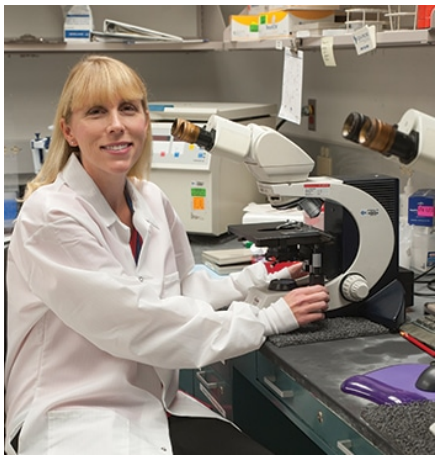
Microbiology’s shifting role in war on sepsis

Anne Paxton

August 2018—If you were casting about for the severest test of a laboratory’s capabilities, day in and day out, sepsis admissions at a pediatric hospital might fit the bill.

At Children’s Hospital of Philadelphia, and at other hospitals, waging war on sepsis requires battles on multiple fronts and clinical pathways that rely on an agile and highly equipped microbiology laboratory.

Three main categories of patients ensure there is no shortage of sepsis cases at CHOP, says Erin H. Graf, PhD, D(ABMM), director of the infectious disease diagnostics laboratory. “We’re a very large oncology center with a lot of children with hematologic malignancies as patients, who are certainly at high risk for sepsis.”



The hospitals treating adults see much more carbapenem-resistant Enterobacteriaceae than the pediatric hospitals, “and it’s just a matter of time before they become more common in our community,” says Dr. Erin Graf of Children’s Hospital of Philadelphia. [Bob Williams]

A large neonatal population makes up the second category, since the 546-bed hospital is also the major center to which other hospitals send their most premature babies. “Low-birthweight infants are at increased risk for sepsis, and we see that fairly often,” Dr. Graf notes. The third population consists of patients with community-acquired sepsis, including, among others, infants with group B strep or *E. coli* acquired from the mother.

Community-acquired infections have gradually declined, thanks to vaccination and successful group B strep screening programs, but the populations of low-birthweight neonates and the immunocompromised continue to grow and help keep the roughly 40 FTEs of the CHOP microbiology laboratory busy. Performing more than 200,000 tests a year, the lab is constantly looking for ways to identify bacteria in the bloodstream faster.

The laboratory uses BD Bactec instruments to run blood cultures, and when a blood culture is positive, there are two mechanisms by which to rapidly identify the organisms, Dr. Graf says. “We run the Luminex Verigene for Gram-positive organisms and find within a couple of hours whether it’s *Staph aureus* that might be resistant, or MRSA or coagulase-negative staphylococci versus a group A or B strep.” A new Accelerate Pheno instrument, soon

to go live, will provide rapid identification within an hour and susceptibility testing within eight hours. “That will speed up the process significantly,” she says.

Also on deck, though still in a research phase, is next-generation sequencing. “The beauty of NGS is you can look for everything, so we don’t have to bias what we’re looking for.” That is one reason her lab has not been as excited about the T2 Biosystems bacteria panel, which the FDA approved this spring, and *Candida* panel. “They only cover five bacteria and *Candida*, but we do not see a lot of *Candida* bacteremia here.”

She would be pleased to see NGS testing become more cost-effective. “Right now it’s not fast enough and not cheap enough, but I think we’ll get there. There is newer technology on its way that is promising at picking up bacteria in the bloodstream. And we can start asking questions about genetic resistance determinants that are present.” She is optimistic, for example, that Oxford Nanopore Technologies instruments will soon bring speed at lower cost.

Pathologists at CHOP have expressed interest in total lab automation, but Dr. Graf is a bit wary. “There’s a large validation that goes along with that because pediatric samples can be so different from adults’. Considering our volume and collection and sample types, that would mean devoting brainpower that we’re currently using on other important projects.”

The laboratory testing that goes along with the several clinical pathways for sepsis includes blood culture and testing of isolates on a MALDI-TOF mass spectrometer, although with the Accelerate Pheno, she notes, it’s not necessary to confirm the identification. Then the lab may run flu or other viral PCRs if there is a need to rule out a viral cause. “The PCRs could include herpes virus, CMV, and EBV, in addition to the standard respiratory viruses that can cause fever and other sepsis-like symptoms,” all of which are laboratory-developed assays, she notes.

“Our chemistry lab runs the standard biomarker tests you’d expect for sepsis, although the clinical staff don’t necessarily use all of those values to decide whether to treat. Treatment is largely based on clinical presentation and meeting SIRS criteria.” The laboratory does run procalcitonin, but there’s uncertainty about predictive use to guide therapy in pediatrics, Dr. Graf says, because the test’s use is not well established and interpretation is hospital- and age-dependent. “So our groups will order it and it is part of the clinical pathway, but they’re not necessarily using that result to make a decision about treatment. It’s more like supportive supplemental information.”

Turnaround time is a constant concern, the great limiting step, she says. “If there is anything we can do to speed up the process from the perspective of whether the child is on the right antibiotic—are we adequately treating organisms they might have, can we narrow the therapy as quickly as possible to be good stewards—we are continuously trying to find ways to get that answer faster.”

Luckily, the hospital is strongly supportive of the laboratory. “We are very well clinically integrated at CHOP—and that’s not necessarily true at every hospital.” For the influenza or meningitis or sepsis pathways, “we are brought to the table to discuss what we can do from the lab’s perspective, what the turnaround time is, what the right specimen type is. And that’s before decisions are made. The lab also has a strong role and strong presence on the hospital’s quality and safety committees. If there’s an instrument the laboratory wants and we can show the clinical value, we usually get it,” Dr. Graf says.

Specimen collection, of course, presents unique problems in the pediatric population and requires the different labs to work together. Clinical protocols require that microbiology receive a specimen first. “Then we will sterilely manipulate it and give it to chemistry; it can go on their nonsterile analyzer there.”

A lot of dialogue takes place between Dr. Graf and the infectious disease clinicians about what should be performed first, when to wait for a positive or negative result, or whether to reflex to other series of tests. “Sometimes it’s a question of sample volume, where we only have enough to do a certain set of tests. Should we save some for downstream testing we may need later? And sometimes it’s a stewardship question. We don’t want to charge patients a ton of money for useless testing, so sometimes we do try to triage testing.”

Sepsis is a clinical diagnosis and does not always include a microbiologic diagnosis, Dr. Graf points out. “So sometimes you’re sending kids home and you don’t really know what you’re treating, because nothing ever grew in culture. That’s why we’re looking at NGS, hoping it might prove to be more sensitive than blood culture.”

Not that NGS will be the be-all and end-all of testing. It has its own limitations, she cautions. In theory, though, it could be more sensitive than culture, particularly in cases in which the child was pretreated with antibiotics and nothing grows in culture. “You could still potentially pick up the DNA of the bacteria floating in the bloodstream.”

With the Accelerate Pheno, Dr. Graf says the laboratory is hoping that by getting results faster, clinicians can say, “‘OK, we need to put in a PICC and we can send you home on *x* antibiotic.’ And if we can make that happen a day or two earlier, it would be wonderful for our patients and families. Other groups are already starting to present data on these types of outcomes, and we hope there is a similar benefit for our population.”

A continuum of care at Geisinger

The approach to sepsis at Geisinger’s Diagnostic Medicine Institute in Danville, Pa., involves a continuum of care, starting with predictive analytics aimed at prevention through rapid diagnosis and efforts to reduce readmissions after discharge, says Donna Wolk, PhD, D(ABMM). As division director for molecular and microbial diagnostics, Dr. Wolk leads a clinical program that combines traditional and molecular testing with her translational research.

The Janet Weis Children’s Hospital at Geisinger was the first rural acute-care pediatric hospital in the U.S. and is unique for its location in rural central Pennsylvania, where it serves 44 counties. “But I think we’re also unique in the U.S. in that we place actionable testing, including rapid diagnostic testing with multiplex PCR, at all of our hospital sites large and small. We function as one system in terms of our microbiology diagnostic service.” Systemwide, Geisinger performs about 45,000 blood cultures a year, a subset of which is collected from pediatric patients.

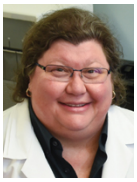
When adopting new technology, such as morphokinetic cellular analysis (MCA) on the new Accelerate Pheno instrument, “we tend to perform pilot testing at our larger hospital sites. After assessing clinical utility and economic benefit, we may expand testing to all 10 hospitals.”

Sepsis diagnostic testing begins with the collection of a traditional blood culture, then a rapid Gram stain, followed by a bacterial or fungal subculture.

Laboratory instrumentation is selected with this in mind: “We believe that microbiology, in terms of bacteremia and sepsis, is a 24/7/365 operation. As such, we deploy rapid molecular testing that is classified as moderate complexity or waived testing so it can be performed anytime by cross-trained second- and third-shift employees who may or may not be microbiologists.” The same is true for performance of their Gram stains, although those are categorized as high-complexity testing. “We consider all positive Gram stains from blood cultures to be stat, and we deployed quality improvement programs that aim to deliver the Gram stain within 30 minutes of the positive blood culture flag at all of our sites around the clock.”

To maintain accuracy for Gram stains, which can be difficult to interpret, the core laboratory offers telemicroscopy consults. All community hospital sites in the system can phone the core laboratory and share electronic images with microbiology staff or submit an image to the doctoral directors via photo sharing. Errors for Gram stains have decreased by about 40 percent since telemicroscopy measures were deployed in 2014, Dr. Wolk reports. Telemicroscopy gives greater confidence to off-campus and alternate-shift staff who may not get to see many positives. “It helps us maintain competency across 10 different hospitals.”

“Unfortunately,” she says, “despite the knowledge that time is of the essence, some hospitals still don’t perform Gram stains on second and third shift or don’t have blood cultures on site. Historically, when turnaround time was measured in weeks, maybe that practice was accepted, but with newer, faster blood culture instruments and rapid molecular testing, we don’t feel that transport to a core laboratory is an acceptable practice anymore.” Geisinger supported research funding to assess the impact of delays in specimen transport, Gram staining, and reporting of results to providers.



Dr. Wolk

Dr. Wolk expresses concern that with more laboratory consolidations in which smaller hospitals are being taken over by larger core laboratories, shipping of blood cultures across the state or even the country is delaying actionable results for bacteremic patients. “If one only examines laboratory costs, consolidation might seem appealing, but there are downstream human and financial costs associated with delays in blood culture processing that lead to delays in adjusting antibiotics. Also, the CMS star ratings and associated reimbursements can suffer if the system’s mortality rate is too high.”

To date, Geisinger has collected evidence of the impact of deploying blood cultures and rapid detection Gram stains at its core hospital sites. But armed with a new research grant, “we’ll be examining the impact of reporting rapid results in all inpatients and pediatric patients as we expand our studies. For instance, if we’re only saving lives in our ICU but not saving extra lives or resources in our general inpatient or pediatric population, then the evidence will tell us how to proceed with deployment of rapid PCR and rapid susceptibility testing. We’ll ask, what patient population is going to benefit from the new technology?”

At Geisinger, pediatric and adult testing are performed with equal speed. “Approximately 60 percent of our sepsis patients are admitted through our emergency rooms, and we focus on every step of the process. Our phlebotomists provide sepsis patients in the ER with an average order-to-collect time for blood cultures of 1.2 minutes. Then, those blood cultures are transported by pneumatic tube system directly to our microbiology laboratory, where they are loaded immediately into our blood culture incubators.” The system maintains quality metrics to document speed at each step of the process. “Gram stains and multiplex PCR results from the BioFire BCID assay are reported as critical values by our phone center within 10 minutes of the laboratory report,” Dr. Wolk says.

In a study performed with an adult ICU sepsis population, results showed a decrease in mortality and total costs per visit. Study of the pediatric population was launched in June and is incomplete. “But our pediatric physicians appear thankful for the improvements in turnaround time for culture, Gram stain results, and multiplex PCR. Anecdotally, they say the faster results help them provide better care on a daily basis.”

With the launch of its MCA instrument, Geisinger performs rapid susceptibility testing for Gram-positive and Gram-negative infections found in pediatric patients and neonates who are at risk for antimicrobial toxicity. “We strive to provide results upon which antibiotics can be rapidly adjusted to limit toxicity, by selecting the best antibiotic based on rapid susceptibility test results,” she says.

She hopes an integrated team approach, supported with data and metrics, “will provide a positive impact in pediatric patients, as it did in adults. If improvements are not documented, we will re-examine our approach.”

Dr. Wolk’s laboratory is performing some NGS testing, more from the adult population than pediatric, and more on tissues and normally sterile sites than from others. “It’s not yet routine for direct specimens, and we have not yet deployed it for blood culture positive samples. It’s used in specialized cases in which the source of the infection cannot be found by routine culture or PCR methods.” The Geisinger algorithm for NGS includes a doctoral microbiology consult along with support by infectious disease physicians. To conserve resources, “We always consider the Gram stain and the stains from pathology tissue blocks to make sure we’re not paying for a sequence analysis on a sample that would yield results in our normal course of events.” In some cases, acridine orange stain is deployed in an attempt to identify microbes that are not evident on the Gram stain. “Using this approach, we’ve identified *Legionella* spp. and *Mycoplasma* spp. in cases of endocarditis.”

Her laboratory has automated molecular testing and specimen inoculation and is planning to deploy total automation when funding is confirmed. For the handling of sepsis specimens, automation doesn't necessarily have an impact on specimen inoculation of the blood culture, "but it does allow the laboratory to aliquot and store positive blood culture broths efficiently and greatly decreases our footprint for storage space for positive broths."

Dr. Wolk makes a case for including pediatric samples in upcoming clinical trials. "From my perspective, it's troublesome that, in some cases, the pediatric blood culture bottles were not included in the original FDA clinical trials for rapid molecular methods, thus requiring individual laboratories to fund the costs of verification. I urge commercial suppliers to consider including pediatric blood culture bottles for rapid diagnostic testing in the pediatric population, so that benefit can be documented." Dr. Wolk is hopeful that the direct-from-whole-blood testing will prove to be beneficial in pediatric patients.

Sepsis in the totally automated lab

Total laboratory automation is a central feature of NorthShore University HealthSystem's approach to sepsis, says Erin McElvania, PhD, D(ABMM), director of microbiology. NorthShore, located in Chicago's northern suburbs, performs about 300,000 microbiology-specific tests per year in its main laboratory.

Dr. McElvania

"Our general protocol is to have our physicians submit blood cultures, then we incubate them in our BD Bactec automated blood culture system until they signal positive, and then we use our Kiestra total laboratory automation to process specimens for culture." In addition, the Verigene assay is used for Gram-negative and Gram-positive organisms. "That gives us rapid identification of the most common agents of sepsis and several resistance determinants as well."

"Because of the smart incubators in our TLA, which are more of a closed system, the agar plates stay at a constant temperature and we see bacterial growth much sooner. We check the plates by imaging them often, and if there is any bacterial growth, we are able to run MALDI-TOF mass spectrometry on those isolates and get a rapid identification when compared to growth of isolates in traditional incubators and biochemical testing."

Optimizing the Kiestra TLA to work better for NorthShore patients is an ongoing project, she says. "We initially did studies to figure out the earliest time point at which we should look at the positive blood cultures we plated, and we settled on four hours. So now that is the first time they are imaged and viewed. Oftentimes that's a little early, but then we follow up with additional imaging every hour or two to see if we can detect growth.

"Since it's automated," she continues, "we can automatically image the plate at many time points so no one has to take anything out of the incubator. The plates are out of the incubator only for about 20 seconds. So with Kiestra TLA we are able to check them frequently without slowing their growth." In fact, Dr. McElvania says she is pleasantly surprised at how fast organisms grow in the Kiestra, contributing to an efficient workflow.

A rapid resistance screen the laboratory has developed and is testing now will be used in addition to traditional susceptibility testing, Dr. McElvania says. "We are able to use our Kiestra TLA with its smart incubators and a heavier inoculum to achieve rapid bacterial growth. By imaging the plates every hour, we are able to determine the earliest time points in which you can reliably read the zone diameters for our modified disk diffusion assay." The cost is reasonable: Even including the agar plates and disks, it amounts to about $1 or $2 per culture.

Verigene is fast, she says, but there are a lot of drugs for which the resistance cannot be identified by a molecular

target. “The Verigene gives the microbiology lab a few resistance determinants where antibiotic decisions may be made. But it’s not a full antimicrobial susceptibility panel, so that’s an area where we’re trying to help physicians and get them information to optimize their antibiotics earlier. For additional information, we plan to use our rapid phenotypic resistance screening assay, and we are in the process of validating it for a number of antibiotics.”

NorthShore’s sepsis population tends to include many older patients from nursing homes or long-term care facilities, often with many comorbidities, along with a smaller pediatric and NICU population. “We generally encounter the routine sepsis-causing organisms, and we have a low rate of antimicrobial resistance compared to other academic centers that have more transplant patients.”

Dr. McElvania has considered the Accelerate Pheno as a way to improve ID and susceptibility testing. “I think it has a lot of promise, but it’s also incredibly expensive and takes up a lot of space. Each instrument can only run one sample at a time, and we would need many of them based on our blood culture volume.”

The laboratory is in contact throughout the day with the clinicians in the infectious disease group and with the infectious disease pharmacists, she says. “Usually, if something isn’t identified by our Verigene, they will call and have us review the Gram stain and see if we have any clues, or they frequently come to the lab to look at Gram stains themselves and discuss patient cultures, so we have a very good relationship with them.”

There is a lot of concern about culture-negative sepsis, of course, and Dr. McElvania says many new culture-independent methods are coming up that could increase sensitivity of pathogen detection. “But they’re not without their own limitations.”

One of her biggest concerns about culture-independent methods is the lack of susceptibility testing on organisms if only molecular methods are used for identification. “They don’t have the ability to do susceptibility testing,” she says.

NGS instrumentation is available in the molecular laboratory, which is adjacent to microbiology. “They’re mainly using it for cancer diagnostics so far, and they are just starting to use it to look for pathogens directly from patient specimens that did not grow in routine bacterial culture.”

Optimizing pathogen recovery through traditional methods and then using molecular testing for culture-independent pathogen detection is a promising strategy, in Dr. McElvania’s view. “I’m most excited and interested in those two things. New technologies, new assays are coming on the market that will help us with these quandaries. But for now, collecting optimal blood volume for culture before antibiotics are initiated is still the gold standard.”

Sepsis diagnosis from whole blood

A notable recent advance was the FDA’s approval, in May, of T2 Biosystems’ bacteria panel. Lee Health in Florida, an early installer of the T2, tests for *Candida* only (the panel was approved in 2014); it has been conducting those tests successfully for more than a year, says Fran Cioffi, MT(ASCP), microbiology supervisor. “There is a T2 instrument that can do both *Candida* and bacteria, and I’m hopeful we will be moving to that.”

Use of the T2 is restricted at Lee Health so that only ID physicians or intensivists can order it for patients in the ICU, those on multiple antibiotics, or cancer and other immunosuppressed patients. “Those are the ones who are going to be subject to having *Candida* in their blood,” Cioffi notes. “Lee Health system placed the T2 in its trauma hospital because its lab had space and the other hospitals didn’t. But if we continue taking the technology, we will have all of our instruments on all campuses. That would be our goal.”

The T2 boasts distinct advantages over traditional blood culture, Cioffi points out. “With T2, you need one to three colony-forming units, which means you can draw right from a blood specimen tube, and in two-and-a-half to three hours you can see whether it’s positive for *Candida*. Yeast grows slowly and on a blood culture you’d need 60 colony-forming units. So we’re able, with the T2 test, to take patients off their antifungal drugs, which are toxic and

expensive, or put them on. And with candidiasis in patients, the mortality rate is high. So this has been very successful for us."

Expense—about $130 per test—can be a stumbling block, she says. "You cannot run the test on every patient. Any test we bring in that's molecular, you have to have control over the ordering. You can't put the tests out there for hospitalists or anybody to order it. Right now, providers will order blood cultures if the patients have an elevated fever. You can't do T2 on those. We have test utilization curbs in place with *Candida* and will do the same with bacteria."

On the flip side, some bacteria would be missed if only a blood culture were done. Organisms like *Acinetobacter* would cause a change in empiric therapy, Cioffi says. "That's an organism you don't get very often." One patient in a study conducted at her hospital was positive for *Acinetobacter* with the T2 but would have been treated only for *S. aureus* with a regular blood culture. "Normal protocol in the lab is that once a blood culture dings positive, you take it out, you subculture it, you know you have the organism, and you treat for it."

"But with sepsis, it used to be that single blood cultures were always single organisms. And that's long gone. Now, with IV drug users, you might get two or three organisms that come up in their blood culture. We've seen that increase over the last few years." In another case, an *E. coli* was never recovered. "Sometimes when a patient is on antibiotics, you have a slower amount of growth with a lower colony count. With blood cultures, because you need a higher colony count, you might not pick that up, but you're going to pick it up with the T2." Still, that the T2 detects only 10 organisms means it is a long way from replacing culture, she says.

Control of contamination is an area in which her lab is making strides. "We run about 6,500 blood cultures a month and our rate of positives is about five to 10 percent. But the CAP standards require that your contamination rate be below three percent, and our rate in the ED, where the nurses were drawing, was getting high."

Installing Magnolia Medical Technologies' Steripath helped reduce contamination, she says. "The technology was discovered by a pathologist who noticed, when he was analyzing bone marrow, there was an epidural plug. He concluded that the same thing probably happens when we do blood cultures. With Steripath, the first 1 or 2 mL of blood when cultures are being drawn goes into a trap and then the rest of the blood goes into the bottle." Use of Steripath has brought the contamination rates for the ED down to the CAP-recommended level, Cioffi reports.

The challenge of increasing resistance

Dr. McElvania, Dr. Wolk, and Dr. Graf agree that for purposes of treating sepsis, the worldwide concern about the emergence of resistant pathogens is warranted.

"The clinicians worry about the increasing pool of bugs that are resistant to frontline agents, and now those are just going to become more and more prevalent," Dr. Graf says. "We are kind of lucky in the pediatric hospital that we don't see carbapenem-resistant *Enterobacteriaceae* that often. But that's likely going to change. The adult hospitals see them a lot more, and it's just a matter of time before they become more common in our community. With a lack of new antibiotics coming down the pipeline, the array of options we will have for treatment of multidrug-resistant bugs like *Candida auris* and carbapenem-resistant *Enterobacteriaceae* is very narrow."

Pair those trends with an increasing population of susceptible hosts and the problem is compounded. "We're all treating kids more and more these days," she says, "and so our population of immunocompromised hosts is just continuing to grow, and they are much more at risk for these kinds of infections."

Anne Paxton is a writer and attorney in Seattle.