In coag collections, every detail counts

Karen Titus

September 2021—Rare wine? Delectable. Rara avis? Magnificent.

Rare blue-top collection tube? Uh oh.

For Richard Marlar, PhD, coming across a non-FDA-approved tube was an unhappy discovery. Dr. Marlar, medical director, coagulation laboratory, University of New Mexico Hospital, says his lab was among the first to encounter one of these rogue tubes, available for purchase on the internet and likely taking wing due to pandemic supply shortages.

When the tube arrived for testing, it quickly kindled concerns, says Dr. Marlar, also a part-time professor of pathology, UNM School of Medicine. “It’s a tube we had never seen before. It looks like it has a CE mark on it, and the Europeans don’t know anything about it. It has a label on it that suggests it’s FDA approved—but the FDA is not aware of it,” he says, adding that his lab has spoken with the agency.

It feels like a “CSI”-tinged moment in a venue that labs would prefer to keep drama-free. It also points to the ongoing need to keep a keen eye on what passes through coagulation laboratories. It’s not so much that the devil is in the details; rather, that’s where accurate results lie.

So important are these details that they’re the focus of a new set of recommendations from the International Council for Standardization in Haematology on collecting blood samples for coagulation testing (Kitchen S, et al. *Int J Lab Hematol.* 2021;43[4]:571–580). As the report notes, one third to three quarters of laboratory errors are linked to the preanalytical phase, with coagulation testing accounting for many of the woes.

The recommendations were last updated in 2008, says Dr. Marlar, who helped author the current document. While numerous individual publications on the topic have emerged in the last decade-plus, “We wanted this to be an evidence-based set” of recommendations, he says. A subsequent document, authored by largely the same group of experts, focuses on processing coagulation samples and is likely to be published by year’s end, if not before.

Collecting and preparing samples for coagulation testing is a surprisingly treacherous endeavor. “Chemistry, immunology, hematology—they’re much more forgiving than what we see in coagulation,” Dr. Marlar says.

The runway leading to the coag lab is long. Ground turbulence can occur anywhere along the way when personnel don’t follow strict protocols regarding fill volumes, matrix, length of time to processing, order of draw, tourniquet use, ordering systems, etc.

“The preanalytic phase is the hardest phase to control because it can occur in so many different regions of patient care,” says ICSH recommendations coauthor Dorothy Adcock, MD, former chief medical officer, Labcorp Diagnostics, and, before that, medical director, Colorado Coagulation.

Dr. Richard Marlar and Dr. Dorothy Adcock helped write the ICSH recommendations for the collection of blood samples for coagulation testing. “I’ve always been surprised, with coagulation testing in particular, at the impact some variable may have,” Dr. Adcock says.

The impact on medical decisions is real. After decades in the field, Dr. Adcock has seen plenty. But her encounters with the unexpected have yet to end. “I’ve always been surprised, with coagulation testing in particular, at the impact some variable may have.” The stories get told in various ways, each with its own particulars, but the theme is constant—pay attention to the fine print.

When she began her career, in the 1990s, “One of our really astute techs—and I love how much I’ve learned from our med techs—mentioned to me that when she got to the patients’ samples soon after collection, the heparin level was significantly higher than if she had the samples sit on the bench for a couple of hours.”

That observation led Drs. Adcock and Marlar to investigate and publish a study (Adcock D, et al. *Blood Coagul Fibrinolysis.* 1998;9[6]:463–470) about the release of platelet factor 4 in citrated blood post-collection and its neutralization of heparin. “Which is why, when you’re collecting a sample, for unfractionated heparin, you need to process it, centrifuge it, within an hour, and test it within four,” says Dr. Adcock.

Cue the chorus: “That was such a surprise to me,” she says. “But it had to be investigated. Because you just never know what the impact will be.”

That story is set nearly a quarter of a century ago, which practically qualifies as ancient history in medicine. But the lesson continues to reverberate—in coagulation testing, doing without thinking can lead to errors. That’s why, says Dr. Adcock, this most recent document is “a good reminder to review your blood collection processes, especially for coagulation samples, and make certain they’re in line with current guidance.”

The recommendations are based on what has become a fairly robust literature, which is a welcome shift. For many years practice has been heavy on tradition. While that might work for, say, the Royal Family, it’s not a basis for high-quality coagulation testing. Or as Dr. Marlar puts it: “We found that that doesn’t necessarily work.” For example, one long-standing habit was to use a discard tube, which, as it turns out, mostly serves to waste patient blood and increase costs.

He and Dr. Adcock, along with others, have published studies on this, as the recommendations make clear (Adcock DM, et al. *Laboratory Medicine.* 1997;28[8]:530–533; Lippi G, et al. *Blood Coagul Fibrinolysis.* 2012;23[6]:572–573), and recommendation No. 5.3 dispenses with a general requirement to discard the first volume of blood collected, unless it’s collected via butterfly needles or indwelling catheters, or when the sample is used for platelet function

studies.

Other studies have looked at the impact on laboratory values of underfilling tubes, “whether it’s by 50 percent, 80 percent, or what have you,” says Dr. Marlar. ”We’ve started doing research, and a lot of issues are emerging. We’re still working on it. We’re still learning.”

Collection tubes might come across as the steady, unsung gal pals of coagulation testing, but in reality, they can be quite the divas. Labs that ignore them do so at their patients’ peril. Tubes can vary slightly—between 3.1 and 3.2 percent—in their exact sodium citrate concentration. They can be made of siliconized glass or different types of plastic. The composition of their stoppers varies, too. As Dr. Adcock notes, some stoppers may leak magnesium, which has been shown to influence results. And not every vacuum is sufficient to draw the correct amount.

It has taken time for laboratories to recognize these differences. When the INR was being rolled out in the late 1980s, Dr. Adcock recalls, two concentrations of sodium citrate were in common use: 3.2 and 3.8 percent. While the big box containing the hundred evacuated tubes was labeled, she says, individual tubes were not. “Some laboratories were not even aware there was a difference between these two concentrations,” and test orders would be randomly placed for each type.

When she and Dr. Marlar published on this in 1997, she says, one major tube manufacturer stepped up and began indicating the citrate concentration on individual tubes. When reagent manufacturers determine the ISI, or International Sensitivity Index, for each reagent, she says, the overwhelming majority of them perform that testing in 3.2 percent citrate or its equivalent. Size may not matter, but concentration does.

The ICSH document reflects this hard-won knowledge. Recommendation No. 3.2 calls for citrated blood to be anticoagulated with 105–109 mmol/L (3.1–3.2 percent) trisodium citrate, barring other indications. The next recommendation, No. 3.3., recommends against using 129 mmol/L (3.8 percent) trisodium citrate.

One of the most common errors, Dr. Adcock says, is using the wrong matrix when submitting samples for hemostasis testing. “That can significantly impact results.” (See “Matrix effect.”)

She describes encountering one case involving a 70-year-old woman, needing a hip replacement, with no previous history of bleeding. The APTT was prolonged and did not correct with a mixing study; it showed some prolongation with incubation, and a low—3.8 percent—factor VIII.

After her lab received the sample for confirmation of a factor VIII inhibitor, it ran a PT and PTT to check the sample integrity. Both were prolonged. “This pattern of results is very typical of EDTA but is not what you would expect to see with a factor VIII inhibitor,” says Dr. Adcock. “And EDTA as a sample matrix can perfectly mimic a factor VIII inhibitor.”

Matrix effect

Assay	Sodium citrate plasma	EDTA plasma	Serum
APTT (sec)	29	68	>180
PT (sec)	12.4	23	>60
FVII Act (%)	115	116	308
FVIII Act (%)	141	4.5	4.5
FIX Act (%)	122	115	350
VWF:Ag (%)	122	143	101
VWF:RCo (%)	114	131	74
PC Act (%)	111	152	< 1
PS Act (%)	96	30	< 1

Courtesy of the special coagulation laboratory, Labcorp.

EDTA plasma

PT & APTT:
- Prolonged but measurable

Mixing studies:
- Lack of correction
- Mimics a time-dependent inhibitor

The ICSH authors note this “important and highly specific preanalytical problem,” explaining that citrated plasma can be contaminated with other additives such as EDTA or activated factors from serum, either through inadvertent cross-contamination between tubes during venipuncture, or by filling a coag tube with EDTA blood or serum.

Dr. Adcock also recalls the case of a medical technologist who had been on warfarin for six months, but four weeks after it had been discontinued, pre-surgery bloodwork indicated a prolonged PT (27 seconds) and PTT (42 seconds). “They asked, could this be some sort of prolonged warfarin effect?” Dr. Adcock says. “Well, no, it couldn’t.”

The technologist, concerned about the results, called Dr. Adcock. After learning that the phlebotomy was difficult and required multiple blue-top tubes, Dr. Adcock suspected the phlebotomist had combined two underfilled tubes. The excess sodium citrate would prolong the results. When the technologist was redrawn—"in our lab, under my own eyes," Dr. Adcock says—the repeat PT and PTT were normal.

The recommendations are a tidy summary of the state of coagulation collection, bringing together the past, present, and future.

"Many of the recommendations have been around for a long time," Dr. Marlar says, but have yet to widely sink in.

"There are stubborn problems," Dr. Adcock agrees. She recites the familiar stanzas: "Hemolysis is a big problem. Sample clotting is a big problem. Inappropriate blood to anticoagulant ratio is another problem."

In some cases, the recommendations break fresh ground. "We've modified our ideas over time, and I think that happens with anything," Dr. Marlar says. In the past, there was an absence of guidance on how long to apply a tourniquet. "Now we're saying no longer than two minutes," says Dr. Marlar, referring to recommendation No. 6.1. When the tourniquet is in place longer, it can affect test results, including hemostatic proteins stored in vascular endothelium.

That two-minute mark is not the goal, adds Dr. Adcock. "Really, it should be taken off as soon as you are able to begin collecting blood through an evacuated tube system."

Laboratories also struggle with fill volumes. Some 5-mL tubes, for example, are designed to take only 3 mLs of blood, giving it the appearance of being just over half filled, says Dr. Marlar. "We occasionally find those tubes where someone says, 'Oh, it's not filled. I'm going to put more blood in it.' And then they overfill it," as if topping off a car's gas tank.

Then there's the order of draw, although Dr. Marlar says it appears the tide is turning on this particular problem. Recommendation No. 7.1 lists the ideal order for filling multiple tubes, starting with the blood culture tube and ending with so-called other tubes (trace elements, for example). This lowers the risk for cross-contamination, although the authors note that the current generation of evacuated blood collection tubes has nearly stamped out this problem.

Remember, says Dr. Adcock, collection devices—the preferred term, she says—should be viewed as an integrated system, comprising the holder, tube, and needle.

Ideally, all these components are from one manufacturer. This, in Dr. Adcock's view, is an important element of coagulation sample collection. "They're actually a pretty complex system. If you change a component of that system, you should test and validate it," which is addressed in recommendation No. 3.5. In that regard, a collection tube is no different from a reagent; just as tests have to be validated with a new reagent, the test also needs to be validated when the collection tube manufacturer changes. "I think that might surprise some people," she says.

"We've found that there are differences between FDA-approved tubes," Dr. Marlar reports. Nevertheless, laboratories may not appreciate those differences if they switch from one manufacturer to another, whether for reasons of scarcity, price, or some other motivation. "We've heard a lot of discussion about, *I can just use any tube out there, and that will be OK.*

"That's not correct," he says.

Summary of recommendations

Section	Recommendations
Ordering tests	Recommendation 2.1: Laboratory management including experienced coagulation laboratory scientists should be involved in the process of developing institutional policies related to test ordering and collection of blood samples for tests being performed in the coagulation laboratory. Recommendation 2.2: Electronic ordering of coagulation tests and electronic reporting of results is recommended, where possible. Recommendation 2.3: Where wrist bands are in use scanning and printing tube labels at the point of collection is recommended over centralized label printing.
Sample collection tube and anticoagulant	Recommendation 3.1: Laboratories must have a written policy on acceptable blood collection systems/tubes for testing performed at their facility. Recommendation 3.2: When citrated blood is recommended for coagulation testing, it should be anticoagulated with 105-109 mmol/L (3.1%-3.2%) trisodium citrate, unless otherwise indicated. Recommendation 3.3: We recommend against use of 129 mmol/L (3.8%) trisodium citrate for collection of blood samples destined for coagulation testing. Recommendation 3.4: Blood samples collected into trisodium citrate for monitoring unfractionated heparin therapy should not have a large air space after addition of blood sample to the tube is completed. Recommendation 3.5: When different blood tubes produced by different manufacturers are expected to be used with the same test system and reagents, the comparability of test results shall be tested and validated prior to their use.
Preparation of the patient prior to collecting a blood sample	Recommendation 4.1: In preparation for collection of a blood sample for coagulation tests, patients should avoid strenuous exercise and stress immediately prior to blood draw.
Blood Sample collection device	Recommendation 5.1: Blood samples for measuring PT, APTT, fibrinogen and other coagulation tests for management of bleeding and clotting disorders should be collected using 19-21 gauge needles for adults with good venous access, and 22-23 gauge needles for others including small children. Recommendation 5.2: Blood samples for assessing some coagulation activation biomarkers should not be collected using indwelling catheters. Recommendation 5.3: There is no requirement to discard the first volume of blood collected prior to collecting coagulation samples via the same needle, except when blood is collected through butterfly needles or indwelling catheters or when the sample is collected for platelet function studies. Recommendation 5.4: The sample should be mixed promptly by multiple gentle inversions (3 or 4 times, or following blood tube manufacturer's indications) immediately after collection.
Venous stasis before collection	Recommendation 6.1: Venous stasis induced by the use of a tourniquet should not normally exceed 2 min during collection of blood for coagulation tests.
Order of drawing when different sample types are collected.	Recommendation 7.1: When multiple types of blood collection tubes are filled from the same venipuncture including when the blood in a single syringe is transferred to multiple tubes, the following order should be used: 1. Blood culture tube 2. Coagulation tube 3. Serum tube with or without clot activators, with or without gel 4. Heparin tubes with or without gel 5. EDTA tubes 6. Glycolytic inhibitor tubes 7. Other tubes (eg trace elements)
Sample labelling	Recommendation 8.1: Blood samples should be labelled immediately before blood collection or immediately after blood collection following the regulatory requirements or policies of the country, region or institution, with patient's first and last name, an identification number and/or date of birth, and the date and time of specimen collection. This should be completed before leaving the side of the patient.
Ratio of blood to anticoagulant	Recommendation 9.1: Blood tubes with <80% of nominal filling volume should be rejected by the laboratory and should not be analysed.
Influence of haematocrit	Recommendation 10.1: The ratio of blood to trisodium citrate anticoagulant should be adjusted for coagulation tests when patients have haematocrit >55% (>0.55) using the formula $C = (1.85 \times 10^{-3})(100\text{-Hct})(V)$ where C is the volume of citrate in mL that should be added to a volume of blood (V) in mL to form an anticoagulated blood sample.
Conclusions	Recommendation 11.1: All laboratories should establish a written policy on what samples can be accepted for coagulation testing and which must be rejected. This policy should be jointly constructed by the laboratory management and clinicians or healthcare providers who make use of that laboratory service.

Kitchen S, Adcock DM, Dauer R, et al. International Council for Standardisation in Haematology (ICSH) recommendations for collection of blood samples for coagulation testing. *Int J Lab Hematol.* 2021;43:571–580. https://doi.org/10.1111/ijlh.13584. Reproduced with permission from John Wiley & Sons Ltd.

Dr. Adcock's encompassing perspective looks ahead as well as back. She predicts coagulation collection issues will

become more weight-bearing as new drugs, especially anticoagulant and factor replacement drugs, become available, just as the field needed to adapt its collection practices when direct oral anticoagulants came on the scene. “That impacted many of our assays,” she says.

The pandemic’s tentacles have latched onto coagulation testing, most obviously with the shortage of blue-top tubes. As labs struggle to acquire citrate tubes, they may also acquire some unintended consequences along with their supplies.

Which brings us back to those aforementioned mystery tubes.

They can be purchased from common online sellers, Dr. Marlar notes, but he and his colleagues haven’t been able to identify the manufacturer.

“Fortunately, we recognized it before we tested it,” he says. The tube made its way into circulation via an outside clinic, which said it purchased the tubes in response to the shortage. Dr. Marlar’s lab was firm in its response: “We told them not to use them anymore.”

In the meantime, his lab made its own purchase to do a comparative study. ”We know nothing about them,” Dr. Marlar says. “We’re not even sure where they’re made.”

If a lab did decide to use these tubes, it would need to be aware the tubes lack FDA approval; any LDT use will require validation. “And they’d have to validate every lot,” Dr. Marlar adds, “because we don’t know that any quality control has been done on these tubes,” or if it has, how.

The easy availability of such tubes “has, I think, been a surprise for many in the lab community,” Dr. Marlar says. “We’re just hoping that we were the unique case, and that it’s not prevalent in the U.S.”

Laboratories aren’t to be faulted for looking for less-expensive tubes—it’s a common enough endeavor. And the pandemic has pushed the search for supplies into the farther latitudes. Given the pressures of the pandemic on supply chain, the FDA has given emergency use authorization for one manufacturer’s citrate tubes, “because we have a massive shortage here,” Dr. Marlar says. “We may be seeing those rogue tubes in the United States now.”

Lab staff shortages, hardly a new problem, have also worsened during the pandemic. The nuances of collection can also get lost in the personnel shuffle. Sometimes technologists will be sent to do blood draws. Or, says Dr. Marlar, “It may get turfed over to the nursing staff,” who oftentimes lack recent experience in the task, or who may take the sample from an IV line or a port, which can lead to errors in sample processing.

When individuals other than trained phlebotomists draw blood, adds Dr. Adcock, hemolyzed samples are more common—they’re more likely to come from emergency departments, for example.

Another surprise could await some labs, although this one will be more welcome: Fixing coagulation collection errors might be easier than finding them.

The recommendations are not high hurdles in need of equally altitudinous leaps. Dr. Marlar advises labs to simply walk through the document and ask themselves if they’re following each recommendation. If not, they need to ask, *Why not?* And then delve into the literature, which, as he reiterates, is sound.

If, for example, it turned out his own lab didn’t follow the recommendation to use 3.2 percent instead of 3.8 percent citrate, “we would look at the literature and the evidence and then make the switch.” It’s not a big ask, he says. “At every lab I’ve worked we’ve made that switch.” Obviously the change requires validation. But, says Dr. Marlar, “It can be a small study. It does not need to be hundreds of patients.” Ten to 20 patients should be sufficient to ensure that replacing 3.8 with 3.2 works and can be incorporated in the lab’s standard operating procedures.

Like restaurant inspections, following the recommendations shouldn’t be a one-and-done, bounce-out-the-door endeavor. “These things change,” Dr. Marlar says. “When these documents come out, every laboratory needs to

look at them and say, *We're doing all of them,* or *we're not.*" As added incentive, he says, laboratory inspectors may ask questions of their own. Though these are not checklist-centric, it's reasonable for labs to ask themselves why they're doing something or not doing something that's supported in the evidence. "As a laboratory director, you have to justify why you're using 3.8 and not 3.2."

Walking through the recommendations might also be a more direct path to discovering problems, rather than waiting for errant results to emerge and (it's hoped) be caught.

Over the course of his career, Dr. Marlar estimates he's seen samples that have violated each one of the recommendations. "But we may not know about it. If we do, we won't send it."

"I've seen things handwritten on tests, or lab labels that have been put on the wrong tube," he says. Nor is his experience unique. "Every laboratorian, supervisor, director has always had these problems," he says. "I think every laboratory needs to be vigilant."

Though there are multiple checks along the way as samples are drawn and sent to the lab, sometimes errors are caught more casually. A technologist may see that a tube is underfilled and reject it, for example. Or a clinician will eye a reported value and realize it's nonsensical. "Sometimes it happens—hopefully, the clinician will notify us that it doesn't look right," Dr. Marlar says, such as a patient receiving heparin whose results have consistently been within the therapeutic range several days and then suddenly are reported as normal.

In Dr. Adcock's experience, it's usually the medical technologist who catches the problem. Or, she might catch a pattern of results that look questionable when reviewing results.

She recalls one case involving a patient who was 24 hours post-surgery. "His PTT was shorter than is probably physiologically possible. So we knew, by looking at the result, that the whole sample was probably suspect," Dr. Adcock says.

In other cases, fills are problematic. She recalls another case, this one involving a phlebotomist who was having trouble filling the blue-top tube completely. Knowing that an underfilled tube would be rejected, he uncapped the tube and added contents from a serum tube. Doing so can shorten the APTT because of the addition of activated coagulation factors.

It can be harrowing to navigate the line between sample quality and need for results, as the authors note.

"From my perspective as a laboratorian, I only want to put out correct results," Dr. Marlar says. At times he'll receive samples in the lab that are not valid; when he speaks with the clinician, they might tell him to run the sample anyway.

As the recommendations point out, rejecting a sample has consequences. "You may not get another one," Dr. Adcock says. It may also delay urgently needed results.

It's an onerous situation. Says Dr. Marlar: "I'd rather take flak from the clinician by asking for another sample. Because when somebody acts on a wrong answer, bad things happen to patients."

Ideally, the recommendations will put an end to these dilemmas. Simply put, says Dr. Marlar, if labs follow the recommendations, those hard conversations are less likely to occur. Inappropriate samples won't land in the lab for testing, and erroneous results won't be sent back. And in another Royal Family tradition, perhaps no one will need to talk about it.

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