Keys to curbing tube interference with test results

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Dr. Donato

November 2013—There's nothing flashy about specimen tubes, which may look like the most mass-produced, commonplace items in the laboratory, but appearances can be deceptive. All tubes are not created equal. "We know that preanalytical errors account for the majority of errors in the laboratory, and many of those errors derive from the tube type in which you collect your sample," says Leslie J. Donato, PhD, co-director of the hospital clinical laboratory and point of care at the Mayo Clinic in Rochester, Minn.

It's a fact that Dr. Donato believes is lost on many phlebotomists and even some laboratorians. As she outlined in a presentation at the American Association for Clinical Chemistry meeting in July and in an interview with CAP TODAY, the specimen tube plays quite a large role in the quality of laboratory results.

Serum and plasma, of course, have different strengths as specimen types, and Dr. Donato categorizes the potential differences in test results they produce in two separate bins. "One is just the inherent difference between analyte concentrations in serum versus plasma. The second is associated with the interference that can be caused by tube additives, mostly those in plasma tubes," she says.

A range of potential problems can arise. For example, different anticoagulants can interfere with certain assays that are run in the laboratory. At Mayo Clinic, heparin tubes are the tube of choice in stat collection areas. "But heparin inhibits DNA polymerase, so for any test that performs PCR, you wouldn't want to use a heparin sample," Dr. Donato says. Another example of interference: With the wrong order of draw for a patient for whom multiple tubes are ordered, additives from one tube may cross to the next tube and affect results.
Dr. Donato describes practical ways in which her laboratory has been able to use specific specimen tubes to remedy common interferences with the quality of test results. One of the most effective solutions was the laboratory's approach to reducing cardiac troponin "flyers," or false-positive results.

The problem came to light because "physicians in our practice were calling with concerns about positive results they felt were not real," or a result didn't match other profile results. For example, a cardiac troponin panel would have a non-detectable result at time zero, a positive result at three hours, and be back to non-detectable at six hours. "This really doesn't make sense, with what we know about the physiology of troponin release upon myocardial infarction, and it doesn't fit with the decrease in troponin after an MI event either."

Both TnT and TnI flyers stem from preanalytic processing issues that interfere with immunoassays designed to measure low abundance proteins, she notes. "The goal of manufacturers is to develop assays that will measure troponin at very low concentrations. We want to detect elevations as sensitively as we can, but because of that desire for increased sensitivity, it has been suggested that these assays are susceptible to interference from debris in the samples, perhaps fibrin strands or activated platelets."

The laboratory initiated re-run processes for all positive TnT plasma baselines and all three- and six-hour samples that had significant deltas (defined as a greater than 0.02 ng/mL difference). Interestingly, when the laboratory would repeat the test on the same plasma sample, "infrequently we would observe a positive plasma sample

would be negative upon repeat at a later time, and we thought this might indicate that debris could be settling in the sample before it was repeated," Dr. Donato says.

The advantage of plasma over serum is that it is readily analyzable, so it's suitable in an emergent situation where a patient might be having a heart attack. But since serum is known to give a cleaner sample, the laboratory thought it might address the problem of false-positives by using rapid serum tubes (RST), a recently developed BD Vacutainer that contains thrombin, a strong clot activator, designed to speed up the clotting process so it occurs within five minutes.

To validate the use of this tube, the laboratory didn't want to interfere with the test order process in the emergency department by asking the ED clinicians to take a duplicate troponin sample in an RST. "What we did instead was to validate the RST for our chemistry testing just for the evaluation period," Dr. Donato says. Since the physician typically orders a troponin sample in a plasma tube, and a chemistry panel at the same time, the laboratory simply used the RST for the chemistry panel and used residual serum to run another troponin. "So we had paired troponin testing, but we weren't asking the ED physicians to change their processes."

In this way, the laboratory was able to compare the TnT run on the routine plasma separator tube with the TnT on the residual RST sample on the chemistry testing (Clin Biochem. 2012 Jul;45[10-11]:842–844). "After implementing the RST for all troponin testing, we then reevaluated our flyer rate by again repeating all positive troponin results for an extended time. When we compared the rates of false-positives [defined as likely to lead to clinical action] in the two matrices, we identified that the RST reduced the rate by about 50 percent over the rate we saw with the plasma separator tube." False-positive errors dropped to about one in 4,000, a .02 percent error rate.

However, the downside is that rapid serum tubes are about four times the price of serum separator tubes or plasma separator tubes. "They are significantly more expensive, and we've only implemented them on our troponin assays, not for any other chemistry that we do," Dr. Donato says. "So it's understandable that other practices may not have the financial resources to use the RST tubes. But with our reduced false-positives, we consider it a justifiable cost in our practice."

In another case, Mayo Clinic again conducted its own research to see why it had such a high rate of hemolysis in its neonatal intensive care unit. The need for redraws of many specimens in the NICU is a systemic issue at all hospitals, Dr. Donato points out. "You're dealing with tiny babies who are very sick, and the chance of having problem draws or unacceptable specimens is very high in this population."

In a 2011 study, Brad Karon, MD, PhD, at Mayo Clinic correlated pediatric cases of in vitro hemolysis with infusions of total parenteral nutrition (TPN) with lipid emulsions (Clin Biochem. 2011;44:254–256). "They added a lipid infusion matrix to patient samples and observed an increased amount of plasma hemoglobin when the sample was placed in an evacuated gel tube as compared to a syringe. So we think the lipid emulsion causes the red blood cells to be more fragile. In our case, using an evacuated gel tube was causing more sheer stress on the RBCs that resulted in hemolyzed samples," Dr. Donato explains.

In a larger evaluation to identify the root cause of hemolyzed specimens in the NICU, two clinical chemistry fellows at Mayo Clinic, Nicole Tolan, PhD, and Erin Kaleta, PhD, also found an association between lipid emulsion and hemolysis. "We found most collections occurred via arterial line draws, a practice we are reducing," Dr. Donato says. "But also, a majority of all the patients with hemolyzed samples were receiving IV fluids. Patients on TPN and lipid infusions, or TPN and lipid infusions with saline, make up most of the cases."

To reduce hemolysis in patients who are receiving lipid emulsion, "we know that the sample is going to be fragile," she says, "and we have tried to implement a variety of methods to reduce disruption of these fragile samples, from hand carrying of those samples to the laboratory to slowing the rate of draw from a line collection." The most beneficial practice change in this setting was asking the care team to pause lipid infusion in NICU patients for an hour before blood is drawn during morning sweeps. "The combination of these efforts has reduced our hemolysis rates significantly, from about 6.4 percent to 4.8 percent."

Like many laboratories, Dr. Donato's has a separate initiative to reduce or eliminate the use of arterial or venous line draws throughout the hospital. "We have found in our practice that these types of collections will result in much higher rates of hemolyzed samples, so we've tried to discourage the practice of drawing from the lines." In general, venipuncture samples are much less at risk than line draws (Clin Biochem. 2012;45:1012–1032). Recently, Darci Block, PhD, director of phlebotomy services at Mayo Clinic, completed a quality improvement project that discontinued the practice of IV start blood collections (with few exceptions), which has resulted in a decrease in the overall redraw rate in the ED from four to five percent to less than two percent.

Throughout her practice, Dr. Donato says, if there is a patient with problems with repeated hemolysis, not just a newborn but anyone with a severe clinical condition where the cells are fragile, "we've implemented the most gentle procedures possible to collect that sample. We instruct our collections phlebotomists to collect in a non-gel evacuated tube or a lithium heparin syringe in which you can gently draw the sample in an effort to prevent in vitro hemolysis. If all other sources of in vitro hemolysis are removed and collecting the sample in this manner doesn't reduce the hemolysis, then in our experience ordering additional redraws is most likely fruitless."

Trace metal testing for such elements as aluminum, chromium, and manganese is another area where Mayo Clinic has found the choice of specimen tube can be critical and testing requires extreme caution. "It's very important if you're doing any metal testing that you collect the sample in a certified metal-free tube, and that's because trace metals are everywhere," Dr. Donato points out. It has long been known that the collection material can be a source of trace metal contaminants (Clin Chem. 1971 Jan;17[1]:61–62). Additionally, potential metal surface contaminants include disinfectants like Betadine, cosmetics, perfumes, deodorants, glass cleaners, and just soil and dust.

Mayo's reference laboratory is one of the few that perform this type of testing, which has been ordered more often in recent years because chromium and cobalt testing, in particular, is used to detect failures of prosthetic devices such as metal-on-metal artificial hips. "Serum chromium testing has gone up quite a bit over concern in the field for whether these devices are in good condition."

Collecting a specimen for such a test, however, can be tricky because there are so many potential contaminating sources for a sample, Dr. Donato says. "To avoid having a contaminated sample, we verify that our collection tubes are metal-free. We send these tubes out to our clients with special instructions for collecting their sample because the materials used in collection and the location of collection are really key to minimize the amount of contaminating trace metals. If it's collected exactly as it should be, and everything is strictly adhered to, then the chances of getting a falsely elevated serum chromium are much reduced." This applies equally to other trace metal testing, she adds.

Finally, to stem some of the possible errors with tube types, one additional strategy is to implement flags in the laboratory information system or middleware. "You could implement rules that detect patterns of extremely high results or extremely low results, to trigger an investigation of a potential problem, such as contamination from wrong tubes, additives from a tube, or even diluted results if it's a line collection." This strategy would be most useful where a combination of results is used, since delaying reporting of results just because one analyte is very high is not always desirable in the critical care setting, she says. "A glucose value of 1,000 should probably trigger an investigation, but a critically low calcium can occur in an extremely sick patient and doesn't necessarily mean the sample is contaminated with EDTA. However, an undetectable calcium, magnesium, and alkaline phosphatase from the same sample might warrant investigation."

The key thing to remember is that order of draw and the correct specimen tube play a huge part in quality lab results, Dr. Donato emphasizes. "We've seen incidents that seem absurd, but do happen, where a collection person notices a test was collected in the wrong tube and simply pours it into the correct tube." Obviously there's a disconnect there that is cause for concern, she says. "We really need to understand that the whole collection process has to be followed correctly to achieve high-quality test results."
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