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Review

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A Comparison of Optical, Electrochemical, Magnetic, and Colorimetric Point-of-Care Biosensors for Infectious Disease Diagnosis

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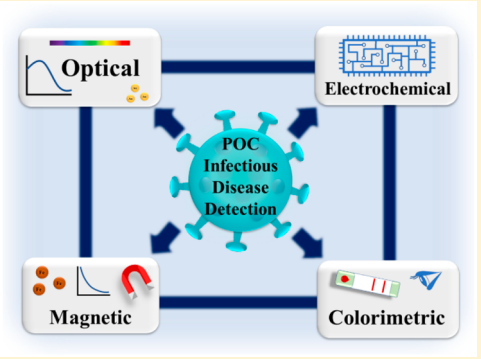
ABSTRACT: Each year, infectious diseases are responsible for millions of deaths, most of which occur in the rural areas of developing countries. Many of the infectious disease diagnostic tools used today require a great deal of time, a laboratory setting, and trained personnel. Due to this, the need for effective point-of-care (POC) diagnostic tools is greatly increasing with an emphasis on affordability, portability, sensitivity, specificity, timeliness, and ease of use. In this Review, we discuss the various diagnostic modalities that have been utilized toward this end and are being further developed to create POC diagnostic technologies, and we focus on potential effectiveness in resource-limited settings. The main modalities discussed herein are optical-, electrochemical-, magnetic-, and colorimetric-based modalities utilized in diagnostic technologies for infectious diseases. Each of these modalities feature pros and cons when considering application in POC settings but, overall, reveal a promising outlook for the future of this field of technological development.



KEYWORDS: *point-of-care, infectious diseases, diagnosis, detection, biosensors*

In the fight against infectious diseases, reaching an accurate and timely diagnosis is often important in order to make an informed decision about the treatment plan. A rapid and accurate diagnosis allows clinicians to prescribe the proper medical treatment and greatly improves patient prognosis overall. When dealing with infectious diseases in particular, a timely diagnosis is even more crucial and may reduce or prevent further infection within the patient population. World Health Organization (WHO) has emphasized the importance of creating point-of-care (POC) tests and created a set of criteria for evaluating POC tests. These criteria are summarized by the acronym ASSURED (Affordable, Sensitive, Specific, User-Friendly, Robust and rapid, Equipment free, Deliverable), which represents the characteristics needed for an ideal POC platform.[1−3] Despite the attention given to the development of novel diagnostic and treatment methods, infectious diseases continue to pose a major threat to the global population.[4−7] Respiratory infections such as pneumonia, influenza, and tuberculosis remain some of the most prolific causes of infectious disease deaths, resulting in nearly 5 million reported deaths each year. This is roughly twice as many as the reported HIV/AIDS-related deaths (2.5 million deaths annually). Malaria and diarrheal diseases account for an additional 1.2 and 2.2 million deaths, respectively, each year.[8−10] Overall, infectious diseases caused by bacteria, viruses, parasites, and fungi result in 15 million deaths each year, and approximately 95% of these deaths occur in low- to middle-income countries.[8,11]

While there are many effective methods for the detection of pathogenic agents, such as culturing, microscopy, genomic amplification (e.g., PCR), and immunoassays (e.g., ELISA), these approaches each have their own shortcomings and are less applicable in resource-limited settings where infectious diseases are more prevalent. Culturing is a very lengthy process, often requiring multiple days or weeks to produce results, in which time the infection can advance within the patient and be transmitted throughout the population. Furthermore, the empirical use of antibiotics is commonly pursued while bacterial culture results are pending, which risks increasing the prevalence of antibiotic resistant bacteria. While more efficient with regards to time, microscopy is limited in both scope and application in POC settings, as it is restricted to pathogens able to be visualized at low magnification.[4,8,12]

Further diagnostic methods such as polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA) are more efficient with regard to time and scope but have several drawbacks. Immunoassays can be successfully utilized to detect infectious diseases if the correct antibody−antigen interaction is determined but are difficult to develop and use for the detection of pathogens with high rates of epitope mutation. Additionally, PCR and ELISA often require the use of expensive laboratory equipment and trained professionals.[13−15] Unfortunately, there are many regions of the world in which this environment is simply not available or sustainable. Due to this, it is common for biological samples to be collected and transported to the nearest laboratory facility or hospital capable of performing such complex laboratory

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Table 1. Information about Optical-Based POC Tests Being Developed Including Technique, Target Pathogen, LOD, Timeliness, and Any Additional Features Mentioned in the Study

technique	target pathogen	LOD	speed	additional features	citation
quantum dot fluorescence polarization analysis using bifunctional aptamer binding	influenza (H1N1 DNA)	detection limit: 3.45 nM	NR	detects DNA sample so prep is needed; not real sample	Zhang et al.[33]
strand displacement amplification with fluorescence polarization	*M. tuberculosis*	1 genome	3 h	detected DNA isolated from culture, sample prep required	Walker et al.[34]
strand displacement amplification with fluorescence polarization	*M. tuberculosis*	10 genomes	20 min	detected DNA isolated from culture, sample prep required	Walker et al.[36]
fluorescent nanoparticle-based indirect immunofluorescence microscopy	*M. tuberculosis*	3.6×10^5 cells/mL	4 h	in mixed bacteria and spiked sputum samples	Qin et al.[35]
fluorescence polarization-based nucleic acid detection	*Salmonella spp.*	1 CFU	20 min	can differentiate between *S. typhi* and *S. paratyphi* in blood samples; cost: $1	Park et al.[30]
fluorescence polarization immunoassay	*Brucella spp.*	NR	5 min	specificity of 97.9% and sensitivity of 96.1%; detects in serum	Lucero et al.[37]
localized SPR-based AuNP-alloyed quantum dot nanobiosensor	influenza (H3N2) influenza (H1N1)	H3N2: 10 PFU/mL H1N1: 0.03 pg/mL in water and 0.4 pg/mL in human serum	5 min	differentiated between H3N2 and H7N7/H9N2; detection in water and human serum	Takemura et al.[41]
quantum dot barcode technology with smartphones and isothermal amplification	HIV or hepatitis B	1000 viral genetic copies per milliliter	1 h	can detect multiple pathogens at once; detects amplified DNA so sample prep needed	Ming et al.[42]
nanobioprobes utilizing fluorescent quantum dots	avian influenza virus (H9N2)	8.94 ng/mL	NR	utilizes *S. aureus* to make the nanobioprobes	Xiong et al.[43]
integrated portable microsystem with PCR amplification and capillary electrophoretic analysis coupled with electrical control and laser-excited fluorescence detection	*E. coli* and *S. aureus*	2−3 bacterial cells	10 min	detected cells suspended in water	Lagelly et al.[47]
microfluidic chip with integrated PCR and electrophoretic analysis	BK virus	1−2 viral copies	NR	identifies viral loads; detected PCR product from urine samples	Kaigala et al.[49]
bead-based immunofluorescence-assay on a microfluidic dielectrophoresis platform	dengue	1×10^4 PFU/mL	5 min	chip is reusable >50×	Iswardy et al.[60]
microfluidic chip capable of performing DNA/RNA amplification, electrokinetic sample injection and separation, and online optical detection of nucleic acid products	*S. pneumoniae* and dengue-2 virus	NR	<5 min		Huang et al.[51]
microfluidic device integrated with microvalves and micropumps for rapid DNA hybridization using shuttle flow	4 serotypes of dengue	100 pM	90 s	sample consumption of 1 μL; could differentiate between the 4 serotypes of dengue; detect each simultaneously	Huang et al.[52]
magnetic immunofluorescence assay using portable device equipped with optical fiber spectrometer and a microfluidic device	avian influenza (H9N2)	3.7×10^4 copy/μL	55 min	sample consumption of 2 μL; complex biological samples including fresh dung, liver, and lung; differentiated H9N2 vs H5N1/H1N1	Zhang et al.[46]
on-chip pressure injection utilizing DNA amplification via noncontact infrared-mediated polymerase PCR and microchip electrophoresis	*S. typhimurium*	<1000 starting copies of DNA	12 min	detected amplified DNA	Easley et al.[53]
microfluidic integration of nanoplasmonic biosensor composed of a microarray of gold nanohole sensors	*C. trachomatis* and *N. gonorrheae*	*Chlamydia trachomatis*: 300 CFU/mL; *Neisseria gonorrheae*: 1500 CFU/mL	<1 h	simultaneous detection in urine samples	Soler et al.[54]
plastic-chip-based magnetophoretic immunoassay using magnetic and gold nanoparticles modified with *M. tuberculosis* antibodies	*M. tuberculosis*	1.8 pg/mL	1 h	detection in sputum; can differentiate between other mycobacterium; cost < $2 per assay	Kim et al.[57]
glass chip optical analytical system developed by integrating microarray and fabricating gold nanoparticles	avian influenza (H5N1 and H9N2)	1 pg/mL	NR		Park et al.[61]
dual-molecular affinity-based Förster (fluorescence) resonance energy transfer platform using fluorescent vancomycin−gold nanoclusters and aptamer−gold nanoparticles	*S. aureus*	10 CFU/mL	30 min	the limit of detections in the diluted milk, orange juice, and human serum sample for *S. aureus* are 300, 100, and 100 cfu/mL	Yu et al.[59]

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procedures, often a great distance away. Once delivered, lab results are obtained, and the results are sent back, often days or weeks later. After receiving the results, the physician must then contact the patient in order begin proper treatment. The inefficiency of this protocol calls for the development of alternative POC diagnostic methods that may be used in smaller, regional medical clinics that do not have the capabilities for traditional diagnostic techniques. To be effective, these methods must be timely, cost-effective, relatively simple, and reasonably portable.[16]

Recently, a number of diagnostic platforms have been developed to detect pathogenic biomarkers (DNA/RNA, glycoproteins, enzymes, antibodies, etc.) with the aforementioned qualities, such as speed, simplicity, and cost-effectiveness.[1,17] These biosensors are often based on nano- or microtechnology platforms, and they commonly rely on the production of optical, electrical, magnetic, or visually detectable signals. Positive detection signals are produced when the sensors encounter pathogens or pathogen byproducts. While the mechanisms used to produce positive signals vary, the goal remains the same across the board: sensitive and timely detection of infectious pathogens. Many of these platforms are undergoing further optimization for use in POC settings, with the goal of being able to detect the desired biomarker within 1 h. In many countries, patients do not live with nearby access to health care, and it is often not feasible for them to come in on two separate days, one to provide a biological sample and a second time to collect results and begin treatment. Additionally, many live far enough away that they may rarely travel to locations with health care access; therefore, diagnostic techniques must be created that can be brought to the patient. While there are several POC diagnostic tools widely used today in the community and clinics with minimal laboratory capabilities, only a small handful of infectious diseases can be detected currently. These include malaria with Binax NOW, tuberculosis (TB) with Xpert MTB/RIF, and HIV with several different lateral flow assays, among others. Furthermore, the use of smartphone-based diagnostics is increasing such as MobiNAAT recently designed to detect *Chlamydia trachomatis*.[2,18−23]

There are many difficulties in developing POC diagnostics that are realistically applicable. In order to increase the number of effective POC diagnostic tools, additional biosensors must be developed with certain capabilities in mind. First, they must be able to detect biomarkers in trace quantities and in complex media such as blood, sputum, or environmental samples, such as food and/or water. Further difficulties arise when considering the complex pathogenesis undergone by many infectious agents, such as bacteria, viruses, or parasites. Certain pathogens may remain in the blood or sputum longer than others and may only produce certain biomarkers at specific stages in their life cycle. For this reason, optimal biosensors are those that can be customized for more than just one biomarker, allowing them to detect multiple pathogens throughout various stages in their life cycle. Furthermore, POC diagnostic techniques aim to be easily utilized with minimal training in a setting that cannot support a full laboratory.

Despite these significant hurdles, much progress has been made in this field. However, realistic implementation is not always the focus of many developing diagnostics, and it is the goal of this paper to outline various novel POC platforms that may soon move from the lab into clinical settings. The biosensors discussed herein are divided into categories based upon the modality used for detection. The ASSURED criteria are often not reported for diagnostic platforms in early development, so those discussed herein are evaluated on the basis of critical POC characteristics such as level of detection (LOD), timelines, cost, detection capabilities in complex media, and other factors relating to the effectiveness of the platforms in resource-limited settings.

DETECTION MODALITIES

There are many different approaches to developing diagnostic platforms, but in this paper, the focus will be on the development of optical-, electrochemical-, magnetic-, and colorimetric-based modalities. Furthermore, there are many different techniques that fall under the modalities listed, but a few of the most commonly studied techniques are highlighted within each section with everything else grouped under the category of *other*. An outline of the various infectious diseases that have been successfully detected in a number of studies can be found in Tables 1−4, with a few described in further detail in the rest of this work.

OPTICAL DETECTION

Optical-based detection of infectious diseases has been utilized in several attempts to design ideal POC platforms. While this modality has shown great potential, there are several pros and cons to be considered. Optical-based diagnostics rely on the sensitive detection of photon emission from dyes and molecules that are excitable by light. Often, fluorescent probes are designed which will only emit photons after interacting with targeted biomarkers, such as antibodies, antigens, or genomic material.[24−26] Compared to other visualization techniques such as microscopy or enzyme-based detection, fluorescence emission allows increased sensitivity, adding to the reliability of this modality. Additionally, there are a wide variety of molecules that have intrinsic fluorescence qualities, which is one of the benefits of this technique that allows some degree of platform customization. However, intrinsic fluorescence properties of nontarget molecules may also cause background noise and false positives; therefore, this method usually requires somewhat clear sample solutions to prevent interference from other molecules.[24,25] While there are many pros to using optical-based platforms, they often require technology not commonly found in resource-limited settings such as filter fluorometers or spectrofluorometers, which increase the platform cost and complexity, and often limit its application in resource-poor settings.[25,27] However, many POC modalities being developed are overcoming this issue by combining optical-based technologies with smartphones to create more portable technologies.[28] Finally, if this approach is used in immunoassays, many florescence molecules utilized as probes may interfere with the binding of antibodies to the antigen, which could prevent this platform from being used with the detection of certain diseases.[25] Additionally, it is critical to control where the fluorescence molecule binds to prevent the antigen binding site from being occupied by the fluorescence molecule; this can be difficult in many cases.[27]

Optical Detection Utilizing Fluorescence Polarization.

A commonly used fluorescence assay, fluorescence polarization (FP), involves the fluorescent tagging of a probe which is then allowed to interact with target molecules, resulting polarized light emission.[25,27,29−31] FP is preferable to many other fluorescence-based immunoassays because little sample prep-

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Table 2. Information about Electrochemical-Based POC Tests Being Developed Including Technique, Target Pathogen, LOD, Timeliness, and Any Additional Features Mentioned in the Study

technique	target pathogen	LOD	speed	additional features	citation
nanowire field effect transistors using nanowire arrays modified with antibodies for influenza A	influenza and adenovirus	50 virons/microliter	NR	can detect two viruses in parallel	Patolsky et al.[68]
glycan-immobilized field effect transistor (FET) biosensor	influenza	60 H5 HA molecules and 6000 H1 HA molecules in 20 μL samples	NR	can differentiate between H1 and H5	Hideshima et al.[66]
direct potentiometric serological diagnosis using extended-gate field-effect transistor containing MOSFET transducer and gold-coated chip	bovine herpes virus-1	1:100 serum dilution	10 min	detection in serum	Tarasov et al.[70]
portable graphene-enabled biosensor using specific immobilized monoclonal antibody	zika	450 pM	5 min		Afsahi et al.[71]
multiplexed detection of bacterial cells with microfluidic chip using antimicrobial peptides	*S. mutans* and *P. aeruginosa*	1×10^5 CFU/mL	25 min		Lillehoj et al.[74]
bead-based microfluidic chip detection using voltammetry and paramagnetic beads made with CdS quantum dots	influenza (H5N1)	NR	45 min		Krejcova et al.[75]
label-free detection using microfluidic chip integrated with an RGO-based electrochemical immunosensor	influenza (H1N1)	0.5 PFU/mL	15 min		Singh et al.[76]
microfluidic detector which employs a high-gradient magnetic field, on-chip RT-PCR, single-stranded DNA generation, and sequence-specific E-DNA detection	influenza (H1N1)	10 $TCID_{50}$	3.5 h	detection from throat swabs	Hsieh et al.[44]
electrochemical DNA sensor in a microfluidic chip format with loop-mediated isothermal amplification	*S. enterica subsp. enterica* serovars *Typhimurium* and *Choleraesuis*	6.9×10^4 CFU/mL	2 h	detection in whole blood and could differentiate between *S. typhimurium* and *S. cholerasuis*	Hsieh et al.[44]
microfluidic electrochemical platform with loop-mediated isothermal amplification	*Salmonella* genomic DNA	16 copies	50 min	can determine viral load	Hsieh et al.[44]
microfluidic immunosensor with cholera toxin subunit B (CTB)-specific antibodies immobilized onto superparamagnetic beads	cholera toxin	9.0 ng/mL	1.5 h	detection in fecal samples	Bunyakul et al.[77]
differentiation of bacteria using multiplex microfluidic chip with on chip loop-mediated isothermal amplification	*M. tuberculosis* *H. influenza* *K. pneumonia*	28 copies/μL 17 copies/μL 16 copies/μL	45 min	detection in bacteria culture samples	Luo et al.[93]
nanoelectrochemical technique using silver nanoparticles	influenza	single virus	NR		Sepunaru et al.[65]
electrochemical bioassay using colloidal gold nanoparticles	*M. tuberculosis*	1 CFU	90 min	can be done using a portable potentiostat	Ng et al.[64]
electrochemical aptasensor developed using coil-like fullerene-doped polyaniline, gold nanoparticles, and aptamers	*M. tuberculosis* (MPT64)	20 fg/mL	NR	detection in human serum	Bai et al.[82]
electrochemical immunoassay using layer-by-layer coating carbon nanotubes and graphene oxide as a nanocarrier	*C. difficile* (toxin B)	0.7 pg/mL	45 min	detection in human stool samples	Fang et al.[83]
electrochemical capacitive sensing	dengue, zika, and chikungunya (NS1)	0.5 ng/mL	NR	detection in serum	Cecchetto et al.[84]
impedimetric glycan-based biosensor	influenza H3N2	13 viral particles in 1 μL	NR	differentiation shown between H3N2 and H7N7	Hushegyi et al.[85]
label free electrochemical immunosensor using an anti-ESAT-6 monoclonal antibody	*M. tuberculosis* (ESAT-6)	7 ng/mL	NR	can discriminate between culture filtrate proteins from pathogenic mycobacteria strains and BCG nonvirulent mycobacteria vaccine strain	Diouani et al.[95]
electrochemical DNA hybridization biosensor using a gold nanotube array electrode platform	*M. tuberculosis*	0.05 ng/μL	NR		Torati et al.[89]
electrochemical detection using multifunctional nanoconjugates and subsequent signal amplification	*E. coli* O157:H7 *V. cholera* O1	39 CFU/mL 32 CFU/mL	NR	can detect 2 types of bacteria simultaneously	Li et al.[90]
label-free biosensor using faradaic electrochemical impedance spectroscopy	*E. coli*	1×10^2 to 1×10^3 CFU/mL	30 min		Guo et al.[87]

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Table 2. continued

technique	target pathogen	LOD	speed	additional features	citation
electrochemical detection using allosteric enzymes and coulometry	anti-HIV antibodies	4 μM	1 h	detection in serum	Laczka et al.[91]
label-free electrochemical sensor using streptavidin-coated magnetic beads	HIV-1 (subtypes A, B, C, D, E, and G)	1.17×10^8–8.39×10^8 copies/mL	30 min		Shafiee et al.[86]
thin-film transistor nanoribbon sensor	*E. coli* and *K. pneumoniae*	<10 copies of genomic DNA	5 min		Hu et al.[92]

aration is needed.[27,32] This assay was utilized in several studies to detect influenza,[33] *Mycobacterium tuberculosis*,[34−36] *Salmonella spp.*,[30] and *Brucellosis spp*.[37] Walker et al. was able to detect *M. tuberculosis* in about 3 h at levels as low as 1 TB genome. Later, they were able to make this test more rapid but slightly less sensitive and were able to detect 10 TB genomes in 20 min.[34,36] FP was utilized by Park et al.[30] for specific detection of *Salmonella spp.*, as well as differentiation between different subtypes of *Salmonella* such as *S. typhimurium* and *S. paratyphimurium*. In blood samples, the bacterial detection reached a sensitivity of about 1 CFU/mL in less than 3 h. They utilized a PCR-based approach with DNA polymerase, complementary DNA to the target STY3007 and STY1121 genes in *Salmonella spp.*, and a reporter probe conjugated with a single fluorophore. When mixed with a sample of *Salmonella spp.*, the complementary DNA bound to the target genes and the free reporter probe emitted a high signal in an FP assay. On the other hand, when the target *Salmonella spp*. genes were not present in the sample, the DNA polymerase catalyzed the cleavage of the fluorophore from the reporter probe, resulting in low values in the FP assay. They calculated the cost of this test to be about $1 per test. This rapid and low-cost design shows great potential for use as a POC platform. However, because this assay requires the detection of intracellular components, additional sample preparation steps are required, such as DNA extraction in this case.

Optical Detection Utilizing Quantum Dots. Quantum dots (QDs) are very small nanoparticles that act as semiconductors that have strong fluorescence properties. QDs have been utilized to develop infectious disease diagnostic platforms in several studies.[38−40]

Takemura et al.[41] demonstrated a detection limit of 0.4pg/mL for H1N1 influenza utilizing CdSeTeS QDs. In another study done by Ming et al.,[42] a quantum dot-based detection platform was combined to develop a portable smartphone diagnostic tool for HIV and hepatitis B with a detection limit of 1000 viral genetic copies/mL and in less than 1 h.

Another interesting application of fluorescence-based detection is demonstrated by Xiong et al. Functionalized *Staphylococcus aureus* cells with fluorescence were turned into nanobioprobes capable of targeting and detecting pathogens.[43] Targeting is made possible through interactions between protein A, which is expressed on the surface of *S. aureus*, and the Fc region of selected monoclonal antibodies (mAbs). Since this assay targets surface proteins, minimal sample preparation is need. These mAbs may be used to target any selected virus or bacteria; however, this study was conducted by specifically detecting H9N2 avian influenza virus. The conjugation of antibodies to the surface of these cellular nanoprobes does not require conjugation chemistry, as the Fc region of the antibodies and protein A have a strong binding affinity. Addition of the fluorescent modality is accomplished by synthesizing $CdS_{0.5}Se_{0.5}$ within the cell. When mixed with targeted viral glycoproteins, these nanobioprobes and immunomagnetic beads formed a sort of "sandwich" around the viral glycoprotein. The magnetic beads allow for the capture of the pathogen while the nanoprobes allow for its detection and subsequent quantification. As the concentration of the targeted viral glycoprotein decreased, the fluorescent signal responded accordingly, and the detection limit determined using this method was 8.94 ng/mL of targeted viral glycoprotein. The true strength of this technique is its ability to be easily targeted against multiple pathogens, due to the simplicity with which

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Table 3. Information about Magnetic-Based POC Tests Being Developed Including Technique, Target Pathogen, LOD, Timeliness, and Any Additional Features Mentioned in the Study

technique	target pathogen	LOD	speed	additional features	citation
magnetic relaxation nanosensor detection using ironoxide nanoparticles	influenza (H5N1 and H1N1)	1 nM	<30 min	differentiation shown between H5N1 and H1N1	Shelby et al.[116]
magneto-fluorescent nanosensors detection ultilizing changes in T2 relaxation time	*E. coli*	1 CFU	30 min		Banerjee et al.[55]
magnetoresistive biosensor	BCG bacteria	1×10^4 cells/mL	NR		Barroso et al.[109]
giant magnetoresistance biosensor using monoclonal antibodies and magnetic nanoparticles	influenza	1.5×10^2 $TCID_{50}$/mL virus	1 min		Krishna et al.[103]
detection using loop-mediated isothermal amplification, AC susceptometry, and magnetic nanoparticles	zika virus oligonucleotide	1 aM	27 min	detection in 20% serum; can use a portable AC susceptometer	Tian et al.[110]
AC magnetic field detection method using oligonucleotide-tagged magnetic beads	*V. cholera* DNA	10 pM	60 min		Bejhed et al.[111]
blu-ray optomagnetic detection	*S. typhimurium* and *E. coli*	8×10^4 CFU/mL	3 h	can simultaneously detect *S. typhimurium* and *E. coli*	Tian et al.[112]
detection using a hand-held diagnostic magnetic resonance system	*S. aureus*	10 bacteria in a 10 μL sample	<15 min		Lee et al.[113]
microchip-based sensor using a magnetic bead bioassay platform	dengue (anti-dengue virus IgG)	100 pg/mL	NR		Aytur et al.[115]
magnetic barcoding detection by nuclear magnetic resonance	*M. tuberculosis*	1×10^3 cells/mL	2.5 h	one-time equipment cost: $4300; per assay: $3; detection in spiked sputum	Liong et al.[114]

mAbs may be targeted to the *S. aureus* surface with no need for conjugation chemistry. These nanobioprobes also provide an approach to biosensor development using a functionalized organism, reducing environmental risks of toxicity often encountered when using inorganic nanomaterials. However, because these nanobioprobes are created with live bacteria, it may be important to handle them with caution. Also, due to the potential mutations and variation in bacteria, the consistency of these products may be reduced.

Optical Detection Utilizing Microfluidic Platforms. Several other studies have utilized optical-based detection methods along with a microfluidic platform. Microfluidic technology has allowed for the creation of disposable, miniaturized devices that combine various steps into a compact space. Microfluidic devices can be customized to do DNA amplification, sample preparation, and detection all in one chip.[17,40,44−46] Researchers have demonstrated the detection of *Escherichia coli*,[47,48] *S. aureus*,[47] BK virus,[49] dengue,[50−52] avian influenza virus,[46] *Streptococcus pneumoniae*,[51] and *S. typhimurium*.[53] Furthermore, one study utilized optical properties along with microfluidic technology to detect *Chlamydia trachomatis* and *Neisseria gonorrheae* at levels as low as 300 and 1500 CFU/mL, respectively.[54] Iswardy et al.[50] utilized a dielectrophoresis chip with antiflavivirus antibody coated bead to detect dengue virus. They reached detection times of about 5 min, and the chip was reusable for more than 50 times. This rapid and low-cost platform demonstrates potentials of this microfluidic chip as a POC diagnostic tool.

Optical Detection with Other Nanotechnologies. Some studies are combining nanotechnology with fluorescent molecules to create diagnostic platforms as was done in the study by Banerjee et al.[55] to detect *E. coli*. Other studies utilize the inherent optical properties, surface plasmon resonance (SPR), of gold nanoparticles (AuNPs).[39,56] Diagnositic techniques utilizing AuNPs have been studied for the detection of *M. tuberculosis*,[57] influenza,[58] and *S. aureus*.[59] In the study done by Yu et al.,[59] a fluorescence resonance energy transfer platform was utilized to detect *S. aureus* as low as 10 CFU/mL in under 30 min.

ELECTROCHEMICAL DETECTION

Electrochemical detection is currently being studied for potential POC diagnosis of infectious diseases. This is one of the most common modalities being researched and developed due to the potential for miniaturization, portability, and cost reductions.[26,27] The glucometer is the most common example that utilizes this modality.[62,63] Electrochemical detection modalities utilize biosensors that are capable of binding to a target molecule associated with the disease. When these probes bind to the target molecule, they undergo a conformational change and create a small current that can be detected. Unlike fluorescence-based detection, this modality is more easily utilized with nonclear samples such as blood.[24] Additionally, electrochemical-based detection does not require complex optical instrumentation that is used in many fluorescence-based detections. Electrochemical-based detection often uses instrumentation such as carbon electrodes and field effect transistor (FET) biosensor along with electrochemical analysis methods such as with differential pulse voltammetry (DPV) platforms. Some instrumentation has been made very portable, such as the hand-held potentiostat. While these methods can be initially costly and complicated to utilize, the technology can evolve into more miniaturized and easy to use platforms as the development progresses.[24,44,57,64−67]

Electrochemical Detection Utilizing Field Effect Transistors. Additionally, various strains of avian influenza were detected using FET sensors with great success.[66,68] The FET method has shown promise due to its portability, sensitivity, and ease of use.[69] Hideshima et al.[66] developed a FET biosensor capable of detecting viral proteins within the attomolar concentration. Ultimately, the developed FET biosensor was able to detect and differentiate between H1 and H5 in attomolar concentrations, which means that this device is capable of detecting a single viral particle, since each viral particle displays roughly 500 hemagglutinin particles on its surface. Bovine herpes virus-1 was detected using FET biosensors in under 10 min; this method could be utilized with other infectious diseases.[70] Furthermore, Afsahi et al.[71]

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Table 4. Information about Colorimetric-Based POC Tests Being Developed Including Technique, Target Pathogen, LOD, Timeliness, and Any Additional Features Mentioned in the Studies

technique	target pathogen	LOD	speed	additional features	citation
lateral flow test using nitrocellulose membrane and adsorbed onto colloidal gold particles	*M. tuberculosis*	NR	15 min	sensitivity and specificity: >90%	Gonzalez et al.[122]
lateral flow assay which pairs a nucleic acid aptamer with an antibody	influenza	2×10^6 viral particles	15 min	can differentiate between subtypes	Le et al.[123]
smartphone-based fluorescent diagnostic device	influenza	H7N1: 5.34×10^2 PFU/mL; H9N2: 1.37×10^1 PFU/mL	15 min	H5N1 sensitivity: 96.55%; specificity: 98.55%	Yeo et al.[124]
lateral flow biosensor using loop-mediated isothermal amplification and gold nanoparticles	*E. faecalis* *S. aureus*	710 CFU/mL 680 CFU/mL	75 min	detection in spiked blood samples	Wang et al.[125]
displacement amplification label-based gold nanoparticle lateral flow biosensor	*L. monocytogenes*	10 fg of genomic templates per reaction in pure culture	1 h	detection in pork samples; one time equipment cost: $600 USD; per assay: $6	Wang et al.[126]
lateral flow assay using label-based gold nanoparticles with a multiple cross displacement amplification technique	*Shigella*	10 fg of genomic templates per reaction in pure culture and 5.86 CFU per tube in human fecal samples	1 h	detection in human fecal samples	Wang et al.[127]
multiplex LAMP label-based lateral flow dipstick biosensor using gold nanoparticles	*Leptospira*	$3.95 \times 1 \times 10^{-1}$ genomic equivalent per reaction	1 h		Nurul Najian et al.[129]
lateral flow assay using multicolored silver nanoparticles	dengue, yellow fever, and ebola virus	150 ng/mL	NR	can differentiate between dengue, yellow fever, and ebola viruses	Yen et al.[128]
naked-eye detection of nucleic acids using isothermal amplification and magnetic particle mediated aggregation	human papillomavirus	5.1 amol	60−120 min	detection in blood sample	Lin et al.[130]
label-free dual fluorescence for the visual product detection isothermal multiple-self-matching-initiated amplified DNA	hepatitis B virus	10 copies of DNA	NR		Ding et al.[131]
visual gene-detecting technique using nanoparticle gene probes and silver staining enhancement	hepatitis B and C viruses	hepatitis B: 3.6×10^{-11} M; hepatitis C: 3.6×10^{-10} M	3 h		Wang et al.[132]
colorimetric assay that utilizes the chemical oxidation of 3,3′,5,5′-tetramethylbenzidine by isothermally functionalized DNA targets	*M. tuberculosis*	1 CFU	75 min	cost: $3 per assay	Ng et al.[133]
probe-based colorimetric assay using gold nanoparticles	*M. tuberculosis*	0.75 μg of DNA	2 h	cost: $0.35 per sample; detection in sputum, bronchial washes, pleural effusion, urine, and blood	Baptista et al.[134]
dipstick visual detection using gold nanoparticles and aptamers	influenza	3×10^8 viral particles	NR		Le et al.[135]
multiplexed kit capable with target-specific fluorescently tagged strand displaceable probes with RT-LAMP	zika dengue chikungunya	~0.71 pfu equivalent viral RNAs ~1.22 pfu equivalent viral RNAs ~38 copies of viral RNA	30 min	detection in urine and plasma	Yaren et al.[136]
immunochromatography dipstick test using monoclonal antibodies	dengue and zika	NR	1 h	distinguishes between the four dengue serotypes and zika; detection in serum; dengue sensitivity/specificity: ranges from 76% to 100%; zika sensitivity/specificity: 81/86%	Bosch et al.[139]

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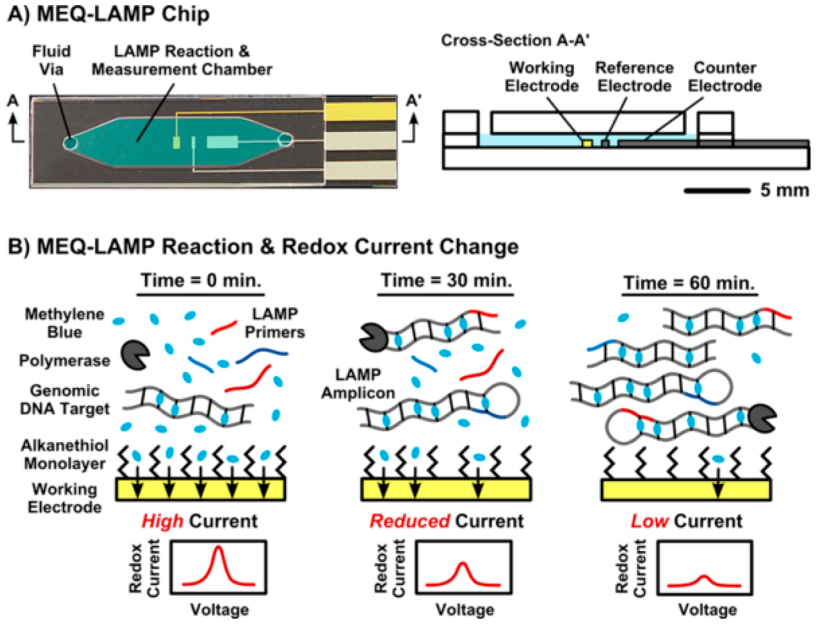

Figure 1. (A) Representation of the MEQ-LAMP chip used by Hsieh et al. for the detection of *Salmonella*.[44] (B) The Methylene Blue molecule in this electrochemical setup allows for real-time detection of the LAMP reaction. Reprinted with permission from ref 79. Hsieh, K., Patterson, A. S., Ferguson, B. S., Plaxco, K. W., and Soh, H. T. (2012) Rapid, sensitive, and quantitative detection of pathogenic DNA at the point of care through microfluidic electrochemical quantitative loop-mediated isothermal amplification. *Angew. Chemie-Int. Ed. 51* (20), 4896−4900. Copyright 2012 John Wiley and Sons.

utilized similar FET methods to detect zika with concentrations as low as 450 pM in about 5 min.

Electrochemical Detection Utilizing Microfluidic Platforms. Microfluidic chips that utilize electrochemistry have become a popular choice for the development of POC diagnostic tools due to their sensitivity, portability, speed, and ease of use.[63,72−79] Several studies have utilized a microfluidic platform along with electrochemistry to detect *Streptococcus mutans* and *Pseudomonas aeruginosa*,[74] several strains of avian influenza,[44,75,76] *Salmonella spp.*,[44] cholera toxin,[77] *M. tuberculosis*, *Haemophilus influenza*, and *K. pneumonia*.[78] In the study done by Hsieh et al.,[44] researchers utilized a DNA probe with a redox reporter bound to a microfluidic chip. When complementary target DNA binds to the DNA probe, it undergoes a conformational change causing a change in the redox rate of the reporter which creates a current that can be detected. This biosensor cannot detect DNA levels naturally found in the blood, so PCR or loop-mediated isothermal amplification (LAMP) is necessary to amplify the DNA. This study integrated LAMP into a microfluidic chip to create a microfluidic electrochemical quantitative LAMP (MEQ-LAMP) device shown in Figure 1, in order to decrease cost and detection time in addition to decreasing the instrumentation needed for the detection. The MEQ-LAMP chip successfully detected *Salmonella spp.* at levels as low as 16 copies of DNA in under 50 min. Combining the preparation, amplification, and detection in one multiplex platform is a step toward an ideal POC platform.

Electrochemical Detection Utilizing Nanotechnology. Several studies utilized nanotechnology along with electrochemistry to increase sensitivity levels in the detection of several infectious diseases.[80,81] These methods have been utilized to detect influenza,[65] *M. tuberculosis*,[64,82] and *Clostridium difficile*.[83] One study utilized electrical signals to detect influenza virus particles using silver nanoparticles and was designed by Sepunaru et al.[65] Silver nanoparticles are first adsorbed onto the virus surface when present together in solution. Upon the addition of a carbon electrode to the solution, current spikes are observed which correlate with the oxidation of the adsorbed nanoparticles. This method produces current spikes which are proportional in magnitude to the concentration of the virus in solution and may therefore be used to quantify viral levels as well as detect the pathogen. While this method is not able to determine the specific viral species, it is able to differentiate between viral and bacterial infections in a simple, POC applicable manner.

Another example of the utilization of electrochemical-based detection of *M. tuberculosis* with AuNPs is demonstrated by Ng et al.[64] This assay took advantage of the high binding affinity between streptavidin (SA) and biotin. *M. tuberculosis* DNA was extracted from *M. tuberculosis* cells and then isothermally amplified with biotin dUTPs to create a biotinylates product and then placed on an SA-coated disposable screen-printed carbon electrode (SPCE). AuNPs are conjugated with streptavidin and washed with BSA/PEG buffer; the BSA binds to any areas left exposed on the AuNPs to prevent unwanted interactions. Finally, these conjugated AuNPs are added to the SPCE, and the more AuNPs are bound to the SPCE, the higher is the DPV signal indicating a positive result. Concentrations as low as 1 CFU of *M. tuberculosis* DNA were detected. Additionally, this assay was tested with a hand-held potentiostat which can be used at POC sights because it can be powered by a laptop. The total cost per assay was about $10, and it could be completed in under 90 min.

Other Electrochemical Detection Methods. Impedance-based electrochemical biosensors were used to detect Flaviviruses such as dengue and zika,[84] influenza,[85] HIV,[86] and *E. coli*.[87] Various electrochemical methods were also utilized to detect several bacterial strains such as *M. tuberculosis*,[88,89] *E. coli*,[90] and *Vibrio cholera*.[90] Another study utilized

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various electrochemical methods to detect HIV using microelectrode arrays.[91]

While detecting infectious diseases has been on the front lines of many research studies, identifying infectious disease resistance is just as important. In one study, thin film transistor sensors were developed by Hu et al.[92] to detect antimicrobial resistance genes for *E. coli* and *Klebsiella pneumoniae*. This method detected 100 copies of the genomic DNA in under 3 min.

MAGNETIC DETECTION

Magnetic detection of infectious disease agents[94] utilizing magnetic nanoparticles (MNPs) is a growing field. This approach usually utilizes binding affinities between conjugated antibodies on MNPs and the target pathogen. There are two popular uses of MNPs for the detection of infectious diseases. The first utilizes the magnetic relaxation properties of the MNPs when they interact with water. MNPs conjugated with antibodies are placed in solution with the target pathogen and bind to one another, causing aggregates. This aggregation alters the spin−spin−spin relaxation time (T2) of the surrounding water protons which can be quantified by a magnetic resonance spectroscopy machine.[4,96−100] The second utilizes magnetoresistive sensors (Figure 2). In this method, the antibody is

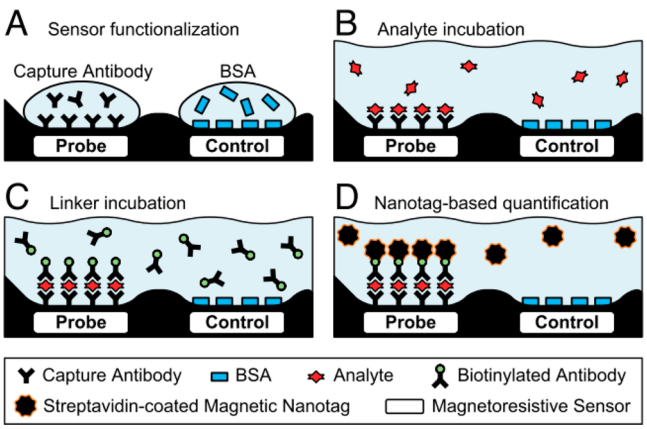

Figure 2. Sandwich type immunoassay-based magnetoresistive sensors which can be utilized to detect various infectious diseases. (A) Antibody is fixed to a surface. (B) Analyte is washed over and allowed to bind to antibody. (C) Biotinylated antibody is washed over to create a sandwich of the analyte. (D) Magnetic nanotags bind to the biotinylated antibody to allow for quantitative detection. Reprinted with permission from ref 101. Osterfeld, S. J., Yu, H., Gaster, R. S., Caramuta, S., Xu, L., Han, S.-J., Hall, D. A., Wilson, R. J., Sun, S., White, R. L., Davis, R. W., Pourmand, N., and Wang, S. X. (2008) Multiplex protein assays based on real-time magnetic nanotag sensing. *Proc. Natl. Acad. Sci. 105* (52), 20637−20640. Copyright 2008 National Academy of Sciences, U.S.A.

fixed to a surface and the sample with the pathogen antigen is washed over it, resulting in binding. Then, MNPs conjugated with the antibody are washed over the same surface and bind to the antigen as well. This second binding of the MNPs alters the magnetic field and gives off an electrical signal that can be detected.[38,96,101,102] Many of these assays are very rapid and take less than an hour for detection. Magnetic-facilitated detection is especially appealing for POC settings due to the nanoparticle's ability to be manipulated in a magnetic field, which can decrease sample preparation time by utilizing magnetic field separation. Additionally, magnetic nanoparticle detection can provide signals in opaque samples such as blood, making this modality superior to light- and visual-based modalities in these cases. Furthermore, biological samples usually do not have ferromagnetism properties. This leads to very minimal background noise, which is commonly found with florescence detection methods.[103] One drawback to most magnetic nanoparticles such as iron oxide MNPs is that they do not possess intrinsic photophysical properties that many other nanoparticles possess, such as gold.[38,104] While the assays are relatively inexpensive to run, the instrumentation needed is not very portable and can be expensive.[38]

While magnetic-based infectious disease detection methods are less commonly studied, there have been a few promising studies conducted that have successfully detected several common infectious diseases, and many of these studies can be adapted to detect many other types of infectious diseases as well.

Magnetic Nanoparticles. Magnetic nanoparticles have been utilized in several studies to create potential POC diagnostic platforms for the detections of *E. coli*,[55,105] influenza,[106] *S. typhimurium*, *S. aureus*,[105] and anthrax.[107]

Using superparamagnetic iron oxide nanoparticles, Shelby et al.[106] designed biosensors capable of detecting and differentiating between influenza glycoproteins. The nanoparticle platform used in this approach is based upon a magnetic iron core surrounded by a poly(acrylic acid) coating. This coating provides stability in addition to the ability to functionalize the nanoparticle via conjugation of targeting molecules, such as mAbs. These molecules allow the nanoparticle to interact in a specific fashion with the desired pathogenic targets, which can then be observed by the collection of sensitive T2 data. In one study, these magnetic relaxation nanosensors (MRnS) were used to detect influenza glycoproteins (H1 and H5) in the nanomolar concentration. In addition, MRnS were able to differentiate between these glycoprotein variants and could therefore be used to distinguish between influenza subtypes. Furthermore, detection of viral contaminants was accomplished within 30 min and is simple and relatively portable, requiring only a benchtop magnetic relaxometer for sample analysis. The MRnS are also stable and cost-effective to produce, making this approach a worthy candidate for POC diagnostics.

In an additional study conducted by Banerjee et al.,[55] this same iron oxide platform was further functionalized with a fluorescent modality, allowing for the detection of pathogenic *E. coli* using both magnetic and fluorescent analysis. In this approach, the poly(acrylic acid) coating was loaded with fluorescent dyes. Following the collection of magnetic relaxation data, bacterial pellets are isolated via centrifugation, resuspended, and analyzed via fluorescence as shown in Figure 3. These magneto-fluorescent nanosensors (MFnS) were able to detect as little as 1 CFU of the desired pathogen (*E. coli* O157:H7) and were able to distinguish between other *E. coli* species, as well as heat-inactivated *E. coli* O157:H7. To further test the realistic effectiveness of MFnS, they were tested in complex media such as milk and lake water and produced similar results, detecting as little as 1 CFU of the desired pathogen within 30 min.

Magnetoresistive Sensors. Huang et al.[108] have been working on developing an immunoassay-based POC diagnostic platform with a giant magnetoresistive biosensor. This technique is also being utilized for the diagnosis of TB with a detection limit of 104 cells/mL of BCG bacteria.[109] Krishna et al.[103] designed a magnetoresistive platform capable of detecting

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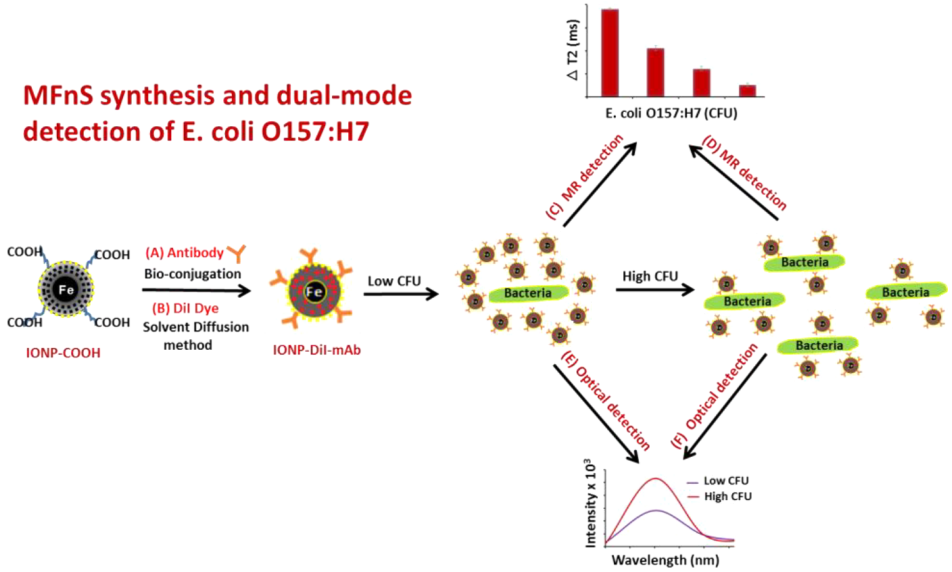

Figure 3. Iron oxide nanoparticles functionalized into MFnS with conjugated antibody and DiI dye when incubated with bacteria allows for the quantitative detection of the bacteria through magnetic resonance and optical detection. Reprinted with from ref 55. Banerjee, T., Sulthana, S., Shelby, T., Heckert, B., Jewell, J., Woody, K., Karimnia, V., McAfee, J., and Santra, S. (2016) Multiparametric Magneto-fluorescent Nanosensors for the Ultrasensitive Detection of *Escherichia coli* O157:H7. *ACS Infect. Dis.* 2 (10), 667−673. Copyright 2016 American Chemical Society.

influenza A virus. A magnetoresistive biosensor chip was designed and functionalized to easily bind amino groups. Influenza A antibodies were fixed to the biosensor, and a sample with influenza A virus was added and allowed to bind. Next, biotinylated antibody was added and allowed to bind to the antigen, and the MNPs were washed over the top and bound to the biotin. Therefore, the number of MNPs was proportional to the amount of influenza A present. A benchtop system was used to detect the changes in magnetic field through a current. This assay detected viral concentrations as low as 1.5×10^2 $TCID_{50}$/mL, which is more sensitive than ELISA. Furthermore, the magnetoresistive chip contains 64 sensor arrays which would allow the potential to conjugate various antibodies and detect different subtypes of virus or even various pathogens in a single assay. While the assay requires time and is somewhat complicated to perform, Krishna et al.[103] foresees the possibility of the assay being further developed into a hand-held device.

Other Magnetic-Based Detection Methods. Although not as common in infectious disease diagnosis, AC susceptometry has been utilized in some studies. AC susceptometry along with LAMP was utilized by Tian et al.[110] to detect synthetic zika oligonucleotides at levels of 1 aM in 20% serum in about 27 min.

Additionally, there have been several other optomagnetic studies that have been demonstrated as well. The detection of *V. cholera* was demonstrated by Bejhed et al.[111] detecting levels of 5 pM in under 20 min. Finally, in another study done by Tian et al.,[112] *Salmonella* was detected using a blu-ray optomagnetic method at levels as low as 8×10^4 CFU/mL in under 3 h.

Kim et al.[57] utilized both magnetic properties and photophysical properties of nanoparticles to detect TB. Magnetic microparticles (MMPs) bought from Invitrogen and synthesized AuNPs were conjugated with two different antibodies that recognize the CFP-10 antigen found in the early stages of TB. The conjugated MMPs and AuNPs aggregated with the CFP-10 antigen to create a type of sandwich, which could then be removed using a neodymium−iron−boron magnet. The absorbance of the remaining AuNPs was then inversely correlated with the concentration of CFP-10 antigen present. Levels as low as 1.8 pg/mL were detected using this method in under an hour. This level of sensitivity and speed, along with the assay's ease of operation and low cost ($2 per assay), make this platform another potential candidate for ideal POC detection. Furthermore, the instrumentation used to run these assays costs roughly $300, which is relatively cost-effective when compared to other laboratory equipment start-up costs.

Finally, a magnetic-based diagnostic technique for *S. aureus*[113] was created with detection levels as low as ten bacteria in a 10 μL sample in under 15 min. Other magnetic-based detection methods for infectious diseases such *M. tuberculosis*[57,114] and dengue[115] have also shown promising results with a level of detection of 1×10^3 cells/mL for *M. tuberculosis* and 100 pg/mL for dengue.

■ COLORIMETRIC AND LATERAL FLOW TESTS

One of the main goals of POC diagnostic research is the development of assays that do not rely on microscopes, relaxometers, a consistent supply of electricity, or expensive and bulky machinery. Toward this end, a number of assays have been developed that ideally allow the collection of detection results using the naked eye or minimal detector technologies and often rely on colorimetric modalities or sedimentation. Lateral flow assays (LFAs) are one such example and have been utilized for pregnancy tests and HIV tests. For this test, a nitrocellulose, nylon, paper, or other composite membrane is usually used in an antigen capture assay. These membranes are capable of capillary action and, therefore, are self-sufficient. Labeled antibodies are bound to the membrane, and the antigen sample is allowed to diffuse along the membrane. When the antigens bind to the labeled antibodies, this results in a color change in the colorimetric molecule being used. This change can be visualized with the naked eye or with minimal assistance. These types of diagnostic techniques are very

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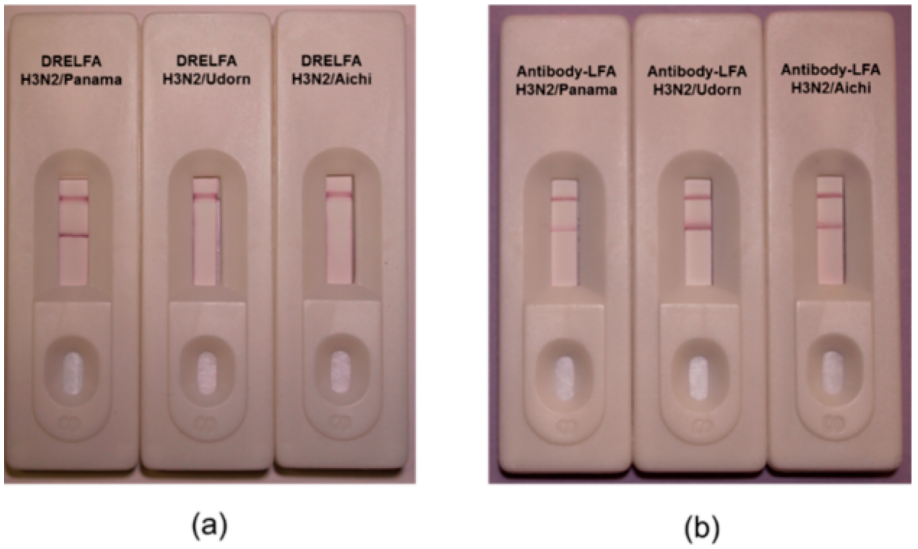

Figure 4. (a) H3N2/Panama was detected using DRELFA but did not cross react with the subtypes H3N2/Udorn and H3N2/Aichi, unlike (b) the antibody-LFA which cross reacted with the other subtypes. Reprinted from ref 123. Le, T. T., Chang, P., Benton, D., John, W., Iqbal, M., Edward, A., and Cass, G. (2017) Dual Recognition Element Lateral Flow Assay (DRELFA) Toward Multiplex Strain Specific Influenza Virus Detection. *Anal. Chem. 89* (12), 6781−6786. Copyright 2017 American Chemical Society, under open access license CC-BY.

attractive because they often utilize very little to no instrumentation, are cost-effective and rapid, and can be easily interpreted with minimal training. On the down side, this technique is solely quantitative and cannot indicate the severity of the infection.[4,25,117,118]

Lateral Flow Detection. While there is a current urine lateral flow test on the market used to detect active TB in HIV-positive adults, it lacks required levels of sensitivity to be fully effective in clinical scenarios.[119−121] In a different study, Gonzales et al.[122] developed a lateral flow test that may be more applicable to POC settings using immunodominant epitopes derived from highly immunogenic *M. tuberculosis* cell-wall proteins. In this technique, peptides were conjugated to BSA and were used along with colloidal gold particles on a nitrocellulose membrane to detect antibodies of TB patients. The prototype utilized a combination of 3 different peptides and had a specificity greater than 90%. This assay was able to determine positive/negative status within roughly 15 min and had minimal reaction toward latent TB and BCG vaccinated patients.

Le et al.[123] continued to develop their avian influenza detection into a lateral flow test prototype. They were able to differentiate between various strains of H3N2 and detect levels as low as 2×10^6 virus particles utilizing the more specific aptamers rather than antibodies along with gold nanoparticles in their dual recognition element lateral flow assay (DRELFA) approach. They compared this approach to the antibody-based LFA to show the superiority of DRELFA to the more common LFA approach (Figure 4). The high portability and ease of use of a lateral flow test makes this a potential POC diagnostic test.

Furthermore, a study conducted by Yeo et al.[124] demonstrated the use of a lateral flow assay in conjunction with a lightweight fluorescence reader connected to a smart phone for the detection of H5N1 avian influenza. A nitrocellulose strip was prepared with anti-influenza antibodies fixed to the test line on the membrane, and antimouse IgG antibodies were fixed to the control line. A bioconjugate was also created which consisted of anti-influenza antibodies conjugated to coumarin-derived dendrimers, which acted as the fluorophore. The bioconjugate and the sample with H5N1 viral particles were allowed to diffuse down the strip, and the H5N1 particles bound to the test line while the bioconjugate bound to the H5N1, creating a sandwich type interaction. This assay is demonstrated in Figure 5. The fluorescence intensity on the test and control lines was then quantified using the smart

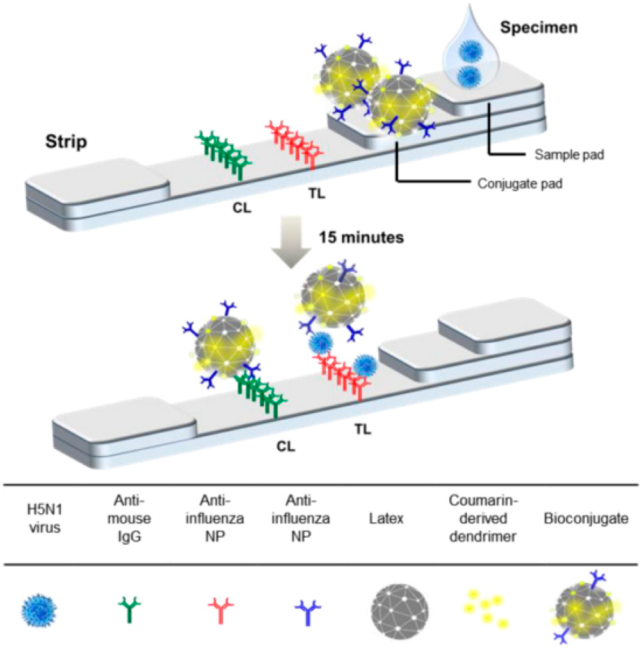

Figure 5. Visual representation of a lateral flow type assay utilized to detect H1N1. The specimen is allowed to flow down the strip, and the bioconjugate binds to the viral particles while the viral particles bind to the test line, creating a sandwich type interaction. Reprinted from ref 124. Yeo, S., Choi, K., Cuc, B. T., Hong, N. N., Bao, D. T., Minh, N., and et al. (2016) Smartphone-Based Fluorescent Diagnostic System for Highly Pathogenic H5N1 Viruses. *Theranostics 6* (2) 231−242. Copyright 2016 Ivyspring International Publisher, under open access license CC BY-NC.

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phone detector. This assay reached sensitivity levels of 97% and detected H5N1 in about 15 min. In addition to these rapid and sensitive characteristics, the high portability of this device makes it a potential POC tool for the diagnosis of H5N1.

Utilizing lateral flow assays, several studies were able to detect several other infectious diseases such as *Enterococcus faecalis*, *S. aureus*,[125] *Listeria monocytogenes*,[126] *Shigella*,[127] dengue, yellow fever, ebola,[128] and *Leptospirosis*.[129] The *E. faecalis* and *S. aureus* lateral flow tests were successfully developed by Wang et al.[125] which could be utilized in about 75 min, detecting levels as low as 710 CFU/mL for *E. faecalis* and 680 CFU/mL for *S. aureus*.

Other Colorimetric Detection Methods. In addition to lateral-flow assays, aggregation and sedimentation-based diagnostic techniques also allow for naked-eye detection. These methods often involve the use of particles which change in color when aggregated, such as gold nanoparticles, or the use of natural coagulants which result in visual sedimentation. Some such studies have developed rapid visual detection of bacteria and viruses such as enterovirus 71,[130] hepatitis B,[131,132] hepatitis C,[132] papillomavirus,[130] *M. tuberculosis*,[122,133,134] avian and human influenza,[124,135] zika, chikungunya, and dengue.[136] Another study utilized engineered phages for colorimetric detection of *E. coli*.[137]

One study visually detected *M. tuberculosis* in 2 h with a cost of $0.35 per test.[134] Furthermore, Driskell et al.[138] utilized a similar technique using AuNPs with dynamic light scattering to detect the influenza virus <100 $TCID_{50}$/mL in under 30 min. Another detection method for influenza was developed by Le et al., who developed a platform utilizing gold nanoparticles that allowed for the visual detection of as little as 3×10^8 viral particles.[135] The gold nanoparticles were assembled with RNA aptamers that allowed for specific affinity with different strains of human influenza. Aptamers were selected in place of targeting mAbs due to cost effectiveness, as they are 1/1000th the price per molecule. In the presence of viral contaminants, the functionalized nanoparticles form a gold nanoshell (AuNS) around the virus. This increase in mass allows for the sedimentation of viral contaminants, which can be facilitated with a portable mini-centrifuge. After 6−10 min of centrifugation at 2000*g*, visual sedimentation of nanoparticle-coated virus was reported within the range of the typical viral load in 1 mL of human respiratory specimens.

While there are tests being developed to detect flaviviruses,[136] there have been problems with differentiating between the various flaviviruses because of their structural similarities. Bosch et al.,[139] however, recently developed a dipstick type detection method to not only detect but also differentiate between zika and dengue in an assay that takes about 20 min.

Another unique POC diagnostic tool being developed uses not visual detection methods but olfactory. This study utilized AuNPs and fragrance substrate molecules to detect *E. coli* by emission of a smell. Levels as low as 1×10^2 CFU/mL were able to be detected in about 15 min.[140]

DISCUSSION AND CONCLUSION

As the need for rapid and accurate POC diagnostic tools continues to become more apparent, many platforms are being developed utilizing various methods and modalities. While many demonstrate impressive capabilities to detect pathogens or biomarkers in a controlled lab environment, it is crucial to consider the characteristics required for realistic implementation throughout the entire development process. Each modality has its own pros and cons, but with careful planning, they all have the potential to facilitate successful POC diagnosis.

POC platforms utilizing optical modalities have a great deal of potential for customization due to the large numbers of fluorescent molecules available. This modality has been used in many of the platforms discussed throughout this Review; however, it also faces some limitations, such as the sample preparation required to eliminate background noise from the intrinsic florescence properties many molecules have. The goal of POC diagnostic tools is to use raw samples such as blood, serum, or urine with minimal to no preparation, so florescence-based platforms are often not ideal in this aspect. Additionally, the benchtop technologies required are costly and require trained personal, further limiting its application in resource-limited settings where equipment is not easy to acquire. The ultimate goal of POC diagnostic technologies is to allow untrained personnel to easily utilize it in a portable manner.

Electrochemical POC diagnostic technology development has the most potential for miniaturization and portability, but its development alone can take a long time and be very costly. A large number of studies have developed potential prototypes that are portable and accurate, but these technologies still have a way to go before they are evaluated for effectiveness in the field. The glucometer is a good example of the potential for electrochemical diagnostic platforms, and it will not be surprising to see similar platforms reach the frontlines of POC detection in the near future.

The utilization of the magnetic properties of some nanoparticles allows for many of the downfalls of fluorescence detection methods to be overcome because this method can be utilized in opaque and nonprocessed solutions. Furthermore, the ability of magnetic nanoparticles to be manipulated by a magnetic field allows for a great deal of customization in development of various diagnostic tools. However, the current lack of portability and cost of the required machinery limit the utilization of many of these platforms as POC diagnostic tools. These hurdles will have to be overcome before they reach their full potential.

The colorimetric and lateral flow tests have had the most success in today's market as can be seen with HIV POC diagnosis and pregnancy tests. Several studies are in the process of developing similar platforms for other infectious diseases and aim to be equally as rapid, accurate, portable, and simple to use. Although lateral flow assays are purely qualitative, they seem to be an attractive option for many developing POC tests due to ease of use and minimal equipment requirements.

Overall, each modality has the potential for becoming an effective POC diagnostic tool but requires further optimization and clinical testing before they reach their full potential. A summary of the pros and cons of each platform discussed within this Review can be found in Tables 1−4. Many POC tests that have recently been implemented in clinical settings are colorimetric-based diagnostic technologies such as dipsticks and lateral flow assays. Currently, there are POC colorimetric platforms being utilized that allow for the detection of HIV, malaria, leptospirosis, typhoid fever, human African trypanosomiasis, and visceral leishmaniasis. Paper-based assays such as many lateral flow assays seem to be an attractive platform because no lab, power source, or sophisticated equipment is required.[2,19−22,141] In addition to the success of these simpler assays, the increasingly rapid advancement of technology promises to offer new solutions to old problems, seen already with the increasing number of phone-based platforms in

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development. While there are many innovative platforms of all kinds currently in development, it remains of utmost importance to consider the qualities needed for realistic implementation of these POC platforms at each stage in development. A focus on such qualities as timeliness, affordability, specificity, sensitivity, simplicity, and portability will increase the rate at which these novel technologies leave the lab and find their place in the real world.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

POC, point-of-care; WHO, World Health Organization; ASSURED, Affordable, Sensitive, Specific, User-Friendly, Robust and rapid, Equipment free, Deliverable; PCR, polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay; TB, tuberculosis; LOD, level of detection; FP, fluorescence polarization; QDs, quantum dots; mAbs, monoclonal antibodies; SPR, surface plasmon resonance; AuNPs, gold nanoparticles; FET, field effect transistor; DPV, differential pulse voltammetry; LAMP, loop-mediated isothermal amplification; MEQ-LAMP, microfluidic electrochemical quantitative LAMP; SA, streptavidin; SPCE, screen-printed carbon electrode; MNPs, magnetic nanoparticles; T2, spin−spin relaxation time; MRnS, magnetic relaxation nanosensors; MFnS, magneto-fluorescent nanosensors; MMPs, magnetic microparticles; LFA, lateral flow assay; DRELFA, dual recognition element lateral flow assay

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