‘Know your panel’: Blood culture ID cautions

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April 2021—The interpretive challenges of blood culture identification panels were the focus of an AMP2020 virtual presentation on false-positives and false-negatives and their sources and solutions.

The spotlight was on *Proteus*, but “it’s not the sole organism we have to worry about,” said Susan Butler-Wu, PhD, D(ABMM), SM(ASCP), director of the clinical microbiology laboratory, LAC+USC Medical Center, Los Angeles, and associate professor of clinical pathology, Keck School of Medicine of USC.

Her co-presenter, speaking on antimicrobial resistance targets, was Richard Davis, PhD, D(ABMM), MLS(ASCP)CM, of Providence Healthcare. (See CAP TODAY, May 2021, for coverage.) Dr. Davis and Dr. Butler-Wu co-wrote a 2020 ASM report titled “Genotypic False Detections from Blood Culture Bottles: Are We Only Seeing the Tip of the Iceberg?”

The *Proteus* problem prompted their report. “*Proteus* was being detected by the BCID,” Dr. Butler-Wu said of the BioFire FilmArray panel, “but no *Proteus* was being isolated from the blood culture bottles.” Such incidents led the FDA to issue a Class II recall.

BD Bactec media were affected in the initial recall in 2018. Subsequent recalls later that year and in 2020 implicated BioMérieux BacT/Alert media.

In a study presented at ASM Microbe 2019, BioFire evaluated the ability of a prototype BCID2 panel with “algorithm and chemistry enhancements to mitigate false-positive results caused by the presence of *Proteus* and *Enterobacteriaceae* nucleic acid in sterile blood culture bottles” (Green J, et al. Poster CPHM-967 presented at: ASM Microbe 2019; San Francisco).

Green, et al., wrote that “sterile blood culture media can contain residual nucleic acid from a variety of bacteria likely introduced from raw materials or manufacturing processes.” They acknowledged that the BCID panel was affected by the presence of nucleic acid that triggers *Proteus* spp. and *Enterobacteriaceae* detection.

In their study, they tested sterile blood culture media bottles from Becton Dickinson (40 unique media lots of six formulations) and BioMérieux (20 unique media lots of five formulations) for *Proteus* spp. and *Enterobacteriaceae* with the BCID and BCID2 panels. Contrived and residual clinical positive blood cultures with *Proteus* spp. were also tested with both panels for comparison. Testing was performed at BioFire and at five clinical pilot sites.

Blood culture media bottles that were positive for *Proteus* by BioFire were extracted and amplified using an independent PCR assay to determine the relative concentration of *Proteus* nucleic acid in the media. Bottles that were negative by BCID were also tested for comparison. The authors found amplification of *Proteus* DNA even in lots that tested negative by the BCID panel. “So it looked like, at least in the lots they tested, that DNA from *Proteus* may be omnipresent,” Dr. Butler-Wu said.

“It’s important to note that this is DNA,” she continued. While the bottles are sterile, DNA present in the blood culture media is being detected. “So this false-positive issue appears to be somewhat of a numbers game. Essentially, if you have enough of the DNA present above the limit of detection, then you’re going to get a positive result.”

The authors found that 29 of 67 sterile media (43 percent) tested positive for *Proteus* with the BCID panel, compared with zero of 67 detections with the BCID2 panel. “These were not detectable with the BCID2 panel because they’ve increased the limit of detection, so it’s not quite as sensitive for the *Proteus* target,” Dr. Butler-Wu said.

Other findings were as follows:

- *Proteus* spp. was detected in 16 of 175 (nine percent) of the additional bottles from development studies at BioFire with the BCID panel, compared with zero of 234 detections with the BCID2 panel.
- *Enterobacteriaceae* was detected in 11 of 175 (six percent) of the additional bottles from development studies at BioFire with the BCID panel, compared with one of 234 (0.4 percent) with BCID2.
- No *Enterobacteriaceae* detections were observed at pilot sites.
- *Proteus* nucleic acid was present at levels ranging from 1×10^2 to 1×10^5 GE/mL in sterile media; levels of 1×10^4 to 1×10^5 were linked to the detection of nucleic acid contamination.
- BioFire testing of contrived *Proteus* positive blood culture samples correctly identified *Proteus* at 1,000- to 10,000-fold below PBC levels ($\sim 1 \times 10^9$ CFU/mL).
- BCID2 detected eight *Proteus* true positive clinical samples confirmed by culture.

Green, et al., concluded that the BCID2 panel was "less vulnerable to false positive detections of *Proteus* and *Enterobacteriaceae* caused by nucleic acid contamination observed in specific lots of sterile blood culture media bottles while retaining a high level of sensitivity that is capable of detecting true *Proteus* PBCs at levels several orders of magnitude below what may be expected in a true clinical sample."

"The unfortunate reality," they added, "is that raw materials used to manufacture media are derived from biological sources that have been shown to contain nucleic acid contamination which may continue to confound molecular diagnostics unless materials are screened for and qualified as nucleic acid free in the future."

Dr. Butler-Wu's laboratory, which uses Bactec media, went live with the BCID panel at about the time of the emerging false-positive *Proteus* problem. "So we had to contend with how to handle this," she said.

Dr. Butler-Wu

Her laboratory's strategy: "We were very, very conservative."

"We don't talk about *Proteus* in our lab when it comes to BCID," Dr. Butler-Wu explained. "There are very few instances where we would even report it. We erred on the totally conservative side. The bottom line was if we were seeing a Gram-negative rod and detecting *Proteus*, we would report it as indeterminate."

Dr. Butler-Wu pointed out that because of the *Enterobacteriaceae* call on the BCID panel, "we were seeing that these would be positive for both *Enterobacteriaceae* and *Proteus*, but it would turn out to be something else entirely."

The lab's conservative strategy worked. By not reporting *Proteus*, "we saved ourselves a lot of angst with respect to use of this panel," Dr. Butler-Wu said. "The first day we went live, we had seven false-positive *Proteus* detections alone. If we had been calling those out on day one of a go-live on a new test, it would have been catastrophic."

Not reporting *Proteus* is not a long-term, sustainable solution, she said, "but it's the one we've employed for now."

In their ASM report, Drs. Butler-Wu and Davis wrote that while a conservative reporting strategy may work for known issues, "it may not be effective when initially encountering a new false positivity issue. Laboratories should therefore always be suspicious for the potential of false positive results any time multiple detections are present and exercise caution when reporting the presence of organisms beyond what is observed by Gram stain."

They suggested that laboratories mitigate the risk of reporting inaccurate molecular blood culture test results by:

- Ensuring the Gram stain matches the results from the molecular test.
- Confirming that organism morphology matches the molecular test results the next day when growth is visible on solid-growth media.
- Reviewing past cultures from the patient (if present) to ensure consistency.
- Reporting any suspected false-positive results to the manufacturer for investigation.

The ultimate solution to the DNA contamination problem is to fix the blood culture media. "However, there's little incentive for manufacturers who are not in the business of producing both blood culture media and identification panels to do something about this," Dr. Butler-Wu said.

Although blood culture manufacturing is required to be sterile, DNA contamination can be introduced into the manufacturing process in many ways, she said. "Blood culture media employ a variety of different plant, yeast, or animal extracts, so there is ample opportunity for organisms' DNA to make it into the product."

The other solution is for manufacturers of amplification-based panels to increase the lower limit of detection, which is what BioFire did with its BCID2 panel.

The GenMark ePlex BCID panel uses nucleic acid amplification and has *Proteus* targets, but "there has not been much indication of a problem," Dr. Butler-Wu said. Still, there are other organisms to worry about.

Several Class II recalls have been issued since 2014 for false-positive microorganism detection associated with certain blood culture media lots when multiplex nucleic acid-based panels are used, she said. The first was "quite worryingly" for *Enterococcus* and *Pseudomonas aeruginosa* detection from BacT/Alert standard anaerobic bottles, and a 2019 recall was for false-positive *E. coli* detections with certain BacT/Alert media.

"This could be complicated by the fact that essentially all commercially available panels can have somewhat variable performance for polymicrobial cultures," Dr. Butler-Wu said. "So typically, we are very conservative in our lab. Essentially, if you can't see an identification by Gram stain, be very wary." Pay attention to the positive results, she said, and look for unusual trends.

To illustrate the challenges associated with false-negative results, Dr. Butler-Wu presented the case of a 72-year-old male who presented to the emergency department with fever and abdominal pain (Fontana L, et al. *J Clin Microbiol.* 2019;57[1]:e00826-18). The patient had a medical history significant for colorectal cancer and had undergone several rounds of chemotherapy and a central hepatectomy with a hepaticojejunostomy. He also had a history of recurrent ascending cholangitis from a biliary stricture.

One month before admission to the ED, the patient had a biliary stent and a percutaneous drain placed. “He has frequently over his history presented with Gram-negative bacteremia that typically was *E. coli* or *Klebsiella pneumoniae*,” Dr. Butler-Wu said.

The patient was febrile on admission and cachectic, lethargic, and diaphoretic on exam. Deep palpation revealed a right, upper quadrant pain but no rebound tenderness.

“They were concerned about obstruction of his biliary drain,” she said. “They obtained blood cultures, and one of the two sets that were sent was positive for plump, enteric-looking Gram-negative rods.”

The laboratory used the Verigene BC-GN panel, “and the organism produced no identification by that panel but was subsequently identified as *Klebsiella pneumoniae* by MALDI-TOF-MS,” she said.

The mystery bug, she said, was *K. variicola,* part of the *K. pneumoniae* complex. “This is an interesting bug because, particularly by phenotypic methods, it’s often called *Klebsiella pneumoniae*, but bloodstream infections appear to have a higher mortality rate than other members of the *Klebsiella pneumoniae* complex.” One study found more than twice the mortality rate (29.4 percent versus 13.5 percent) compared with other members of the complex (Rodríguez-Medina N, et al. *Emerg Microbes Infect.* 2019;8[1]:973–988).

“There has also been an emergence of multidrug resistant strains among the *Klebsiella variicola* species,” Dr. Butler-Wu said. In several published papers, “it’s estimated that *Klebsiella variicola* may actually account for about 10 percent of bloodstream isolates that are identified as *Klebsiella pneumoniae*.”

That the Verigene Gram-negative panel was unable to detect *K. variicola* “was a significant issue,” she said, leading to false-negative results for this organism. “It’s important to know your panel and its limitations.”

A retrospective study performed over 15 months at Huntington Hospital in California found that six percent of 1,044 positive blood cultures were negative by the FilmArray BCID (Ny P, et al. *J Clin Microbiol.* 2019;57[5]:e01941-18).

Those BCID-negative blood cultures were then tested by standard phenotypic identification procedures using the BD Phoenix, and the results included many different anaerobic species, Dr. Butler-Wu said. Among the aerobic organisms the BD Phoenix identified were “quite a few coag-negative staph and a smattering of other organisms.”

“This mattered,” she said. When the authors looked at the time-to-effective therapy and mortality among these patients, they found that those whose blood cultures were positive for on-target organisms had better outcomes. Patients who had bloodstream infections with off-target organisms had lower rates of effective antimicrobial therapy, compared with patients who had on-target organisms, in terms of timeliness, she said.

“And, critically, they also observed a higher mortality rate among patients who had off-target bloodstream infections compared with on-target bloodstream infections”—26 percent versus eight percent.

“You can argue this means we need to, as they say, build a bigger boat,” Dr. Butler-Wu said, noting the latest generations of the Verigene BC-GN, ePlex BCID-GN, and FilmArray BCID and BCID2 panels have dramatically increased the number of organism targets.

But is the boat big enough? she asks, and then answers her own question: It may never be. Huang, et al., studied the ePlex BCID system, which Dr. Butler-Wu noted has the most comprehensive of the available panels. “And even with that, they were still observing six percent of isolates growing in blood culture that were not identified by these highly multiplexed panels” (Huang TD, et al. *J Clin Microbiol.* 2019;57[2]:e01597-18).

“You can never cover every possible organism,” Dr. Butler-Wu said, “but you can cover the majority of them.”

Dr. Butler-Wu would like to see a greater role for clinical microbiology in antimicrobial stewardship, citing two studies in particular. “They involved an antimicrobial stewardship pharmacist in micro rounds, and observed improved patient outcomes, improved time to effective antimicrobial therapy, and several other measures,” she

said of the studies (Sapozhnikov J, et al. *Am J Clin Pathol.* 2021;155[3]:455–460; MacVane SH, et al. *Open Forum Infect Dis.* 2016;3[4]:ofw201).

But she understands the reality. “Long-term, daily involvement in antimicrobial stewardship pharmacy in microbiology rounds may not always be feasible,” Dr. Butler-Wu said. “Not all labs do microbiology rounds, and often clinical teams may be too busy to attend.”

She therefore proposes another option: targeted discussions focused on positive blood cultures with off-panel positive results. “They can be reviewed carefully by a microbiologist and that information provided back to the stewardship team.”

She asks: “Could there be greater involvement of clinical microbiology directors in interpreting Gram stain results in the context of these off-panel positive blood cultures? And is this a way to meet that gap, where we know we’ll never have panels that can catch absolutely everything, but we, through our own skills, can get a sense of what might be going on with the specimen and relay that information to our colleagues in stewardship?”

A meta-analysis (31 studies consisting of 5,920 patients) by Timbrook, et al., revealed that the odds ratio for mortality risk was significantly lower for patients who had undergone rapid blood culture identification (Timbrook TT, et al. *Clin Infect Dis.* 2017;64[1]:15–23).

“This includes detection of resistance markers in addition to the identification,” Dr. Butler-Wu added. “And a critical point for labs is that this benefit, in terms of reducing the odds ratio for mortality risk, was only observed [with] and appeared to be dependent on antimicrobial stewardship. There’s no point in doing rapid testing in a vacuum if it’s not combined with our ID pharmacy and stewardship colleagues. It’s going to be a waste of time and have minimal impact.”

“So rapid identification and timeliness of results matter, but only if you’re combining it by getting the results to the right people who can act on it.”

Dr. Butler-Wu concluded with a “shout-out” to the Gram stain: “The Gram stain is our very best friend. As clinical microbiologists, we have to balance what we can see versus what we can detect, know our panels, and relay that information effectively to our clinician colleagues.”

“You have to be ready for anything in the blood culture identification business,” she said.

Amy Carpenter Aquino is CAP TODAY senior editor.