Chapter 33
Technical and Clinical Niches for Point of Care Molecular Devices

Ellen Jo Baron and Sheldon Campbell

Definition and Limitation of Point of Care Tests

A point of care (POC) device is one that is used outside of a central laboratory environment; generally near, or at the site of the patient/client. Point of care testing (POCT) varies from tests performed in physician's office labs, or "satellite" or "stat" labs, to tests performed on tabletop instruments in a clinic area, to testing performed with hand-held instruments at the bedside. In peripheral lab settings, POCT may be performed by trained laboratory staff, but clinic and bedside POCT is frequently performed by staff who lack specialized laboratory training and whose primary job is something other than doing lab tests.

In the industrialized world POCT is most commonly used to provide results within medical decision-making or infection control intervention actionable timeframes in order to accelerate and streamline care. That timeframe differs depending on the nature and seriousness of the infectious process, but for purposes of this discussion, a maximum of 4 h may be a reasonable upper limit from sample collection to results delivery within the scope of a POC test. In some clinical situations, this will be too long, and clinical decisions must be made without test results for guidance. POCT is also used (especially in resource-limited settings) to provide laboratory results unavailable in any other way. This may be provided by simple test systems that use stable reagents and provide rapid results. Novel systems for remote

E.J. Baron (✉)
Department of Pathology, Stanford University School of Medicine,
Palo Alto, CA 94304, USA

Research and Development, Cepheid, Sunnyvale, CA 94089, USA
e-mail: ejbaron@stanford.edu

S. Campbell
Department of Laboratory Medicine, Yale School of Medicine,
New Haven, CT 06520, USA

Y.-W. Tang and C.W. Stratton (eds.), *Advanced Techniques in Diagnostic Microbiology*,
DOI 10.1007/978-1-4614-3970-7_33,

testing (e.g., viewing of malaria smears via mobile phones) are also coming into use which might fit an operational definition of POCT [1].

There are several reasons to develop a POC test for an infectious disease. These include:

- The need to quickly provide highly targeted therapy. Current algorithms for seriously ill patients depend on empiric treatment based on the most likely pathogens for a given clinical presentation; however, this method involves broad-spectrum therapy to cover the likely contingencies. Knowing the exact identification of the pathogen will allow more focused therapeutic decisions. If a molecular method also detects important resistance factors in the pathogen, then a therapeutic decision can be made specifically to both treat the pathogen and limit development of resistant organisms.
- POCT infectious disease molecular assays may be developed to detect specific infections for which a rapid response is desirable. Examples include common outpatient infections such as group A streptococcal pharyngitis where immediate diagnosis saves follow-up effort; or *Chlamydia* and gonorrhea, where rapid results may allow immediate treatment of patients who might otherwise be lost to follow-up. There is the potential for both clinical and public health benefits from this class of test.
- Another potential objective of a POCT is to recognize quickly which patients require infection control precautions as they are admitted to the healthcare institution to prevent the spread of the agent to other patients or to caregivers. Some POC assays are meant for surveillance only and in such cases, interventions are taken to break transmission routes and prevent the development of infections. Increasingly, healthcare institutions are being asked to become more cost-effective, and rapid applications of infection control activities have been shown to be most effective. The potential for POCT to impact on infection-control is particularly significant for long-term care facilities and other health care settings without on-site laboratories.

Clinical Situations for Which POC Molecular Tests Are Currently Available

Platforms employing molecular technology which are simple enough for potential POC use are just beginning to come to market. No molecular test has yet achieved CLIA "waived" status (although some are being developed for FDA submission), so POC molecular tests are still physician's office lab or "satellite lab" tests rather than true bedside methods. Several comparatively simple, rapid molecular methods, though, are available and increasingly used.

The most widely used molecular test at patient POC sites is the real-time PCR (RT-PCR) assay for detection of nasal colonization of methicillin-resistant *Staphylococcus aureus* (MRSA) [2]. Colonized patients can be placed into contact isolation, decolonization protocols can be initiated, and appropriate surgical

prophylaxis can be used [3]. The use of this assay within the United States Veterans' Administration hospitals is one factor credited with lowering healthcare-associated MRSA infections 59 % since universal screening and additional infection control interventions were implemented. Selected nosocomial infections due to *Clostridium difficile* and vancomycin-resistant enterococci (VRE) also decreased [4]. Two further reports on the use of surveillance for MRSA illustrate the effectiveness of this intervention. With rapid results available within hours of patient admission, the Northshore Hospital System showed 69.6 % decrease in hospital-associated MRSA disease over the study period [5]. In contrast, another healthcare institution used a slower method for MRSA nasal surveillance with results available more than a day later and results were disappointing [6]. Molecular POC tools are virtually the only method possible to achieve the most effective infection control. Additional assays that detect both MRSA and methicillin-susceptible staphylococci in patients' skin and soft tissue wound sites and in nares are also available [7].

Testing feces for the presence of toxigenic *C. difficile* is another use of rapid molecular technology today [8]. An RT-PCR platform and a loop-mediated isothermal amplification (LAMP) platform are FDA-cleared. They each employ different targets. The LAMP assay seeks a genetic locus in the TcdA gene of *C. difficile* whereas the PCR assays either identify a portion of the toxin B gene (TcdB) [9] or a second FDA-cleared assay presumptively identifies the epidemic, hypervirulent 027 strain by detecting both a binary toxin sequence and a deletion in the toxin regulatory gene, in addition to the TcdB gene [10, 11].

The same RT-PCR platform is FDA-cleared for detection of influenza A, B, and influenza A H1N1 novel 2009 in respiratory secretions, which is a modification of a previous test available for a limited time during the 2009 H1N1 Influenza A outbreak [12]. In addition, a self-contained PCR technology using packets of reagents in plastic pouches has also been FDA-cleared for detection of respiratory viruses [13]. Another rapid molecular test has been FDA-cleared for multiplex detection of 15 respiratory viruses, including adenovirus, 2 coronavirus strains, 5 influenza strains, human metapneumovirus, parainfluenza virus types 1–4, RSV, and rhino-enterovirus, with a time-to-result of 1–1.5 h using a novel multiplex PCR and array detection format [14]. Although other PCR methods for virus detection and identification in respiratory secretions are available and show excellent sensitivities and specificities, they are not candidates for POC tests due to their complexity, long performance time, or format that leads to inefficiencies when performing non-batch (such as stat) testing [14].

An FDA-cleared RT-PCR assay can be used to detect gastrointestinal colonization with VRE using rectal swabs [15]. The US version of the test was developed to detect the vanA gene only because vanB VREs are uncommon in the United States today, and because there are more vanB-containing non-enterococci than enterococci in feces. A commonly used FDA-cleared PCR platform has another enterococcal assay that detects vanA and vanB, but specificity of the vanB marker is poor and the format is not optimized for POC [16].

The same platform as described for VRE and staphylococci is FDA-cleared for direct detection of group B streptococci (GBS) in vaginal/rectal swabs [17].

This assay has been used at the time of delivery to test women who never received antenatal surveillance cultures for GBS, or for women who tested negative in their surveillance cultures but whose colonization status may have changed between the time of the culture and presentation in labor [18].

In addition, this platform has an FDA-cleared test for the presence of enterovirus in cerebrospinal fluid [19]. Unlike all the other tests available on this platform, the CSF enterovirus assay is designated "high complexity" due to the need for the testing person to pipette a specific 200 μL volume of CSF into the cartridge.

Lightcycler® and other RT-PCR platforms have been used to develop tests for herpes simplex and varicella zoster virus in cerebrospinal fluid. They could be considered borderline POC tests because if ordered stat, a highly trained laboratory scientist could theoretically perform the test and report results within the 4 h timeframe [20]. It is unlikely, however, that in cases of severe disease, the clinicians treating the patient would be willing to wait that long before treating based on clinical presentation and CSF cell count separate from microbiology laboratory results.

A direct DNA hybridization assay for identification of *Gardnerella vaginalis* (as a marker for bacterial vaginosis), *Candida albicans*, and *Trichomonas vaginalis* has been in use in large physician offices and reference laboratories for many years [21]. Tests can be run in batches of 6 or fewer samples and results are available within 2 h.

In countries other than the United States, the RT-PCR method is used to detect *Mycobacterium tuberculosis* and rifampin-resistance in *M. tuberculosis* using a hemi-nested PCR protocol that uses five molecular beacons to bind to different regions of the ribosomal polymerase B gene in which most mutations conferring rifampin resistance are found [22]. If all five regions bind to their specifically colored fluorescent beacons, the organism is a wild-type *M. tuberculosis*. If one or more of the regions fails to bind its specific beacon, but at least two regions are present, the *M. tuberculosis* strain is reported as resistant [23]. This test can be used with unprocessed respiratory tract secretions at the patient location and results are available within 2 h. Studies have shown that even unskilled workers can achieve high levels of accuracy with this assay [24]. After achieving endorsement by the World Health Organization, it is being broadly disseminated throughout the resource-poor world.

It should not be overlooked that there are several POC diagnostics for detection of agents of bioterrorism. Anthrax, *Yersinia pestis*, and *Francisella tularensis* are all easily weaponized agents, and tests have been developed and field tested for their detection in both the environment (e.g., powders) and in or on patients [25].

Clinical Situations for Which POC Tests Should Be Developed in Future

Several attractive targets for POC infectious disease diagnostics exist. These include:

- Diagnosis of bacteremia and fungemia. Current culture-based technology requires incubation for at least 8 hours before the first indication of a positive result, after which some organisms can be rapidly identified using molecular

methods [26]. However, appropriate therapy within the first few hours often makes the difference between severe morbidity or death and recovery [27]. Because the numbers of circulating bacteria or yeast in the bloodstream of septicemic patients can be low, the volume of blood necessary to detect small numbers of organisms has limited the application of molecular methods. Once an effective front-end concentration system is developed, the diagnosis of these extremely severe infections can be approached as a POC test.

- Meningitis and encephalitis are potentially severe infections that benefit from early diagnosis so that patients can be appropriately managed. The limited number of common pathogens associated with CNS infections makes development of such tests feasible. Additional agents for which rapid, simple molecular tests are needed include *Neisseria meningitidis*, *Streptococcus pneumoniae*, GBS, *Listeria*, *Haemophilus influenzae*, herpes simplex and varicella zoster virus.
- Tests for diagnosis and management of diseases seen in outpatients, particularly those in hard-to-manage populations. POC tests for STDs, for respiratory viruses and group A streptococci, and for HIV and HCV viral load can potentially streamline care for these conditions, allowing same-visit management and decreasing both the effort of follow-up and the potential public health impact of patients who cannot be contacted to deliver their results.
- Many clinicians and infection preventionists are concerned about the rising incidence of multidrug-resistant gram-negative rods. Metallo-beta-lactamases, carbapenemases, cephalosporinases, etc., pose risks to patients and problems for infection control. A rapid molecular test to detect major determinants of resistance, regardless of the organism carrying them, would be a desirable rapid or POC test.

Limitations and Current POC Technologies

Several pathogens can be detected in patient samples using molecular tests within 4 h. However, there are formidable obstacles to moving molecular diagnostics into the POC setting. There include:

- The impracticality of performing some methods in a random access, non-batched mode.
- The need for highly trained individuals to perform the test; and if they must be located at the POC, the inefficiency of having such individuals waiting during the time between test requests.
- The need for space for instruments and other supplies and physical infrastructure that do not exist at most POC locations.
- The delay incurred when an additional sample is received for testing once a testing process has commenced that cannot be stopped in the middle.
- The need to test all necessary controls with individual samples rather than groups of patient samples.
- The need for additional instruments for sample preparation or pre-amplification.
- The possibility of contamination.

After these factors have been considered, the remaining current technologies include polymerase chain reaction, isothermal loop-mediated amplification, and direct DNA hybridization. Other methods are in earlier stages of development but may show potential in the future.

Molecular methods, when brought to routine POC use, have the potential to provide performance equivalent to that of laboratory-based methods. Methods must be chosen to have extreme sensitivity to detect small numbers of organisms in limited sample volumes, and further automation and miniaturization of platforms is desirable [28]. This is the situation in a number of infectious diseases; for example, tuberculous meningitis, where the paucibacillary nature of the cerebrospinal fluid has challenged the development of effective molecular assays [29].

Molecular methods at POC will bring new challenges to those who administer and perform POCT. In addition to the usual QA and QC associated with any POCT, molecular POCT will require procedures for controlling contamination with both amplified material and patient-derived materials. QC of each stage of the analytical procedure; extraction, amplification, and detection, may make trouble-shooting more challenging. The phenomenon of inhibited specimens may require operators to report more complex results than "positive" or "negative." POC molecular instruments are likely to be more complex than current systems such as glucose testing systems and may, initially, lack some of the sophisticated POC management tools associated with traditional POC platforms [30].

Molecular diagnostic technologies are transforming the diagnosis of infectious diseases. Current and emerging clinical needs; increased acuity of inpatient care, expanded outpatient care, and an increasingly mobile population; the need to control healthcare-acquired infections, and novel antibiotic resistance mechanisms, will all drive molecular microbiology to the POC [31].

References

1. Breslauer DN, Maamari RN, Switz NA, Lam WA, Fletcher DA (2009) Mobile phone based clinical microscopy for global health applications. PLoS One 4:e6320
2. Kim DH, Spencer M, Davidson SM et al (2009) Institutional prescreening for detection and eradication of methicillin-resistant *Staphylococcus aureus* in patients undergoing elective orthopaedic surgery. J Bone Joint Surg Am 92:1820–1826
3. Bode LG, Kluytmans JA, Wertheim HF et al (2010) Preventing surgical-site infections in nasal carriers of *Staphylococcus aureus*. N Engl J Med 362:9–17
4. Jain R, Kralovic SM, Evans ME et al (2011) Veterans Affairs initiative to prevent methicillin-resistant *Staphylococcus aureus* infections. N Engl J Med 364:1419–1430
5. Robicsek A, Beaumont JL, Paule SM et al (2008) Universal surveillance for methicillin-resistant *Staphylococcus aureus* in 3 affiliated hospitals. Ann Intern Med 148:409–418
6. Huskins WC, Huckabee CM, O'Grady NP et al (2011) Intervention to reduce transmission of resistant bacteria in intensive care. N Engl J Med 364:1407–1418
7. Wolk DM, Picton E, Johnson D et al (2009) Multicenter evaluation of the Cepheid Xpert methicillin-resistant *Staphylococcus aureus* (MRSA) test as a rapid screening method for detection of MRSA in nares. J Clin Microbiol 47:758–764

8. Voelker R (2010) Increased *Clostridium difficile* virulence demands new treatment approach. JAMA 303:2017–2019
9. Noren T, Alriksson I, Andersson J, Akerlund T, Unemo M (2011) Rapid and sensitive loop-mediated isothermal amplification (LAMP) test for *Clostridium difficile* diagnosis challenges cytotoxin b cell test and culture as gold standard. J Clin Microbiol 49(2):710–711
10. O'Connor JR, Johnson S, Gerding DN (2009) *Clostridium difficile* infection caused by the epidemic BI/NAP1/027 strain. Gastroenterology 136:1913–1924
11. Tenover FC, Akerlund T, Gerding DN et al (2011) Comparison of strain typing results for *Clostridium difficile* isolates from North America. J Clin Microbiol 49:1831–1837
12. Sambol AR, Iwen PC, Pieretti M et al (2010) Validation of the Cepheid Xpert Flu A real time RT-PCR detection panel for emergency use authorization. J Clin Virol 48:234–238
13. Rand KH, Rampersaud H, Houck HJ (2011) Comparison of two multiplex methods for detection of respiratory viruses: FilmArray RP and xTAG RVP. J Clin Microbiol 49(7):2449–2453
14. Ginocchio CC (2011) Strengths and weaknesses of FDA-approved/cleared diagnostic devices for the molecular detection of respiratory pathogens. Clin Infect Dis 52(Suppl 4):S312–S325
15. Gazin M, Lammens C, Goossens H, Malhotra-Kumar S (2012) Evaluation of GeneOhm VanR and Xpert vanA/vanB molecular assays for the rapid detection of vancomycin-resistant enterococci. Eur J Clin Microbiol Infect Dis 31(3):273–276
16. Stamper PD, Cai M, Lema C, Eskey K, Carroll KC (2007) Comparison of the BD GeneOhm VanR assay to culture for identification of vancomycin-resistant enterococci in rectal and stool specimens. J Clin Microbiol 45:3360–3365
17. Church DL, Baxter H, Lloyd T, Miller B, Gregson DB (2011) Evaluation of the Xpert(R) group B streptococcus real-time polymerase chain reaction assay compared to StrepB Carrot Broth for the rapid intrapartum detection of group B streptococcus colonization. Diagn Microbiol Infect Dis 69:460–462
18. El Helali N, Nguyen JC, Ly A, Giovangrandi Y, Trinquart L (2009) Diagnostic accuracy of a rapid real-time polymerase chain reaction assay for universal intrapartum group B streptococcus screening. Clin Infect Dis 49:417–423
19. Kost CB, Rogers B, Oberste MS et al (2007) Multicenter beta trial of the GeneXpert enterovirus assay. J Clin Microbiol 45:1081–1086
20. Petitjean J, Vabret A, Dina J, Gouarin S, Freymuth F (2006) Development and evaluation of a real-time RT-PCR assay on the LightCycler for the rapid detection of enterovirus in cerebrospinal fluid specimens. J Clin Virol 35:278–284
21. Brown HL, Fuller DD, Jasper LT, Davis TE, Wright JD (2004) Clinical evaluation of affirm VPIII in the detection and identification of *Trichomonas vaginalis*, *Gardnerella vaginalis*, and *Candida* species in vaginitis/vaginosis. Infect Dis Obstet Gynecol 12:17–21
22. Boehme CC, Nabeta P, Hillemann D et al (2010) Rapid molecular detection of tuberculosis and rifampin resistance. N Engl J Med 363:1005–1015
23. Helb D, Jones M, Story E et al (2010) Rapid detection of *Mycobacterium tuberculosis* and rifampin resistance by use of on-demand, near-patient technology. J Clin Microbiol 48: 229–237
24. Boehme CC, Nicol MP, Nabeta P et al (2011) Feasibility, diagnostic accuracy, and effectiveness of decentralised use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: a multicentre implementation study. Lancet 377:1495–1505
25. Lim DV, Simpson JM, Kearns EA, Kramer MF (2005) Current and developing technologies for monitoring agents of bioterrorism and biowarfare. Clin Microbiol Rev 18:583–607
26. Loiez C, Herwegh S, Courcol RJ (2011) Usefulness of real-time PCR for the diagnosis of sepsis in ICU-acquired infections. Infect Disord Drug Targets 11(4):348–353
27. Garnacho-Montero J, Aldabo-Pallas T, Garnacho-Montero C et al (2006) Timing of adequate antibiotic therapy is a greater determinant of outcome than are TNF and IL-10 polymorphisms in patients with sepsis. Crit Care 10:R111
28. Kiechle FL, Holland CA (2009) Point-of-care testing and molecular diagnostics: miniaturization required. Clin Lab Med 29:555–560

29. Baron EJ, Carroll K (2005) Rapid diagnosis of tuberculous meningitis: what is the optimal method? Infect Med 22:633–637
30. Kim JY, Lewandrowski K (2009) Point-of-care testing informatics. Clin Lab Med 29:449–461
31. American Academy of Microbiology Report (2008) Clinical microbiology in the 21st century: keeping the pace. American Academy of Microbiology, Washington, DC. Available at http://www.escmid.org/fileadmin/src/media/PDFs/4ESCMID_Library/5Directives_Reports/other_reports/Clinical_Microbiology_in_the_21st_Century.pdf