Steps to preventing coag test processing error

Anne Paxton

February 2022—It was Isaac Asimov who surmised: “The most exciting phrase to hear in science is not ‘Eureka!’ but ‘That’s funny . . .’” And it was coagulation processing in the clinical laboratory that, in a small way, illustrated Asimov’s axiom for Dorothy Adcock, MD, former chief medical officer, Labcorp Diagnostics.

One of her favorite stories is about an astute medical technologist who told her that when a patient who was on unfractionated heparin came in and was drawn and the tech wasn’t busy, the patient was always therapeutic. But if the technologist was busy and the sample sat around, the patient was always subtherapeutic.

“That’s when we did a study showing that unfractionated heparin is not stable in the primary tube, because the platelets release a neutralizing factor that neutralizes the heparin,” Dr. Adcock says. It was a useful finding for laboratory quality control—and confirmation of something else, she adds: “Coagulation can be tricky, and sometimes the unexpected happens,” especially in the preanalytic phase.

“Sometimes you have to be like Sherlock Holmes in the coagulation laboratory,” Dr. Adcock says. “You have to look. You have to be wary.” As a rule, “you will catch more errors if you are suspicious.”

From ambient temperature variations and centrifuging delays to interfering substances, jostling, and G-forces, coagulation specimens can encounter a plethora of post-collection hazards that can, figuratively speaking, make tests run aground, leading to inaccurate results.

As noted in a set of recommendations published in the *International Journal of Laboratory Hematology*, coauthored by Dr. Adcock and based on published data in peer-reviewed literature and expert opinion, preanalytical coagulation testing errors, including inappropriate or problematic blood sample processing, are surprisingly common (Kitchen S, et al. *Int J Lab Hematol.* 2021;43[6]:1272–1283).

The new recommendations for reducing preanalytical error that occurs post-collection, developed by the International Council for Standardization in Haematology (ICSH), highlights the most common risks in transport, storage, and processing of citrated coagulation specimens and provides guidance on how to avoid them.

Areas covered in the 23 recommendations include pneumatic tube systems; clots in citrated samples; centrifugation; primary tube storage and stability; interfering substances including HIL (hemolysis, icterus, and lipemia); secondary aliquots and their transport, storage, and processing; and preanalytical variables for platelet function testing. (The authors’ recommendations on collecting blood samples for coag testing were published first: Kitchen S, et al. *Int J Lab Hematol.* 2021;43[4]:571–580.)

Compared with specimen collection, “there are a lot more variables” in the processing stage of coagulation testing, says coauthor Richard A. Marlar, PhD, medical director, coagulation laboratory, University of New Mexico Hospital.

Still, the laboratory can monitor and adjust more steps of processing than of collection. “The majority of laboratory testing errors occur at the preanalytical phase,” Dr. Adcock says, “because it’s a complex, multistep process and it can’t be controlled at each step.” Once a specimen gets to the laboratory, “we can control more things there. We have instruments that are quality controlled. We can look at results post-analysis.”

There are objective standards that should be followed, she notes. “The sample should be transported at ambient temperature, not refrigerated. It should be spun to be platelet-poor. And you shouldn’t have a clot in the sample.” Things that can be done to reduce error by automated methods are valuable in making sure standards are adhered to, she adds. “The majority of our errors are caught that way, the tricky errors certainly.”

But, as with collection, important parts of the processing phase occur outside the laboratory’s control. For example, some processing errors stem from specimen collection, Dr. Adcock says. “Probably the most common issue we see

is that the sample has been incorrectly collected in the wrong primary tube, and then it's put into a secondary tube. We receive it at the reference laboratory and we're not aware of whether the correct tube was initially collected, and that can dramatically influence the results."

Most coagulation specimens should be collected into sodium citrate tubes, the light blue top tube, she says. "If, however, you are collecting plasma, there are other tubes, not just citrate tubes. And another that's commonly used is an EDTA tube. So if the sample is incorrectly collected into EDTA to obtain plasma and sent to the coagulation laboratory, that EDTA plasma can perfectly mimic a factor VIII inhibitor. And that's a serious diagnosis that can be a life-threatening disorder. We've seen this happen multiple times."

"A primary tube may come in as the red serum tube or the blue top tube, but as soon as they take it out of the primary tube and put it in that generic secondary tube, we don't know what the primary tube was," she says. Some laboratories, including Labcorp, characterize samples that come to the lab in secondary aliquot tubes, Dr. Adcock notes. "But most labs don't do that."

Because EDTA samples are characterized by having an extremely low calcium, a simple way that Labcorp screens them is to look for the presence of calcium in the sample. "If there's essentially no calcium present, then we don't test the sample for coagulation. We know it's EDTA. We run a PT and a PTT on every sample as well to look for other possible interfering substances."

Labcorp adopted this practice when it acquired Colorado Coagulation where Dr. Adcock was formerly medical director. "Colorado Coagulation was very boutique. We could handle each specimen with kid gloves because we had a limited number of samples and very experienced techs. And we took some of our high-quality processes to Labcorp and implemented them in a high-throughput coagulation laboratory. So that's when we built these algorithms in the LIS to avoid errors from testing the wrong sample type."

To avoid those errors, vigilance is also a necessity, she says. "Factor VIII inhibitor or factor VIII deficiency, one or the other, could cause prolongation of the PTT, and the PT should be normal. But if the PT is prolonged, something's wrong. And in an EDTA sample, both the PT and PTT are prolonged. So the medical technologist's sensor should go up that something is wrong." Sometimes the laboratory information system can use algorithms set up to catch certain combinations of results.

Dr. Adcock has long recognized the importance of international standards to make certain that such errors are caught and corrected. She started publishing on standardization in coagulation in the 1990s. "It appeared to me that many of the variables that we accepted as truth are not evidence based. And I wanted to make sure there was evidence behind each practice."

She and Dr. Marlar were involved in developing the last set of standards, a CLSI guideline published in 2008. "It was an acceptable document," she says. But trying to make good laboratory medicine practices available worldwide has always been the goal. And with the new ICSH recommendations, she is of the view that the goal has been accomplished. "I have been working with many of the individuals who wrote the ICSH document for decades, and we've in fact corroborated our findings across continents," she says. Dr. Marlar agrees: "These are basically standards for the world," he says.

Transport of coagulation specimens to the laboratory presents many risk factors, especially when pneumatic tubes are used, Dr. Marlar notes. At his previous institution, there were two hospitals with one pneumatic tube system between them. "It was quite a distance that the samples had to be transported, so we had to make sure they were packed tight and wouldn't wiggle around in the tube. Of course, sending by tube shakes everything up. I think some tubes go faster and harder and stop faster and harder than others. So every laboratory needs to validate or at least verify the samples coming through."

Anything dealing with platelets or platelet release products should not be sent through a pneumatic tube, he says. That's one reason he sometimes gets concerned when samples are sent through for heparin analysis. "Because if the platelets rupture and platelet factor 4 is released, you can end up neutralizing some of the heparin. So every

lab needs to validate or verify that their tube system is working and still giving correct answers."

Hospital architecture can significantly affect the transport component, Dr. Marlar says. "We used to have more vertical hospitals where you were moving the specimen from the fifth floor or seventh floor to the second floor. But now, as hospitals get more horizontal and you have outpatient clinics that are a ways away and the specimens are still being transported by tube systems, you may have more issues."

The current plan at his hospital is to add a new seven-story tower. "The tubes will now need to travel down seven flights, then another 200 meters over to the lab. So we're talking quite a bit of distance, and we'll have to revalidate our pneumatic tube system when we start that."

In developing nations, there can be even bigger challenges. A massive hospital that he visited in Asia, the largest he had ever seen, drew all blood in a building a quarter mile away from the lab and, lacking a pneumatic tube system, relied on hand-carrying for transport—unfortunately through heat often in the 90s.

"So if the samples weren't adequately packed to maintain room temperature," he says, "who knows what happens to the samples?" The laboratory should have some say in laboratory site decisions, and further recommendations on the distance between draw site and processing site might be important to make as well, Dr. Marlar says.

Laboratories may want to test two specimens, he says, one hand-transported and the other through the system. "Some studies have encountered a greater than 10 percent difference" in the result, he says. "If the difference is less than 10 percent, it's considered the same. But you have to do a very good study to make sure you're not getting erroneous results. You have to pick the tests that are the most fragile, if you will, and make sure they are not disrupted or erroneous in nature."

Dr. Marlar

A number of post-collection factors can lead to hemolysis. Too much shaking of the sample or getting it too hot or cold may cause damage, and Dr. Marlar believes all tubes should be checked if possible. "Sometimes that's impractical now that we have very large hospitals, very large labs. But we have to be aware of problems when a PTT sample is short. For example, we try to check every sample that comes in if we can, and I think every lab should try to do that. Unfortunately, that requires popping the top off the tube, which can be a hassle."

But, he adds, "We don't have a lot of ability to say there's a clot present in the sample, without automated instruments that may be able to detect clots. If the sample doesn't make sense, if it's shorter than they expected it to be, if it's a heparinized sample and the PTT should be up in the 90s but it's normal, the technologists should be checking into what's going on."

Hemolysis affects PTT results more often, Dr. Adcock says. "Most factors, factors that are PTT based, have a limited stability compared to PT and that's also true of hemolysis. It can affect both PTT and PT, although PTT tends to be affected more."

After the freeze-thaw cycle is when a fibrin clot typically appears, she notes. "A red clot is a typical clot and you often see one in the primary tube. But if you receive your samples as a secondary frozen aliquot, and many do, you can still potentially reject a sample because of a fibrin clot, which is a pale clot. If you see that pale gelatinous clot post-thawing, that's also cause for rejection."

Interfering substances or HIL—hemolysis, icterus, and lipemia—"are one of the biggest problems we have," Dr.

Marlar says. One of the nice things about the newer instruments now coming out is that they have a system for detecting the levels of HIL, he notes. “If the instrument says the icterus is above the range that the manufacturer provides, the machine will flag it and you can set it up to ‘pending’ and it won’t run at all. Or it can be run and the technologists can make the decision whether to release the result.”

Adjustments may be needed depending on patient population. Because many liver patients are seen at the University of New Mexico Hospital, “our instrument had a lower threshold than what we would like for icterus,” Dr. Marlar says. “So we validated samples with high icterus levels so we can now report out results that are higher for these liver patients who have much higher bilirubin levels.” It was a big plus for the gastroenterologists and patients to have those formerly flagged samples analyzed, he adds, “so we would be able to report them.”

A relatively new addition to the latest ICSH guidance is platelet function testing. When performing platelet function testing, “you’re no longer dealing with proteins; you’re dealing with cells, which are a lot more finicky than a protein. You can disrupt a platelet much more easily than you would a protein,” Dr. Marlar says.

Most previous guidance documents didn’t discuss platelet processing, he says. “We kind of just ignored that and stuck with coagulation, but now it’s becoming an important testing area and we have to do it right.” Specifically concerning the processing phase of platelet function testing, the ICSH guidance includes recommendations on centrifugation, ambient temperature, and time limits.

For centrifugation generally, the biggest problem is not sufficiently spinning down specimens and having too many platelets, Dr. Marlar says. A sample with 20 to 25,000 platelets per mL in it can sit for three or four hours and the platelets may break and the heparin gets neutralized. “We can have artifacts generated that way too,” and if the centrifuge has a cooling system and it cools the sample down too low, “then we get an underestimation on the heparin sample.”

That’s where the standards come in, he says. “We try to make every sample process the same way. That doesn’t always happen but that’s our goal.”

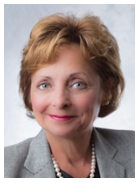
Some coagulation factors are not stable over time, Dr. Adcock notes. “For instance, if you receive a sample on Monday and the doctor calls you on Friday to ask, ‘Can you add a factor VIII to that sample?’ Well, no, you can’t. Because factor VIII is not stable. It is notorious for decreasing over time. So you have to have stability information in your standard operating procedures. Certain assays can be performed and certain assays can’t be performed, because some factors are stable and some are not.”

If a sample is processed within the laboratory’s validation, or the verification based on the literature, “I think you’re okay using the primary tube for storage up to that point,” Dr. Marlar says. “After that, I am reluctant to go get a sample out of the refrigerator that’s been there for 24 hours and come back and do specialized testing.”

In their conclusion, the authors of the ICSH recommendations call for acceptance/rejection protocols that are balanced. Not all questionable results should go unreleased, Dr. Adcock explains. “Even if a sample doesn’t meet your criteria, there can be exceptions. The lab needs to balance the risk of releasing unsafe or misleading results against the risks associated with rejection. So you could go ahead and release the results in several instances.”

“For example, PTT-based samples are subject to more variation than PT-based results. Say a sample coming to you for PT INR rolled off the lab table and was found on the floor 24 to 48 hours later, so it’s out of stability. The lab tech tests it anyway. The patient’s always been therapeutic and you look at previous results. It may be best just to report that therapeutic result rather than have the patient come in again.”

Another example is antithrombin. “Antithrombin is really stable, and if a sample is eight days old, rather than the stated typical stability of seven days, the antithrombin result is still good. Also, if you know that a sample beyond stability will falsely elevate D-dimer and the D-dimer result is below the laboratory’s or manufacturer’s determined cutoff, you’re still good.” Those types of situations should always be taken to the medical director, she says, so they can make the ultimate decision about reporting.



Dr. Adcock

One could reject a sample that doesn't meet criteria, Dr. Adcock notes, "but our *International Journal of Laboratory Hematology* article is saying that there's always an exception and you have to consider the impact on the patient of performing the assay or rejecting the sample."

The last recommendation of the ICSH guidance is that all coagulation labs establish written policies on transport, storage, and processing of both primary tubes and secondary aliquots. Dr. Adcock does not see that as having much impact since it is part of good laboratory practice to have such policies. But now that this new guidance is out, she suggests that laboratories review current practices to be certain they are in keeping with the guidance.

The ICSH recommendations are based on evidence, on published, peer-reviewed research by experts, Dr. Marlar says. Clinical labs processing citrated blood samples for coagulation tests need to look at these recommendations, check the accreditation requirements for their country or state, then focus on what they are doing that is different. "Then they need to find out how to change what they're doing, or they should document that it's being done correctly and they are not getting potential erroneous results."

The recommendations are meant to apply in all regions of the world, Dr. Adcock says, and she has found that reaching an international consensus has not been problematic. In working with standards experts in Italy and Australia, she has found that "we speak the same language. The samples are the same, as are things that we've verified in our laboratory and they've verified in their laboratories. And it was rewarding early on in our interactions because we had the same battles, the same issues, the same resolutions."

How much adjustment will be needed by laboratories to meet the new ICSH recommendations? "If institutions have well-described processes and if they're consistent with the current guidance, then all should be good," Dr. Adcock says.

But, she says, laboratories will have the best results if they don't make assumptions. To ensure that coagulation processing is up to the mark, she says: "Read the guidance document, look at your processes, and make sure they are being followed as part of good ISO practice. And always do what you say you do."

Anne Paxton is a writer and attorney in Seattle.