AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Clinical Microbiology®

MINIREVIEW

Point-of-Care Testing for Infectious Diseases: Past, Present, and Future

Thomas R. Kozel, Amanda R. Burnham-Marusich

Department of Microbiology & Immunology, University of Nevada, Reno School of Medicine, Reno, Nevada, USA; DxDiscovery, Inc., Reno, Nevada, USA

ABSTRACT Point-of-care (POC) diagnostics provide rapid actionable information for patient care at the time and site of an encounter with the health care system. The usual platform has been the lateral flow immunoassay. Recently, emerging molecular diagnostics have met requirements for speed, low cost, and ease of use for POC applications. A major driver for POC development is the ability to diagnose infectious diseases at sites with a limited infrastructure. The potential use in both wealthy and resource-limited settings has fueled an intense effort to build on existing technologies and to generate new technologies for the diagnosis of a broad spectrum of infectious diseases.

KEYWORDS infectious disease, lateral flow immunoassay, molecular diagnostic, point-of-care, rapid tests

Accepted manuscript posted online 24 May 2017

Citation Kozel TR, Burnham-Marusich AR. 2017. Point-of-care testing for infectious diseases: past, present, and future. J Clin Microbiol 55:2313–2320. https://doi.org/10.1128/JCM.00476-17.

Editor Colleen Suzanne Kraft, Emory University

Address correspondence to Thomas R. Kozel, tkozel@med.unr.edu.

A point-of-care (POC) test is performed at or near the site where a patient initially encounters the health care system, has a rapid turnaround time (approximately 15 min), and provides actionable information that can lead to a change in patient management. Rapid results reduce the need for multiple patient visits, enable timely treatment, and facilitate the containment of infectious disease outbreaks. POC diagnostics also reduce the reliance on presumptive treatment and thereby facilitate antibiotic stewardship. Rapid diagnostic tests work by detecting analytes that are found in or extracted from clinical samples. There are two primary types of analytes: microbial antigens and patient antibodies that are specific for microbial antigens. However, there are emerging molecular technologies that enable nucleic acid-based approaches at the POC. In this minireview, we describe the origins and evolution of rapid POC tests, highlight several recent developments, and identify future directions that will move the field forward.

PAST

Perhaps the first large-scale use of the immunoassay for the diagnosis of infectious disease was in a report in 1917 by Dochez and Avery that pneumococcal polysaccharide can be detected by immunoassay of serum and urine from patients with lobar pneumonia (1). In a prescient comment, the authors suggested that antigen detection could enable a rapid diagnosis of infection. Interest in the immunoassay for an antigen or antibody for the diagnosis of disease was accelerated with the high sensitivity provided by the radioimmunoassay (RIA) in 1960 (2) and the enzyme-linked immunoassay (ELISA) in 1971 (3, 4). Indeed, the ELISA remains the dominant immunoassay platform technology in the non-POC central laboratory setting. Moreover, with automation, the ELISA technology also enables high-throughput sample processing. However, the ELISA and RIA platforms are also time consuming, have moderate or high complexity that requires trained laboratory personnel, and are typically equipment intensive. As a consequence, these technologies are not suited for POC use.

The promise of immunoassays such as ELISA and RIA for the diagnosis of disease prompted numerous individuals and biotechnology companies to find the means to

TABLE 1 Examples of CLIA-waived tests for infectious diseases[a]

Disease or pathogen	Principle	Measurand	No. of tests[b]
Group A *Streptococcus* (GAS)	LFIA	GAS antigen	79
	Molecular	Bacterial DNA	2
Infectious mononucleosis	LFIA	Heterophile antibodies	44
Helicobacter pylori	LFIA	IgG antibodies to *H. pylori*	35
	Biochemical[c]	Urease enzyme activity	7
	LFIA	*H. pylori* antigen	1
Influenza types A and B	LFIA	Influenza type A and B antigens	12
	Molecular	Viral RNA	2
	Biochemical	Neuraminidase enzyme activity	1
Respiratory syncytial virus	LFIA	Respiratory syncytial virus antigen	9
HIV-1 and HIV-2	LFIA	Antibodies to HIV-1/2	4
	LFIA	HIV-1 antigen, antibodies to HIV-1/2	1
HIV-1	LFIA	Antibodies to HIV-1	4
Influenza type A	LFIA	Influenza type A antigen	4
Influenza type B	LFIA	Influenza type B antigen	4
Urinary tract infections[d]	Biochemical	Catalase enzyme activity	2
Influenza A/B and RSV	Molecular	Viral RNA	2
Trichomonas vaginalis	LFIA	*T. vaginalis* antigen	2
Adenovirus	LFIA	Adenoviral antigen	2
Borrelia burgdorferi (Lyme disease)	LFIA	IgG and IgM antibodies to *B. burgdorferi*	1
Treponema pallidum (syphilis)	LFIA	Antibodies to *T. pallidum*	1
Hepatitis C virus	LFIA	Antibodies to hepatitis C virus	1
Gardnerella vaginalis, *Bacteroides* spp., *Prevotella* spp., and *Mobiluncus* spp.	Biochemical	Sialidase enzyme activity	1

[a]Adapted from reference 10.
[b]Number of CLIA-waived tests for each disease/pathogen.
[c]Biochemical tests measure the production of the products of the enzymatic action.
[d]There are numerous additional tests for urinalysis, including tests for nitrite, pH, protein, leukocytes, etc., that are not included in this table. For details, see Centers for Medicare and Medicaid Services current procedural terminology (CPT) code 81002.

perform rapid tests at the POC. Early steps included the use of capillary migration in cellulose acetate sheets as the structural foundation for an immunoassay (5) and the ability to couple antibodies to colloidal gold or latex particles (6). Several companies then developed technologies that led to the present lateral flow immunoassay (LFIA) platform (7–9). The home pregnancy test that uses the lateral flow format provided clear evidence of the value of the format for at-home use of antigen testing. In turn, the rapid test for the diagnosis of streptococcal pharyngitis popularized the LFIA technology for the diagnosis of infectious diseases.

PRESENT

Most POC rapid diagnostics use the LFIA platform. The LFIA platform is extremely versatile. The detection of high-molecular-weight antigens requires an antibody pair where an antibody to one analyte epitope is labeled with a reporter, such as colloidal gold, and a capture antibody to a second epitope on the same analyte is immobilized on the lateral flow strip. In an antigen-capture sandwich format, the intensity of the signal at the test line is proportional to the concentration of the analyte. Sandwich immunoassays are the foundation for POC tests for infectious diseases that detect microbial products in clinical samples, e.g., the group A streptococcal cell wall carbohydrate (Table 1). The detection of low-molecular-weight analytes with a single antigenic determinant requires a competitive format. In these assays, the intensity of the test line is inversely proportional to the analyte concentration. Examples of assays using competitive formats include many immunoassays for the detection of drugs of abuse. Finally, the LFIA format can be used for the detection of patient antibodies to target antigens. In this instance, the target antigen is immobilized on the strip, and the

binding of patient antibody is detected by the use of a labeled reporter, such as a second antibody. Examples of serological assays in the LFIA format include tests for HIV-1/2 or hepatitis C virus (Table 1).

Many tests have received Clinical Laboratory Improvement Amendments (CLIA) waivers that enable POC use (Table 1) (10). CLIA-waived tests are typically simple and have a low risk for an incorrect result. By contrast, tests that are categorized by the FDA as having a moderate or high complexity are typically done at a central laboratory. The categorization criteria include the knowledge needed to perform a test, the needed training and experience, the need for reagent preparation, the number and complexity of operational steps, the extent of calibration and quality control, the equipment maintenance, and the need for independent interpretation and judgment (11). Not surprisingly, the ability to use LFIA technology at the POC is playing an important role in resource-limited countries. In addition, a limited number of POC tests that utilize molecular approaches have been developed. Several POC tests that illustrate the range of current applications are described below.

Strategic screening—CrAg LFIA for cryptococcal meningitis. The latex agglutination assay for the detection of cryptococcal polysaccharide (cryptococcal antigen [CrAg]) was one of the first immunoassays for the diagnosis of infectious disease (12). Recently, an LFIA was developed and FDA-cleared as a prescription-use laboratory assay for the detection of CrAg in serum (13). Although the CrAg LFA is a laboratory-based assay in developed countries, a report from the World Health Organization (WHO) recently noted that the low cost, rapid results, the lack of required infrastructure, and the ability to be performed by personnel with little training satisfies most of the WHO affordable, sensitive, specific, user-friendly, rapid/robust, equipment-free and deliverable to end users (ASSURED) criteria for POC tests (14). To this end, Williams et al. recently reported that CrAg lateral flow assay (LFA) testing with fingerstick whole blood in resource-limited settings can facilitate the prioritization of patients on whom to perform a diagnostic lumbar puncture with the measurement of opening pressure (15).

An important development in CrAg testing for patients with AIDS was the discovery that screening for CrAg in plasma to detect subclinical disease in patients presenting for antiretroviral treatment (ART) can identify patients at the highest risk for developing cryptococcal meningitis (16). This finding led to a WHO recommendation that serum or plasma CrAg screening be considered prior to ART initiation in patients with a CD4 count less than 100 cells/mm^3 in those regions with a high prevalence of cryptococcal antigenemia. A positive reaction would trigger preemptive antifungal therapy (14).

Ease of sample collection—detection of HIV antibodies using oral fluids. Several LFIAs have been CLIA waived for the detection of HIV antibodies in fingerstick or venipuncture whole blood (Table 1). In addition, LFIAs for HIV antibodies have been developed for use with oral specimens, e.g., the OraQuick Advance rapid HIV-1/2 antibody test. The test was initially approved by the US Food and Drug Administration in 2004 for professional use with oral fluid, fingerstick whole blood, venipuncture whole blood, and plasma specimens. The test was subsequently approved in 2012 as an over-the-counter test for use with oral fluid specimens. Oral fluids may be more acceptable to patients due to the noninvasive nature of the specimen collection. At-home use also offers an option for individuals who do not wish to be tested in public health settings. Finally, the evaluation of oral fluids reduces blood exposure for health care workers.

Despite the many advantages of a rapid HIV test that can be performed by nonprofessionals or by home use, there are also limitations to the use of oral fluids. A systemic review and meta-analysis of the diagnostic accuracy of the test found that the use of the OraQuick test with oral specimens had a pooled sensitivity that was approximately 2% lower than the test's sensitivity with fingerstick specimens (17). The study of a longitudinal Nigerian cohort using the Avioq HIV-1 Microelisa system found a reduced sensitivity of oral fluid testing for antibody detection compared with that of blood-based testing when specimens are obtained early after HIV infection (18). Curlin et al. also reported that the oral fluid OraQuick test may fail to detect HIV-1 infection

in some cases (19). The reasons for failure were multifactorial and included the lack of sensitivity compared with that of laboratory-based testing for antibodies in blood and operator proficiency or variability. Finally, tests for HIV antibodies may fail to identify acute early HIV-1 infection when the risk of HIV-1 transmission is much higher than with established infection (reviewed in reference 20). Despite such limitations with HIV testing, the OraQuick test for oral specimens or home pregnancy tests or tests for drugs of abuse for use with urine illustrate the potential of the LFIA platform for alternative sample types for home testing or for use in resource-limited settings.

Diagnostic accessibility—malaria. The majority of malaria cases occur in areas with limited health care resources and infrastructure. Thus, a malaria diagnostic's ability to impact public health heavily depends on the diagnostic's accessibility (21). Moreover, when the influence of individual variables was ranked in a model of the public health impact of new theoretical malaria diagnostics, diagnostic accessibility was found to be a more influential parameter on total lives saved than diagnostic sensitivity or specificity (22). For example, a field-based POC malaria diagnostic with 90% sensitivity and specificity was estimated to save over 2.2 million adjusted lives and prevent 450 million unnecessary treatments annually, but a test with 95% sensitivity and specificity that required even minimal laboratory infrastructure would save only 1.8 million adjusted lives and prevent only 400 million unnecessary treatments (22). Accordingly, LFIAs that detect various *Plasmodium* sp. protein antigens are now among the most accessible and heavily used laboratory diagnostics for malaria worldwide. Rapid tests for the plasmodium antigen are also valuable in non-POC laboratory settings due to their ease of use and ability to provide round-the-clock first-line triage results; positive results can then be confirmed by expert microscopy during standard business hours.

High sensitivity at the POC—molecular diagnostics. The performance of LFIAs for antigen detection is critically dependent on the concentration of the analyte in a clinical sample. Analyte concentrations below the assay limit of detection for the test may produce a false-negative result. For example, the CDC recently expressed concerns over the limited sensitivity of rapid influenza diagnostic tests compared to reverse transcription-PCR (RT-PCR) or viral culture and noted that negative rapid test results should be interpreted with caution, particularly when influenza activity is high (23). In another example, a meta-analysis of rapid diagnostic tests for group A streptococcal pharyngitis found a sensitivity of approximately 86% (24, 25). As a consequence, a negative rapid test for streptococcal pharyngitis is followed up by culture in children and adolescents (26).

Concerns over the low clinical sensitivity of some antigen detection LFIAs led to a considerable effort to develop molecular diagnostics that can provide high sensitivity and a rapid diagnosis at the POC. Although the field of POC molecular diagnostics is young, there are already several molecular tests that meet the criteria for POC use, e.g., rapid and CLIA waived. The first test based on nucleic acid amplification to be granted a CLIA waiver was the Alere i influenza A & B test, which was approved in January 2015. The test uses a variation on isothermal DNA amplification technology, termed nicking enzyme amplification reaction (NEAR), to detect RNA gene targets from influenza A and B viruses (27). The turnaround time is 15 min, and the approved sample for CLIA-waived use is a nasal swab. The assay sensitivity and specificity compared with that of viral cell culture in a seven-site clinical study were 97.8% and 85.6%, respectively, for influenza type A and 91.8% and 96.3%, respectively, for influenza type B (27).

Since approval of the Alere i influenza A & B test, several other molecular tests have been CLIA waived, including a test for group A *Streptococcus* (GAS) on the Alere i platform that uses throat swabs and provides results in approximately 8 min (28) and three tests on the Cobas Liat platform (Roche Diagnostics). The Cobas Liat system is based on real-time PCR detection of bacterial DNA targets or real-time reverse transcriptase PCR detection of viral RNA targets. CLIA-waived tests currently available on the Cobas Liat system include influenza A/B, GAS, and influenza A/B plus respiratory syncytial virus (RSV) (29, 30). The turnaround time for all Cobas Liat platform tests is 15

to 20 min for approved sample types that include nasopharyngeal swabs (influenza A/B and influenza A/B plus RSV) and throat swabs for GAS. Both Cobas Liat and Alere i platform tests are run in an on-demand (i.e., random-access) format. Both platforms require specialized equipment in the form of an Alere i or a Cobas Liat benchtop machine for amplification and analysis. The Xpert Flu/RSV Xpress test by Cepheid is also CLIA waived, but requires 1 h for sample preparation and real-time reverse transcriptase PCR. As an indicator of how early in the development curve the field of POC molecular diagnosis is and how much potential remains for rapid growth, there are only two CLIA-waived molecular GAS tests, yet there are 79 CLIA-waived GAS lateral flow immunoassays (Table 1).

FUTURE

Although POC testing is now widely accepted and the LFIA platform is mature, there are many technologies on the horizon that will improve accessibility, test performance, and adoption by the end user. Some of the potential developments and driving forces are described below.

Microfluidics. Microfluidic devices can provide a fully integrated POC device for sample processing, fluid handling, and signal generation. A major goal is a low-cost diagnostic for use in remote settings. Microfluidics-based devices use channels to transport small amounts of fluid by actuation forces. On-chip immunoassays have many similarities to the standard LFIA, ELISA, or molecular diagnostics platforms; however, the use of microfluidic technologies reduces assay complexity and enables multiplex analysis and high-throughput screening. On-chip nucleic acid analysis is particularly promising because it miniaturizes and integrates the various assay steps, including (i) the lysis or extraction of target cells to yield their genetic contents, (ii) the purification of nucleic acids, (iii) the amplification of nucleic acids, and (iv) on-chip detection of reaction products.

Current efforts in the development of lab-on-chip diagnostics include the identification of new biomarkers, as well as integrated microfluidic design, construction materials, and detector technologies. At this point, it is difficult to predict which technologies will emerge as commercially viable products. A particular concern is the per-test cost and the need for instrumentation to drive the devices and product detection. One novel approach to assay construction is the use of layered paper to construct three-dimensional microfluidic devices that can distribute fluids vertically and horizontally and enable streams of fluid to cross one another without mixing (31). With regard to detector technologies, a universal mobile electrochemical detector was recently described that can communicate results to distant sites using a mobile phone (32). These and similar developments will be critical for lab-on-chip diagnostics for resource-limited settings.

Communicability. The immediate goal of a POC assay is to use the information gained from the test to impact the care of the patient. In the case of the LFIA, results can often be obtained by visual inspection. However, for many diseases, particularly, communicable diseases such as influenza or emerging infectious diseases, the use of POC assays can provide a key element of disease surveillance. Linking data to specific geographical locations via global positioning system (GPS) can provide information regarding disease emergence, disease spread, or progress toward control.

The communication of results from typical lateral flow POC assays will require the abilities to digitally capture data and to communicate results to a central database. Many electronic readers are currently available. However, a particularly attractive option is the modification of smartphones for use as readers in resource-limited settings. Effective communication of results for disease surveillance can be best accomplished if there is standardization for result recording and reporting. Ideally, multiple detection technologies might be combined in a single instrument. There are three major caveats to the goal of connectivity and agreed standards. First, reader technology adds to test cost. This may put such advanced result processing out of reach for resource-limited settings. Second, using an electronic reader to scan POC tests and store or transmit

patient data presents a concern for data privacy and security. Lastly, the timing of standards development is critical. If standards are developed too early, the result might be the institutionalization of weaker technologies and the stifling of innovation.

Molecular diagnostics. The high sensitivity and specificity of molecular diagnostics have historically come at the cost of a long turnaround time (hours), investment in expensive equipment, and a need for user training. However, recent developments in isothermal DNA amplification, such as the Meridian Biosciences Illumigene or Quidel AmpliVue diagnostic platforms, which are available for the detection of herpes simplex virus, *Clostridium difficile*, *Bordetella pertussis*, *group* B *Streptococcus*, etc., have made great strides in streamlining the workflow of molecular diagnostics. For example, in an interesting twist, the AmpliVue platform uses a hybrid strategy composed of new molecular and classic LFIA approaches that avoids the need for an electronic reader. In this strategy, two tags (e.g., biotin and 6-carboxyfluorescein [FAM]) are incorporated into the DNA amplicon during the isothermal DNA amplification step, and then the presence of the amplified DNA is detected by LFIA (e.g., anti-FAM antibodies at the test line and streptavidin-conjugated colored particles as the detector) (33). Although they are not POC or CLIA waived, tests like these have made molecular test results available in $<$2 h, with a relatively large proportion of that time available as "walk away" time that frees the laboratory technologist for efforts elsewhere.

But, until 2015, there were no CLIA-waived molecular diagnostics that could bring the high diagnostic sensitivity and specificity of molecular testing to the POC. Given the largely untapped commercial opportunities in this area, the near-term future will likely bring rapid expansion of CLIA-waived POC molecular diagnostics similar in format to the existing CLIA-waived molecular platforms (e.g., Alere i or Cobas Liat). The longer-term future may bring novel POC molecular platforms that match the affordability and sample-to-answer user simplicity of traditional LFIAs.

Ultimately, bringing molecular diagnosis to the POC in even extremely rugged, resource-limited field settings may be feasible. The successful migration of high-sensitivity molecular diagnostics from the reference lab to the field could dramatically improve the accuracy and sensitivity of POC diagnosis over existing rapid (i.e., LFIA) tests, enhance public health reporting, and facilitate outbreak containment in these difficult settings. To that end, a malaria molecular diagnostic (Illumigene malaria LAMP) was recently field tested in Senegal (34). The assay provided results within 1 h (including sample preparation) that were at or below the WHO recommended threshold of 2 parasites/μl. The key design features for POC field use of the assay in resource-limited settings included: loop-mediated isothermal amplification to eliminate the need for a thermocycler, lyophilized reagents for long-term stability at high temperature, and relatively simple procedures for ease-of-use by operators in a field laboratory.

Lastly, one of the inherent strengths of molecular diagnostics is their ability to multiplex. Molecular diagnostic panels that differentiate multiple pathogens already exist for central and reference labs. The development of new molecular panel diagnostics that are CLIA waived and can provide results in $\leq$15 min could bring large-scale multiplexing to the POC. Determining how such POC molecular panels would be best incorporated into clinical diagnostic algorithms and the clinical and economic benefits of a multiplexed syndrome-based testing approach will become important issues (35, 36).

Host biomarkers. An alternative to the detection of microbial antigens as indicators of infection by specific pathogens is the use of host biomarkers to distinguish classes of infecting microbes, e.g., to distinguish bacterial from viral infections. Such tests can have great value in a biodefense countermeasures strategy, as well as for POC use in resource-limited countries, e.g., to distinguish acute febrile illness due to malaria from bacterial infection. Kapasi et al. recently reviewed 59 studies that evaluated more than 112 host biomarkers for distinguishing bacterial from nonbacterial causes of acute febrile illness (37). This review identified several host biomarkers with the potential for

high diagnostic performance, including heparin binding protein, C-reactive protein plus interferon gamma-inducible protein 10 plus tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), and procalcitonin, among others. None of these host proteins can currently be identified at the POC using FDA-approved tests, but a migration to POC formats such as LFIA is quite possible. In an alternative approach to the detection of host biomarkers, Herberg et al. examined RNA expression by microarray to differentiate bacterial from viral infections in febrile children (38). The results identified a 2-transcript RNA biosignature that could differentiate bacterial from viral infection. The study had a number of limitations, but the results are promising. If studies aimed at microfluidics and the use of molecular technologies at the POC are successful, such host biomarker profiling is a potentially powerful approach to a difficult problem.

SUMMARY

In this minireview, we have surveyed the landscape of POC testing, its origins, examples of the scope of current applications, and the needs for future development. The major strength of LFIAs and POC molecular assays is their ability to provide diagnostic information during the initial patient visit. These tests are most valuable in cases where the choice of treatment for a patient is time sensitive and where particular treatments or actions would be triggered by the test results (e.g., fluconazole treatment for a CrAg-positive AIDS patient or hospital admittance for very young infants with RSV). The major weaknesses of current POC tests are sometimes low clinical sensitivity in the case of the LFIA and both cost and infrastructure requirements in the case of POC molecular assays. However, as Urdea et al. elegantly stated, "one does not have to wait for the ultimate technical solutions to begin saving lives" (21). Accessible and rapid tests that can provide an initial diagnosis at the POC are a powerful tool for effective patient care, antibiotic stewardship, and outbreak containment.

ACKNOWLEDGMENTS

We thank Jacqueline Scarbrough for assistance in extracting data on CLIA-waived tests from the CMS database.

This work was funded in part by NIH grants R33AI085548 (to T.R.K.), R01AI093365 (to T.R.K.), R41AI108114 (to T.R.K.), R44AI109891 (to A.R.B.-M. and T.R.K.), and R43EB023408 (to A.R.B.-M. and T.R.K.). The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

The University of Nevada, Reno has licensed monoclonal antibodies to Immuno-Mycologics, Inc. that are used for the construction of the CrAg LFA. According to university policy, a portion of the royalties are shared with T.R.K. T.R.K. has an equity interest in DxDiscovery, a university start-up focused on early-stage diagnostics development. A.R.B.-M. is a principal scientist at DxDiscovery.

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