PCR for Everything—Seeking Value in Speed

With faster and more comprehensive diagnostic tests becoming widely available, we need to think more carefully about whether they truly improve patient care.

Bert K. Lopansri

Medicine is a science of uncertainty and an art of probability.
— Sir William Osler

In the summer of 2013, a patient arrived in our emergency department with complaints of high fever and abdominal pain. She came to us straight from the airport following her return from a 3-month visit to India. Identified as septic, she was “pan-cultured,” meaning readily obtained fluids and substances were sent immediately to begin cultures. Due to concerns for bacterial gastroenteritis, she received ceftriaxone in the emergency department and ciprofloxacin after admission. FEVERS continued and blood cultures yielded Escherichia coli, which was phenotypically resistant to most antibiotics, including ceftriaxone and ciprofloxacin, but susceptible to “last-resort” carbapenems, which were then used to treat her.

Before that stage, however, we asked whether she might be infected with a carbapenemase-producing E. coli—such as a New Delhi metallo-β-lactamase, which would inactivate the “last-resort” antibiotics. Pending clinical lab findings, should this patient be placed into contact isolation and consigned to yellow-gowned health care workers to prevent spread to other hospitalized patients? Should her antibiotics be switched to an expanded-spectrum cephalosporin, carbapenem, or colistin, an uncommonly used antibiotic with significant risk for nephrotoxicity?

These questions were swirling before antibiotic susceptibility results became available. Fortunately, conventional microbiology methods confirmed that, although the microorganism infecting her was resistant to most antibiotics, it produced “only” an extended-spectrum β-lactamase (ESBL), one that cleaves most such antibiotics as well as aztreonam but spares carbapenems.

When we encountered this patient, we were participating in a clinical trial evaluating a commercially produced molecular diagnostic instrument—the Verigene® Gram-Negative Blood Culture Test (BC-GN), by Nanosphere, Inc. of Northbrook, Ill.—that identifies bacterial pathogens and many of the resistance markers that they carry within 3 hours of a blood culture turning positive. Our retrospective testing of this isolate revealed the absence of carbapenemase genes and the presence of the CTX-M type ESBL, which was consistent with results obtained by phenotypic testing.

After achieving microbial clarity, we posed another critical question: would the patient have improved more quickly and could the length of stay have been shortened if we had known sooner that she was infected with an ESBL?

Antibiotic-Resistant Bacteria Challenge Clinical Medicine in Many Ways

This vignette illustrates the latest challenges and uncertainties we face in clinical medicine from antibiotic-resistant bacteria. Methicillin-resistant Staphylococcus aureus (MRSA) was long the poster child for the “resistance movement.”

SUMMARY

➤ When evaluating patients, clinicians and clinical microbiologists should consider whether results from rapid diagnostic tests will improve how those patients are treated.
➤ Molecular diagnostic tests shorten turnaround times but also can increase costs.
➤ Rapid organism identification with molecular tests can improve antibiotic use and patient outcome with bloodstream infection, but increase cost and add little value without an infrastructure to translate rapid results into action.
➤ The newest syndromic tools, designed to detect pathogens that cause gastroenteritis, may reduce many uncertainties but also can confuse clinicians, especially when several targets test positive.
However, the unholy band of drug-resistant nasty germs continues to grow. Vancomycin-resistant *Enterococcus faecium* remains a problem among some patients, such as those who are immunocompromised. The numbers of carbapenem-resistant *Acinetobacter baumannii*, ESBL- and carbapenemase-producing Enterobacteriaceae (CRE) exploded during the past decade—further reinforcing the standard clinical practice of treating patients with broad-spectrum antibiotics before their infecting pathogens are identified. As history shows us repeatedly, this practice contributes to antibiotic resistance and increases patients’ risk for becoming infected with *Clostridium difficile*.

What is the alternative? One major step is to treat infected patients from the outset with effective and appropriate antibiotics—a step that requires rapid, reliable, and accurate detection of the specific pathogen responsible for each patient’s illness. In other words, we need to take better advantage of advances in diagnostic microbiology.

Three major developments are helping to shorten the time to identifying infecting pathogens: (i) molecular methods that identify bacteria within 1–3 hours of a positive blood culture bottle turning positive (Table 1); (ii) matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) microorganism identification using mass spectrometry (MS); and (iii) syndrome-specific molecular methods that detect pathogens directly from primary specimens. Although these methods minimize sample preparation steps and shorten turnaround times, the requisite instruments are expensive and, in the case of molecular testing, each test comes with an added unit cost.

### Rapidly Identifying Organisms and Assessing the Value of That Speediness

One important advance is shortening the time to identify what might be causing bacteremia or fungemia, whether by MALDI-TOF MS or by molecular methods. Traditional methods for de-

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**TABLE 1. Commercially Available Tests for Identifying Bacteria in Positive Blood Culture Bottles**

<table>
<thead>
<tr>
<th>Single Test Methods</th>
<th>Organisms and Resistance Markers Detected</th>
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<tbody>
<tr>
<td>Peptide Nucleic Acid FISH QuickFish (OpGen; Gaithersburg, MD)</td>
<td><em>Staphylococcus</em> spp., <em>Enterococcus</em> spp., GNR Yeast</td>
</tr>
<tr>
<td>BD GeneOhm™ Staph SR (Becton Dickinson Diagnostics; Sparks, MD)</td>
<td>MRSA, MSSA</td>
</tr>
<tr>
<td>Cepheid Xpert (Cepheid, Inc; Sunnyvale, CA)</td>
<td>MRSA, MSSA</td>
</tr>
</tbody>
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Outside of U.S. only.

Does not distinguish between *E. faecalis* and *E. faecium*.

Does not distinguish *vanA* from *vanB*.

a Does not distinguish between *E. faecalis* and *E. faecium*.

b Does not distinguish *vanA* from *vanB*.

c Does not distinguish *vanA* from *vanB*.
Detecting pathogens in blood cultures and determining their drug susceptibilities require many steps and can take 72 hours (Fig. 1). Newer technologies shorten that turnaround time to one to three hours after a blood culture turns positive (Table 1). Although not cleared by the Food and Drug Administration (FDA) for direct use on positive blood culture broth, many labs, including ours, have internally validated and are using MALDI-TOF MS for this purpose. Its main drawbacks are an inability to detect antibiotic resistance markers sooner and low sensitivity for identifying organisms in polymicrobial samples.

What is the value of these advances, and does this increased speed matter? If the main value from faster diagnostic tests is improving overall antibiotic use, the answer is unequivocally yes. The emerging consensus is that rapid testing, when paired with antimicrobial stewardship, will improve antibiotic use, especially in cases where patients are carrying antibiotic-resistant bacteria, according to Debra Goff of the Ohio State University Medical Center, James Musser of the Methodist Hospital in Houston, Melissa Miller of the University of North Carolina, and their respective collaborators.

However, without ways to promptly communicate rapid test results to take action on the clinical side, such molecular testing would increase health care costs but add little value in terms of how patients are being treated, cau-

**TABLE 2.**
**Proposed Benefits of Rapid Bacterial Identification, Resistance Detection and Improved Antibiotic Use**

<table>
<thead>
<tr>
<th>Benefit</th>
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<tbody>
<tr>
<td>Decreased mortality</td>
</tr>
<tr>
<td>Decreased length of stay</td>
</tr>
<tr>
<td>Decreased risk for <em>Clostridium difficile</em> infection</td>
</tr>
<tr>
<td>Decreased antibiotic exposure for blood cultures deemed to be clinically insignificant or a contaminated specimen</td>
</tr>
<tr>
<td>Decreased time to implementation of infection control procedures for antibiotic resistant bacteria</td>
</tr>
<tr>
<td>Reduced selection pressure for development of antibiotic resistance</td>
</tr>
</tbody>
</table>

**FIGURE 1**

Traditional process for blood cultures which require incubation of blood culture bottles, subculture onto solid media and identification with antibiotic susceptibility testing using automated methods. Rapid tests by differing methods allow for identification directly from positive blood culture broths. To date the only rapid test that identifies organisms before blood cultures turn positive is for *Candida* spp. (T2 Biosystems; Lexington, MA).
Lopansri: Between Thailand and the U.S., Focused on Infectious Disease and Diagnosis

Bert Lopansri’s parents raised him and his two brothers to believe that they could be anything they wanted to be—as long as they became doctors. “Fortunately for my parents, one of the three developed an interest in science and medicine,” he says, referring to his career in medicine, specializing in infectious diseases and microbiology.

Today, Lopansri, 45, is an infectious diseases expert who spends his time doing patient care, research, and teaching. He is clinical associate professor at the University of Utah School of Medicine, and Chief of the Division of Clinical Epidemiology and Infectious Diseases at LDS Hospital and Intermountain Medical Center in Salt Lake City. His research now encompasses health care epidemiology and clinical microbiology, particularly the impact of rapid diagnostics and novel ways of communicating rapid results to clinicians. He also has a longtime interest in global health, including tropical diseases, which has involved numerous visits to Africa and Southeast Asia.

Born in Chiang Mai, a city in mountainous northern Thailand, Lopansri moved with his family to the Midwestern United States when he was a baby. However, he visited Thailand every summer until he started college. “On summer trips during high school and undergraduate studies, my aunt would take me to the teaching hospital where she worked and had me round with her,” he says. “She would tell me about patients who died from things like rabies, tetanus, malaria.” Later, seeing hospitalized patients with dengue, yellow fever, measles, HIV, and dysentery, he adds, “as an undergraduate, I was amazed by how the clinicians pieced together the clinical presentation to make a diagnosis, and lab tests were not the major pieces of the equation.”

Lopansri received his B.S. degree in biology in 1993 from the University of Illinois at Urbana-Champaign, and his M.D. in 1997 from Loyola University Stritch School of Medicine in Maywood, a suburb of Chicago, where he also completed his internship and residency in internal medicine. Later, he completed an Infectious Diseases fellowship at the University of Utah, where he joined the faculty as an assistant professor in 2003 and was part of a team investigating the pathophysiology of severe malaria in African children and Asian adults. He developed a passion for clinical research during his Fellowship when he was tasked to measure arginine levels in very small volumes of plasma collected from African children suffering from cerebral malaria. Once he solved a self-induced technical problem, he says, “While processing samples in a blinded manner, I quickly realized that I could predict which ones were from healthy children and which ones were from those with cerebral malaria. It was at that moment that I realized … a career in research was in the cards.”

He left in 2007 for Chicago to pursue a career in academia where he continued malaria research and was involved with hospital epidemiology and infection control. He returned to Salt Lake City in 2011 and, in addition to his patient care duties, has been involved in clinical microbiology as a medical director. “I have had the good fortune to be involved with transformations occurring in diagnostic microbiology and evaluating how cutting-edge technology can improve patient care.”

Lopansri’s wife is a director of project management for the pharmaceutical company Quintiles. They have an 11-year-old son and a 12-year-old daughter. “I enjoy athletics greatly and now spend time watching my kids compete and coaching them. I also enjoy the outdoors—hiking, camping, skiing—anything that gets me outside, although I do draw the line at anything that gets me outside, although I do draw the line at activities in which death is a potential outcome.” He plans to travel with his wife and children to explore parts of the world they have not seen. “We’ve only made it to Thailand, so we have a long way to go,” he says. “My kids let me know about it regularly.”

Marlene Cimons
Marlene Cimons lives and writes in Bethesda, Md.

It is difficult to evaluate the impact that these molecular tests might have on patient outcomes and health care costs for several reasons, including heterogeneities in patient comorbidities and among different pathogens. Additionally, past studies are of small sample size, mostly conducted at single health centers and without rigorous experimental designs. Hence, available evidence varies widely, with proposed overall cost savings ranging from none to more than $60,000 per patient depending on the microorganism being studied.

In one such study, there appeared to be a mortality benefit in patients with gram-negative bacteremia when MALDI-TOF MS was performed directly from positive blood culture bottles, ac-
according to Goff, Musser, and their respective collaborators. However, there was no benefit in other types of bacteremia, according to Miller of the University of North Carolina and her collaborators. We conducted a small quality study to assess the impact of a multiplex panel in patients with *S. aureus* bacteremia using matched, historical controls as comparators. In our study, we showed improvement in antibiotic use with a roughly $4,500 cost savings per patient for those with MSSA bacteremia (unpublished). Based on available studies and personal experiences with rapid diagnostic testing, I conclude that some but not all patients with septicemia will experience a cost reduction when rapid diagnostic tests are applied and acted on. The key question is how many patients need to benefit to prove value when using cost and mortality as metrics.

The final value consideration is not readily measurable and relates to the influence on a clinician’s approach to a patient with septicemia. Knowing the identity of a pathogen sooner has tremendous value to a clinician as it can set the course not only for antibiotic treatment but for identifying unsuspected sources of infection. As an example, *S. aureus* septicemia has different implications than other causes of bacteremia, including other *Staphylococcus* spp., and often requires more aggressive search and control strategies. A 24-hour head start makes a major difference to a hospitalized patient as many things can occur within that short period.

**Questions about Multiplex, Molecular Syndromic Panels**

Last summer, I was asked to see a patient admitted to the hospital with sepsis, severe abdominal pain and cramping, profuse diarrhea, and severe inflammation of the colon. We learned later that she was one of several individuals who developed diarrhea after attending an event the evening before. A multiplex, respiratory panel by PCR, which detects 20 respiratory pathogens in a single test, was negative. A stool sample tested positive for leukocytes, indicating an inflammatory diarrhea, but cultures were negative. In the ER, she was treated initially with ceftriaxone and, later after being admitted, ciprofloxacin, on which she was treated initially with ceftriaxone and, later.

After being admitted, ciprofloxacin, on which she was treated initially with ceftriaxone and, later, was treated with ciprofloxacin, on which she was treated initially with ceftriaxone and, later. A multiplex, respiratory panel by PCR, which detects 20 respiratory pathogens in a single test, was negative. A stool sample tested positive for leukocytes, indicating an inflammatory diarrhea, but cultures were negative. In the ER, she was treated initially with ceftriaxone and, later after being admitted, ciprofloxacin, on which she improved.

At the time of this encounter, we were validating two FDA-cleared molecular panels for detecting gastrointestinal pathogens, including *Shigella* species. Even with this pathogen in mind, our repeat cultures of the initial sample were negative. However, an independent lab with a multiplex panel confirmed *Shigella*. If we had known earlier that this patient was infected with *Shigella*, could we have treated her with different antibiotics, and avoided admitting her to the hospital? Why was a respiratory panel ordered initially, and how many times have respiratory panels been used in this capacity?

This vignette reflects the trend in diagnostic molecular microbiology to follow a multiplex “syndromic” approach. It also perfectly captures how my enthusiasm for these tests weighs against my reservations about their potential overuse and misuse.

The newest syndromic tools are designed to detect pathogens that cause gastroenteritis (Table 3). Unlike bloodstream infection panels, which start with a positive culture and when positive almost always provide actionable information, syndromic tests start with primary specimens. Proposed advantages with the gastrointestinal (GI) panels include automation with >90% sensitivity and specificity and a turnaround time 2–3 days faster than stool cultures. When compared to antigen detection tests that are available for many GI pathogens, and other singleplex stool tests such as *Clostridium difficile* tests by nucleic acid detection, however, multiplex molecular panels provide little benefit with respect to turnaround time.

When viewed from a laboratory perspective, one important value from using these panels is that they greatly improve efficiency of laboratory workflow, especially for stool cultures. For example, clinicians who submit stool samples typically order multiple tests. Thus, taking the syndromic approach can be more cost-effective and efficient. Why order a stool culture, multiple antigen tests, and a *C. difficile* test when you can order a single test covering all these targets?

When viewed from a clinical perspective, however, the value of these broad panels becomes less certain. Keeping patients from being admitted and shortening their hospital stays are important value metrics. However, I am less certain about the impact such testing can have on antibiotic use and clinical outcomes. Supportive care with fluid replacement is most important for treating patients with diarrhea, while antibiotics play a limited role. Unlike blood culture panels,
which can influence antibiotic use, the GI panels are configured to diagnose infections that either cannot be treated with antibiotics (rotavirus, norovirus, sapovirus, adenovirus, astrovirus), should not be treated (STEC, E. coli O157), may or may not be treated depending on severity of illness (Campylobacter spp., Salmonella spp., Plesiomonas shigelloides, Yersinia enterocolitica, Vibrio spp., Clostridium difficile toxin A/B, E. coli O157, STEC), or Enteroaggregative E. coli, Enteropathogenic E. coli, Enteroaggregative E. coli, Shiga Toxin 1, Shiga Toxin 2.

One other important consideration is that clinical presentations and epidemiologic factors differ among gastrointestinal pathogens, even when symptoms overlap. While these tests will reduce many uncertainties, they also can confuse clinicians, especially when several targets test positive. For example, what does a clinician do with test results that are positive for enteroaggregative E. coli and C. difficile, when there are no risk factors for the latter?

A colleague recently described a patient whom she suspected to be infected with norovirus (personal communication from Dr. Dascomb). However, the multiplex GI panel confirmed norovirus but was also positive for C. difficile and E. coli O157, with negative shiga toxins. Is the C. difficile result real? What is the significance of O157 positive with a negative shiga toxin? She elected to trust the clinical presentation, ignore the O157 result, and not to act on the C difficile result. The patient improved without antibiotic treatment for C. difficile.

Important questions to consider going forward are: must we test simply because we have the molecular means to identify more enteric pathogens, and how will these tests integrate with other tests currently being used in most labs such as culture, singleplex molecular and antigen-based detection methods? In the United States, there are about 179 million cases of diarrhea each year, according to Herbert DuPont of the University of Texas School of Public Health and Medical School in Houston. Testing a mere 5% of those affected individuals, at $75-$300 per episode depending on what tests are ordered, will come with a hefty price tag to the system. To control such costs, we need to consider patient needs first. Development of care process models to guide the approach to a patient with acute diarrhea has the potential to influence use of diagnostic tests. We must also carefully monitor how these new multiplex molecular diagnostic panels are being used and how these tests integrate with other diagnostic tests available.

In the PCR-for-everything age, clinicians need to know what they are looking for when ordering a test. They are still trained to take detailed histories and to perform physical examinations in order to understand what might be causing a patient’s illness. Laboratory testing is an adjunct that may throw light on suspicions and guide therapy but should not become the sole means for diagnosis. Despite value in using rapid methods to identify bacteria and drug resistance markers in patients with bloodstream infections, an appropriate infra-

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**TABLE 3. Clinically Available Commercial, Multiplex, Molecular Gastrointestinal Pathogens Tests**

<table>
<thead>
<tr>
<th>Test</th>
<th>Bacteria</th>
<th>Viruses</th>
<th>Parasites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verigene Enteric Pathogens Test</td>
<td>Salmonella spp., Shigella spp., Campylobacter spp., Vibrio Group, Yersinia enterocolitica, Shiga Toxin 1, Shiga Toxin 2</td>
<td>Norovirus GI/GII, Rotavirus A</td>
<td>None</td>
</tr>
<tr>
<td>Luminex xTAG® (Luminex, Corp; Austin, TX)</td>
<td>Salmonella spp., Shigella spp., Campylobacter spp., Clostridium difficile toxin A/B, STEC, E. coli O157, Enterotoxigenic E. coli</td>
<td>Norovirus GI/GII, Rotavirus A</td>
<td>Giardia lamblia, Cryptosporidium</td>
</tr>
<tr>
<td>BD Max™</td>
<td>Salmonella spp., Shigella spp., Enteroinvasive E. coli, Campylobacter spp., STEC</td>
<td>Norovirus GI/GII, Rotavirus</td>
<td>Cryptosporidium, Entamoeba histolytica, Giardia lamblia</td>
</tr>
</tbody>
</table>

*STEC, shiga toxin-producing E. coli*
structure is needed, such as an antimicrobial stewardship program, to allow prompt action based on results from such testing. The value of multiplex molecular GI panels is less certain and needs to be evaluated. As we navigate the ever-expanding menu of broad, multiplex panels, we must be cautious about letting the test-for-everything approach become the norm.

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Suggested Reading


