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Point-of-Care Testing for Group A *Streptococcus* Infection and Influenza

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Abstract

Point of care (POC) testing has emerged as a critical tool in the early and rapid diagnosis and treatment of infectious diseases. While the mainstay of these POC tests has been lateral-flow-based antigen detection assays, recent technological advances in nucleic acid detection combined with regulatory changes has allowed more sensitive detection of infectious etiologies in the near-patient setting. This advancement is particularly impactful in the ambulatory setting, where rapid diagnosis can ensure appropriate treatment at the early stages of infection, both preventing more serious sequelae and also improving physician workflow and patient satisfaction. Along with this new technology come concerns about quality of testing, nucleic acid contamination, and the appropriate use of POC tests. This review covers the clinical manifestations of disease, the current state of POC testing, and the impact of molecular testing for both group A *Streptococcus* infection and influenza.

Introduction

Point of care (POC) diagnostic testing has made a significant impact on the early diagnosis and treatment of infectious disease. Prompt and accurate diagnosis of treatable and common infectious diseases has clear benefits, such as early and appropriate treatment, prevention of significant infectious and immune-mediated sequelae, increased patient satisfaction, decreased turnaround time for laboratory testing, and an overall increased efficiency of diagnostic workflow [1-4]. Furthermore, it has been found that early identification of the microbiological cause of disease limits excessive laboratory testing [4] and allows rapid implementation of the appropriate antibiotic or antiviral treatment. Despite the promise and ease of POC testing, its widespread use has been hampered by concerns about the test's sensitivity and specificity, increased costs, and quality concerns relative to diagnostic tests performed within a clinical microbiology laboratory [5-8].

POC tests are designed to be as simple as possible. The Food and Drug Administration (FDA) defines two different regulations in regard to tests performed outside of a high-complexity laboratory setting. POC tests can obtain a Clinical Laboratory Improvement Amendments (CLIA) waiver from the FDA to be performed in CLIA-waived settings, such as ambulatory and urgent care clinics. These tests can be performed and interpreted by any user who is trained and deemed competent. There are very few requirements for quality control. Alternatively, moderately complex POC testing can be performed in certain POC settings by clinically licensed personal. These testing areas must have a full laboratory CLIA license and are typically found in hospitals and emergency rooms. POC tests for infectious disease (specifically, group A *Streptococcus* [GAS] infection and influenza) are usually waived tests based on lateral-flow technology.

Lateral-flow tests use monoclonal antibodies to recognize the presence of an analyte within a specimen. The monoclonal antibodies are typically labeled with a colored compound and then captured on a test line by an immobilized secondary antibody. A control band that binds to excess detector antibodies is present. The test gives a

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qualitative assessment of the presence of the analyte. The presence or absence of the test line is open to the subjectivity (and level of training) of the user. In general, these tests suffer from lack of sensitivity compared to culture or PCR methods.

Here, we outline the clinical need for and use of some of the most common POC diagnostic tests for GAS and influenza A/B virus. Diseases caused by these two organisms represent many visits to primary care providers, urgent care clinics, and emergency departments. The large number of clinical visits has allowed rigorous comparisons of these POC tests to the reference standard testing performed in the clinical microbiology laboratory. Finally, recent advances in nucleic acid technologies has propelled more sensitive and specific tests into the market, requiring outpatient settings to reevaluate the potential costs and benefits of a more sensitive diagnostic assay at the bedside.

GAS (*Streptococcus pyogenes*)

Clinical background

Streptococcus pyogenes, or group A beta-hemolytic *Streptococcus* (GAS), causes a myriad of infectious syndromes, ranging from mild to severe, that are either directly caused by a suppurative infection or due to post-infectious immune dysregulation. GAS can be found as a colonizing bacterium on the skin around the nares and in the nasopharynx in a low percentage of the population. Different strains of GAS exhibit different virulence factors that, together with the host immune system, influence both the invasiveness and risk of post-infectious sequelae. Early diagnosis through POC testing allows early antibiotic treatment, drastically reducing the risk of serious complications, the duration of infection, and the spread of pathogenic GAS in the community.

In children and adults, GAS is the most common cause of bacterial pharyngitis, making up approximately 30% of all cases of pharyngitis. In adults, the rate of GAS pharyngitis is lower, estimated to be 10 to 15% of all pharyngitis infections [9]. The differential diagnosis for GAS infection includes the common viral infectious agents, as well as other bacteria (group C and group G *Streptococcus*). Making a rapid and accurate diagnosis is essential to ensure that (i) the viral causes of pharyngitis are not treated with antibiotics and (ii) true GAS is properly treated to decrease the duration of symptoms and complications.

Clinically, it is very difficult to distinguish GAS pharyngitis from pharyngitis due to viruses or other bacteria. The presence of specific clinical findings, such as tonsillar enlargement, vomiting, tender cervical nodes, palatal petechiae, or a scarlatiniform rash, increases the probability of GAS pharyngitis, but overall, these are non-specific. Independently, none are sensitive enough to rule out the need for specific GAS microbiological testing. The clinical dilemma, particularly in an era with emerging antibiotic-resistant superbugs, is to provide precise treatment in cases of GAS pharyngitis without inadvertently treating viral etiologies of pharyngitis, which can contribute to increasing antibiotic resistance among bacteria. Inappropriate use of antibiotics has also been linked to other complications, including childhood obesity [10,11].

Treatment is critical to prevent the serious purulent and non-purulent complications of GAS infections. Direct worsening of the GAS bacterial infection can lead to extension of infection into nearby structure or hematogenous spread to other soft tissue sites. This can have severe manifestations, including necrotizing fasciitis, peritonsillar cellulitis or abscess, otitis media, and sinusitis. In addition to extensive spread of the GAS bacteria, there are also non-suppurative complications that occur due to the robust immune response to GAS infections. The more severe post-infectious complications include acute rheumatic fever, post-streptococcal glomerulonephritis, and pediatric autoimmune neuropsychiatric disorders associated with streptococcus (PANDAS) [12-14] (Table 1).

Due to the plethora of GAS pharyngitis-like syndromes due to viral and non-GAS bacteria, diagnosis cannot be performed accurately based on clinical symptoms alone. Studies have shown that there are no individual signs or symptoms that have been effective at ruling in or out streptococcal pharyngitis [13]. Therefore, in addition to the clinical picture, a rapid diagnostic assay to easily distinguish between GAS- and non-GAS causes of pharyngitis would allow more precise prescription of antibiotic regimens, increased patient satisfaction, and improved clinic workflow.

POC testing for GAS

In a randomized trial of children presenting with pharyngitis, physician access to rapid antigen detection tests (RADTs) were shown to decrease the rate of antibiotic prescriptions across the board. These findings suggest that RADTs can be an integral component to guide decisions for antibiotic administration, limiting the number of unnecessary antibiotic prescriptions [15].

Current CDC recommendations are for initial clinical assessment based on a streptococcal score in which points are awarded for clinical and demographic characteristics that increase the pre-test probability of pharyngitis due to GAS. A point is awarded for age between 5 and 15 years, assessment in winter, evidence of acute pharyngitis on examination, tender and enlarged anterior cervical lymph nodes, middle-grade fever, and absence of signs and symptoms of upper respiratory tract infections [16,17]. In children, the RADTs have low sensitivity, particularly in cases with low streptococcal scores. Therefore, in pediatric populations, the CDC and Infectious Disease Society of America guidelines recommend that in cases with negative results by RADT the specimen be reflexed or sent to a clinical laboratory for culture, which is the gold standard [18]. The challenge with this recommendation is that culture requires 24 to 72 hours for a result, and many physicians either ignore or are unaware of the recommendations for culture backup in the setting of a negative RADT result.

The long history of POC for GAS has illuminated key considerations regarding its use in the outpatient CLIA-waived settings. First, although the test is very simple and straightforward to use, there have been reports of inappropriate performance of the RADT leading to an extremely high rates of false positives [5]. Ultimately, it was found that personnel at the test site were improperly trained and had not been reading the test results at

the proper time interval, which increased the risk of false-positive test results. This report highlights the perils of relying heavily on POC tests in the absence of any quality assurances in the outpatient clinical setting.

The sensitivity and specificity of the RADTs are contingent on the professional performing the test explicitly following the manufacturer's instructions. In addition, many outpatient CLIA-waived settings often do not have the appropriate resources, space, or trained personnel to perform multiple tests to this standard. Inadequate training in general laboratory practices increases the probability of specimen mix up and false-positive or false-negative results.

Molecular POC tests for GAS

Recently, the FDA has granted CLIA waivers for two nucleic acid amplification-based POC tests for GAS. To this point, there have been a limited number analytical studies looking at the sensitivity and specificity of these tests compared to culture. One study on the Cobas Liat system (Roche Molecular Systems, Pleasanton, CA) prospectively tested throat swabs from 427 patients (96% were from 3 to 21 years of age) compared to a RADT and bacterial culture [19]. The Liat assay system demonstrated high sensitivity (97.7%) and specificity (93.3%) compared to the reference culture in the context of a 15-minute turnaround time; the RADT in this study had a sensitivity of only 84.5% [19]. Another study on the Alere i system (Alere, Inc., Waltham, MA) using prospectively tested throat swabs from 481 patients (74% were 18 years old or less) showed 98.7% sensitivity and 98.5% specificity compared to culture [20]. In this study, there were 13 subjects who were positive by the Alere i test but negative by culture and were shown to be positive by an alternative PCR-based method through discrepant analysis [20]. These data suggest that the improvement in these measures may obviate the need for reflexive backup testing for samples found to be negative for GAS by the POC PCR method to the clinical microbiology laboratory. Lacking in the literature are studies examining the clinical outcome and health economics of implementing a GAS molecular POC testing strategy.

The increased cost of nucleic acid-based testing is often cited as a major reason that organizations do not move toward these more sensitive and specific tests. It is important to consider the cost and labor of clinical microbiology culture and the number of negative tests that would be reflexed to the clinical laboratory. Laboratory directors at the Mayo Clinic report that, in certain seasons, over 70% of RADTs are negative and would require reflex culture within the clinical laboratory, adding a significant workload and cost to this "cheaper" test. This reflexive testing algorithm not only delays diagnosis and treatment, it subjects patients to longer duration of illness and increases the potential for the spread of disease [21].

Influenza

Clinical background

Although influenza viruses cause self-limited acute febrile respiratory illness for the general population, high-risk populations, such as those at the extremes of age (≤2 and ≥65 years), pregnant individuals, and the immunocompromised, are at especially increased risk for significant morbidity and mortality. Early detection of influenza viruses can guide clinical and therapeutic decisions, as the evidence for benefit with anti-influenza medications (e.g., zanamivir and oseltamivir) is strongest in studies where treatment is initiated within 48 hours of symptom onset. Rapid respiratory viral diagnosis, including influenza virus, has also shown significantly lower odds ratios for admission, length of stay, duration of antimicrobial use, and number of chest radiographs [4]. Current recommendations for the treatment of influenza therefore emphasize early clinical consideration of influenza, early laboratory testing, and early initiation of empirical treatment, particularly for patients in high-risk categories.

Table 1. Severe Complications due to untreated GAS Tonsillopharyngitis

Sequela	**Mechanism**
Streptococcal toxic shock syndrome	Enterotoxin produced by specific strains of GAS cause capillary leakage and tissue damage due to hyperactivation of inflammatory cytokines; clinically presents as systemic and multiorgan failure
Scarlet fever	Delayed-type hypersensitivity reaction to previous encounter with GAS toxin; clinically causes a diffuse erythematous and papular rash starting from the groin and progressing toward extremities, sparing the palms; the rash is followed by desquamation and often accompanied by a strawberry tongue.
Acute glomerulonephritis	Infection with nephritogenic strains of GAS (types 12 and 49) can result in glomerulonephritis with severity ranging from microscopic hematuria to nephritic syndrome and proteinuria.
Acute rheumatic fever	Delayed sequela of GAS pharyngitis presenting 3 weeks postinfection, with arthritis, carditis, chorea, subcutaneous nodules, and erythema marginatum [25]
PANDAS	Controversial association between GAS and exacerbation of neuropsychiatric disease; limited to children [11,12]
Sinusitis/otitis media	Common extension of GAS organisms from the nasopharynx up the ostiomeatal complex and into the sinuses or from the pharynx to the ear via the eustachian tube
Bacteremia	Rare sequela of GAS pharyngitis, but if no other infectious source can be identified, may be due to hematogenous spread of GAS

Influenza A and B viruses are the two influenza virus subtypes that are largely responsible for seasonal influenza epidemics. The most sensitive and specific laboratory test for influenza A and B viruses, as well as the laboratory reference standard, is reverse transcription-polymerase chain reaction (RT-PCR). This molecular-based method has the ability for high throughput testing (depending on the testing plaform employed) and detects viral RNA from influenza A and B virus conserved gene targets from the respiratory specimen tested (both lower and upper respiratory tract). Depending on the testing method, RT-PCR methods may determine qualitative differentiation of influenza viruses (e.g., influenza A virus versus influenza B virus) and subtyping of influenza A virus strains. The superior sensitivity and specificity of RT-PCR-based tests result in accurate results [4,22]. Most molecular-based methods can deliver results within 4 to 8 hours; however, the total turnaround time may be longer, depending on whether these methodologies are available on site, as the tests are usually performed within the centralized diagnostic laboratory setting, where moderate- to high-complexity testing is performed. Therefore, despite excellent sensitivity and specificity, the delay of influenza test results due to a centralized testing model adversely affects timely, clinically relevant decision making.

POC testing in influenza diagnosis

POC testing methods for influenza have attempted to address the issue of rapid laboratory diagnosis for the purpose of expedited clinical decision making, in addition to population health-related outbreak control. POC tests for influenza, which detect influenza viral antigens in respiratory tract specimens, also referred to as rapid influenza diagnostic tests (RIDTs), have long been utilized in the clinical setting as a screening tool for the diagnosis of influenza. They are characteristically CLIA-waived tests that detect influenza A and/or B viral nucleoprotein antigens by immunoassay techniques, such as chromatographic lateral flow. The results are provided in a qualitative manner (positive versus negative) and, more importantly, yield quick results (typically <15 minutes). Requirements for appropriate specimens may differ between RIDTs but generally involve collection from the upper respiratory tract, such as a swab from the nasopharynx. Most tests require a health care professional to manually transcribe test results into the patient's health record. RIDTs have the advantage of providing rapid results in a clinically relevant time frame to guide clinical management compared to the in-laboratory molecular-based method, as described above.

Despite benefiting from their ability to yield quick results, RIDTs, like most POC microbiology immunoassays, suffer from poor sensitivity. The sensitivities of immunoassay-based RIDTs for influenza A and B viruses generally range from 50 to 70%; however, compared to laboratory standard methods, such viral culture or RT-PCR, the sensitivity ranges from 10 to 80%. When comparing the sensitivities for influenza A versus B viruses, the sensitivity range for influenza B virus is consistently lower [23]. On the other hand, the specificities of RIDTs are relatively high, approximately 90 to 95%. The poor sensitivity and high specificity of RIDTs imply that false-negative results occur more commonly than false-positive results. Influenza virus prevalence also affects the positive predictive value (PPV) and negative predictive value (NPV) of RIDTs. The PPV of RIDTs is lowest when influenza virus activity is low. The NPV is lowest when influenza virus activity is high, meaning that false-negative results are more likely to occur when influenza virus prevalence is high [22]. Given the exceptionally poor sensitivity, a negative-value result by RIDT does not preclude the diagnosis of influenza. It may be misleading for clinicians to rely on such results and thus either miss or delay treatment; therefore, many institutions may choose not to perform RIDTs in their practice settings. Instead, clinical judgment has been recommended in these scenarios for diagnosing influenza virus infection. One study performed in a large urban emergency department and urgent care ambulatory clinic demonstrated that a physician's clinical judgment (sensitivity, 29%; specificity, 93%) was no better than the performance of an RIDT lateral-flow immunoassay (sensitivity, 33%; specificity, 98%), suggesting that the use of clinical judgment to make decisions to treat patients for influenza is still a poor replacement for an in-laboratory molecular assay [22]. In a computer simulation model study evaluating the potential economic value of several diagnostic strategies for influenza, it was found that clinical judgment, followed by PCR and POC testing, was most cost-effective, given high influenza probability [24].

Molecular POC tests for influenza

Until recently, the majority of RIDTs utilized immunoassay techniques to detect influenza virus nucleoprotein antigens. Novel rapid molecular tests have since emerged in the field of influenza POC testing to detect viral RNA in upper respiratory tract specimens, with promising sensitivity and specificity for influenza A and B viruses compared to the superior performance of moderately complex molecular influenza tests. In January 2015, the FDA granted the first CLIA waiver for any nucleic-acid-based POC test for the Alere i influenza A and B test (Alere, Inc., Waltham, MA), an isothermal nucleic acid amplification platform utilizing nasal swabs as the collection device. This landmark decision paved the way for the emergence of other CLIA-waived nucleic-acid-based platforms in the market, including the Roche Cobas Liat and the bioMérieux FilmArray respiratory panel EZ, both approved for nasopharyngeal swab samples. Syndromic-panel testing is beyond the scope of this article and is not discussed further. The rapid molecular tests from Alere and Roche are CLIA waived and benefit from ease of use; they can be performed in under 20 minutes. Each test is performed one at a time and also provides discrete results (positive, negative, or invalid) that have the potential to be interfaced with a electronic health record, thereby eliminating user interpretation and transcription errors respectively.

The majority of studies on rapid molecular testing have focused on performance characteristics of these tests compared to either viral culture or non-rapid molecular-based testing. The Cobas Liat has shown excellent sensitivity and specificity of ≥90% for both influenza A and B viruses (sensitivity and specificity range for influenza A virus, 96 to100% and 97.9 to 100%, respectively; sensitivity and specificity range for influenza B virus, 96.9 to 100%

and 97.9 to 100%, respectively) [25-28]. The Alere i has shown a wider range of sensitivity and specificity (sensitivity and specificity range for influenza A virus, 65.96 to 93.5% and 62.5 to 100%, respectively; sensitivity and specificity range for influenza B virus, 45.2 to 100% and 53.6 to 100%, respectively) (**Table 2**) [29-37]. Both tests demonstrated performance superior to that of immunoassay RIDTs, suggesting stronger utility of rapid molecular tests for clinical management decision making.

A recent study prospectively evaluated the impact of rapid molecular tests for influenza in the pediatric setting by physician interview to ascertain real-time diagnostic and disposition plans if given immediate influenza virus/respiratory syncytial virus PCR results by rapid molecular testing. The results of the interview showed that physicians would have decreased emergency department length of stay by 33 minutes, ordered fewer tests, and prescribed fewer antibiotics among discharged patients, with increased appropriate antiviral use [4]. Further outcome studies evaluating the impact on patient care of rapid molecular tests are needed to fully understand the utility of these tests in the management of influenza in various clinical settings.

Given the promising sensitivity and specificity of rapid molecular testing for influenza, the introduction of these tests will certainly change the landscape of diagnostic testing. Although these tests can offer a rapid diagnosis and can be performed easily by any member of the health care staff, it is important to note that rapid molecular tests are expensive and do not have high throughput. Thus, the placement of the technology and the flow of patients will need to be evaluated. Since these POC platforms can perform

Table 2. Sensitivities and specificities of molecular POC influenza tests

Design	Reference standard	Platform tested	Sensitivity (%)	Specificity (%)	Reference
121 frozen respiratory samples	RT-PCR	Cobas Liat	Influenza A virus (96); Influenza B virus (100)	Influenza A virus (100); Influenza B virus (100)	27
129 frozen respiratory samples	RT-PCR	Cobas Liat and Alere i	Cobas Liat, Influenza A virus (100), Influenza B virus (100); Alere i, Influenza A virus (71.3), Influenza B virus (93.8)	Cobas Liat, Influenza A virus (100), Influenza B virus (100); Alere i, Influenza A virus (100), Influenza B virus (100)	28
842 frozen nasopharyngeal samples	RT-PCR and viral culture	Cobas Liat	Compared to RT-PCR, Influenza A virus (97.7), Influenza B virus (98.6); compared to viral culture, Influenza A virus (97.5), Influenza B virus (96.9)	Compared to RT-PCR, Influenza A virus (99.2), Influenza B virus (99.4); compared to viral culture, Influenza A virus (97.9), Influenza B virus (97.9)	26
197 frozen respiratory samples	RT-PCR	Cobas Liat	Influenza A virus (99.2); Influenza B virus (100)	Influenza A virus (100); Influenza B virus (100)	25
267 prospective respiratory samples	RT-PCR	Alere i	Influenza A virus (91.4); Influenza B virus (54.5)	Influenza A virus (97.6), Influenza B virus (98.8)	29
119 frozen nasopharyngeal samples	RT-PCR	Alere i	Influenza A virus (65.96); Influenza B virus (53.3)	Influenza A virus (98.51); Influenza B virus (95.96)	30
96 prospective nasal swabs	RT-PCR	Alere i	Influenza A virus (95); Influenza B virus (95)	Influenza A virus (100); Influenza B virus (100)	31
140 frozen nasopharyngeal swabs	RT-PCR	Alere i	Influenza A virus (80); Influenza B virus (45.2)	Influenza A virus (98.1); Influenza B virus (98.2)	35
202 nasopharyngeal samples	RT-PCR	Alere i	Influenza A virus (77.8); Influenza B virus (75)	Influenza A virus (100); Influenza B virus (99)	33
98 respiratory samples	RT-PCR	Alere i	Influenza A virus (93.8), Influenza B virus (94.1)	Influenza A virus (100); Influenza B virus (100)	34
291 frozen nasopharyngeal samples	RT-PCR	Alere i	Influenza A virus (93.8); Influenza B virus (91.8)	Influenza A virus (62.5); Influenza B virus (53.6)	32
236 frozen respiratory samples	RT-PCR	Alere i	Influenza A virus (93.3); Influenza B virus (100)	Influenza A virus (94.5); Influenza B virus (100)	36
360 frozen respiratory samples	RT-PCR	Alere i	Influenza A virus (73.2); Influenza B virus (97.4)	Influenza A virus (100); Influenza B virus (100)	37

only one test at a time, limitations will be placed on daily throughput. Furthermore, the cost-effectiveness of rapid molecular testing for influenza will need to be determined based on placement and impact on the continuum of patient care.

Summary

GAS and influenza virus diagnosis presents a clear clinical need for POC testing in certain clinical settings (urgent care clinics, primary care offices, and emergency departments). It can be very difficult for centralized hospital and reference microbiology laboratories to provide diagnostic testing within a clinically actionable time frame. Historically, rapid diagnostic tests for these pathogens have been built on a lateral-flow technology that suffered from reduced sensitivity and increased subjectivity in result interpretation compared to the laboratory reference standard. Recent advances in POC molecular diagnostics, with the addition of CLIA waiver, have made it possible to bring molecular testing to the POC.

Along with new POC technologies come additional concerns about quality of testing outside of a microbiology laboratory. One major difference between POC testing and formal testing in clinical laboratories is in the rigor of the quality control measures and levels of federal regulation that ensure the highest quality of clinical laboratory testing. POC tests are valid only as dictated by the manufacturers' package inserts and reference ranges. Deviation from protocols and reagent expiration dates can result in errors that put patients at risk. Audits of POC sites with CLIA waiver certification identified serious issues with quality of testing [6], such as inadequate training, inability to locate procedure protocols, and failure to follow manufacturers' instructions. Physician knowledge about the limits of POC tests can be insufficient and lead to poor patient care. POC testing in the ambulatory setting has several factors complicating standardization and quality control metrics. Testing can occur at a significant distance from laboratory experts who are adept in diagnostic testing, standardized protocols, and result interpretation. While the tests performed in an outpatient setting are CLIA waived and considered low complexity, they are not immune from pre-analytical, analytical, and post-analytical errors. In the absence of standardized workflows outlining a directed protocol, there may be inaccurate test results and interpretation and an increased risk to patient safety. Further complications include manual test reporting in the electronic medical record that can have transcription errors, using the tests on inappropriate specimen sources (e.g., GAS antigen tests used on skin swabs), and not following guidelines for follow-up testing.

It is the responsibility of the leadership of the microbiology laboratory to participate in the organization and use of POC tests for infectious disease within a health care system. That way, the concerns outlined above can be avoided. Recently the American Academy of Microbiology released a report entitled *Changing Diagnostic Paradigms for Microbiology* that highlighted recommendations for POC testing, including considerations of patient flow within a clinical setting, the need for oversight of POC testing by the clinical microbiology laboratory, and the lack of outcome studies analyzing the impact of POC testing for microbiology [38]. High-quality POC testing for infectious diseases beyond GAS and influenza will continue to evolve. Hospital systems and ambulatory clinics can expect to see a continuous growth in POC testing. With the laboratory's help, these new POC molecular tests can have a positive impact on patient care.

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