognition element for clinical diagnostic purposes. The reasons for this phenomenon are found in the aptamers themselves as well as in their competitor. On the one hand, functional aptamers are only available for a few clinically relevant analytes. This may be due to their very confined biophysical properties impairing binding to hydrophobic or acidic protein targets. Even with having the proper aptamer in one's hand, knowledge about suitable surface immobilization techniques in the research and development community is limited. Considerable patent restrictions in the early years also hindered a quick and broad adoption of this innovative biorecognition element. On the other hand, antibodies constitute a mighty competitor. Antibodies entered the market more than 20 years before the aptamers and were able to provide reliable, well-evaluated, and reasonably priced tests for a plethora of analytes. As a consequence, Ab technologies are well-established and constitute a considerable financial commitment for industry. Temptation of major companies to switch to aptamer technology is therefore limited [74].

The smartphone-based POCT technology can be considered as a prime example for the translational trend of preemptive diagnostics from a physician-centered process to a self-responsible task of the individual patient. This will have a global impact on the healthcare sectors.

Thus, high-sensitive diagnostics at the point of care and directly in the hands of patients are no hype or momentary trend. To help the individual patient with chronic diseases such as diabetes mellitus, arthritis, chronic kidney disease, depression, chronic obstructive pulmonary disease, and others will constantly stimulate diagnostic research and development. Even when no revolutionary leap is to be expected, the described emerging analytical and information technologies will continuously be adopted globally in the various healthcare systems. What is crucial to remember is that specifically developing countries will benefit from POCT as feasible, cost-effective, and often sole diagnostic modality.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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