Quick on the draw—coagulation tube response

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October 2002—As the sensitivity of coagulation testing has increased, the preanalytical phase has been getting more attention as a potential source of error.

Variables that have long been known to affect the accuracy of activated partial thromboplastin times are the reagents and instruments used in testing and the delays between acquiring and processing a blood sample.

More recently, the coagulation tube itself has been found to be a clinically significant source of variability. So significant, in fact, that it led the largest supplier of sodium citrate tubes worldwide to withdraw a line of tubes from the market in September 2000.

Becton Dickinson, Franklin Lakes, NJ, stopped manufacturing its partial-draw sodium citrate tubes after several clinical studies found APTT times were artificially shortened in patients on unfractionated heparin. Platelet counts also tended to be falsely low.

“Partial-draw tubes were introduced into the market because people wanted to draw a lower blood volume, but everything had to be standardized around a certain tube size, 13 x 75 mm,” says Jeffry B. Lawrence, MD, vice president for medical and scientific affairs, BD Clinical Laboratory Solutions. “The idea was you would have the best of both worlds: You would be drawing less blood but you would also be able to use your same instruments and centrifuge and blood collection equipment.”

This simple design meant that the 13 x 75 mm glass tube contained significantly less blood than the standard full-draw tube. The partial-draw tubes were manufactured with less vacuum, resulting in 1.8-mL or 2.7-mL blood draws.

“At the time we withdrew the product, we got our engineers together to fix the problem because the marketplace needed tubes in the 13 x 75 mm configuration, but we couldn’t use the partial-draw design because it caused erroneous results,” says Dr. Lawrence. “The lower blood volume in the tube was associated with more headspace—the vertical distance between the top of the column of blood and the stopper.

“Partial-draw tubes fill more slowly than full-draw tubes,” he adds, “and when these tubes are filled, the platelets get activated by prolonged exposure to shear forces arising from drawing blood into the increased headspace. The activated platelets then release platelet factor 4, which neutralizes heparin in the samples.” This results in a lower effective concentration of heparin in the citrate tube than is present in the patient, which artificially shortens the APTT in patients on heparin therapy.

BD is also eager to “get out of glass wherever clinically feasible,” says Dr. Lawrence. “We were increasingly concerned about the risk of health care worker injury from the breakage of glass tubes.”

In May, the company launched a new line of low-volume, plastic coagulation tubes that are being marketed as BD Vacutainer Plus plastic citrate tubes. They consist of a tube within a tube. The outer tube is made of polyethylene terephthalate and has standard 13 x 75 mm external dimensions. By varying the diameter of the polypropylene inner tube, a minimized headspace is maintained in both the 1.8-mL and 2.7-mL draw volumes.

“We studied more than 1,000 patients with these new Plus plastic tubes,” says Dr. Lawrence. “We didn’t approve their release until we showed that across all patient populations there wasn’t any effect on the test results.”

People have long undergone coag ulation testing for clinical indications such as abnormal bleeding and excessive clotting and for screening prior to eye, brain, and other major surgeries in which excessive blood loss could cause problems. More recently, labs that perform coagulation testing have been focusing on monitoring patients on anticoagulation therapy, either intravenous heparin or oral warfarin.

“One of the reasons that this has become more important from a clinical perspective,” says Dr. Lawrence, “is that people are using a lot more anticoagulants. It’s been shown that you can really reduce the morbidity and mortality rate from heart attacks and from certain other kinds of clotting problems if you anticoagulate these patients.”

Because it’s impossible to predict the anticoagulant dose needed to treat a patient, that person is typically given a dose of intravenous heparin, for example, and six hours later an APTT is performed to see if it falls within a certain therapeutic range. “The reason why it is important to standardize tube performance is that the therapeutic ranges of heparin and warfarin are the best compromise between a lower dose that may not prevent clotting and a higher dose that may cause bleeding,” Dr. Lawrence says.

A published review of several clinical trials has found that four percent of patients on intravenous heparin therapy for thrombotic disease will have major bleeding as a consequence. “No one knows what number occurs because of inaccurate coagulation results, but I think a significant number of them could be due to issues such as the partial-draw tubes,” Dr. Lawrence says.

BD sponsored clinical studies in laboratories nationwide to test the performance of its new tubes on healthy subjects and patients with coagulation disorders on anticoagulant therapy. The assays performed were anti-factor Xa heparin, APTT, fibrinogen, platelet counts, and prothrombin time.

In each case, the performance of the new 1.8-mL and 2.7-mL low-volume plastic tubes was compared to the full-draw, 4.5-mL glass tube. “We refer to that as the gold standard,” says Dr. Lawrence, “because the only information that exists about what dose a patient should have based on their PT or APTT [comes from studies] done with BD full-draw glass tubes.”

Furthermore, because the degree of bias observed with the partial-draw tubes varied depending on the reagents and instruments used by a laboratory, the tubes were tested with the following reagents: Dade Actin (FS, FSL), Dade Innovin, Dade Thromboplastin C Plus, Hemoliance Brain Thromboplastin, Hemoliance Thrombosil 1, IL Test APTT-SP, IL Test PT-Fibrinogen HS Plus, Stachrom Heparin Xa reagent, and STA Fibrinogen. The tubes were also tested with the following instruments: Coulter STKS, Dade Behring BCS, Diagnostica Stago STA, IL Electra 1400C, IL Futura, Sysmex 1500C, and Sysmex K1000. “We tried to look at the market share in and outside the United States to find the most prevalent reagents and common instruments being used,” Dr. Lawrence says.

Julie Wengert, MT(ASCP), a blood bank/hematology supervisor at Southeast Missouri Hospital, Cape Girardeau, Mo., was involved in testing BD’s partial-draw tubes before they were withdrawn from the market and in testing the new low-volume tubes.

“Basically, their tube is a tube in a tube. They made them so that the tubes are completely full, there is no dead space, and there is no platelet activation,” she says. The studies in Wengert’s laboratory found that the APTTs and PTs obtained with the new 1.8-mL and 2.7-mL tubes were comparable to those obtained with the full-draw 4.5-mL tube.

The results paralleled those from another site where BD sponsored studies of the new tubes: No remarkable difference between them and the full-draw tube was found, says David R. Chance, BS, SH(ASCP), technical supervisor at the Saint Louis (Mo.) University Hospital Coagulation Reference Laboratory. Furthermore, he adds, “There is the safety factor of not having to worry about broken tubes.”

Another advantage of plastic is lower disposal costs, says Norman Anderson, MT(ASCP), laboratory director at Southeast Missouri Hospital. “When you look at glass versus plastic and disposal and waste, you’re looking at volume, and [plastic] would really help costs because glass weighs more.”

Dr. Lawrence believes the clinical trials sponsored by BD can satisfy the Clinical Laboratory Improvement Amendments of 1988 as well as CAP requirements for documenting the performance of the various tools used in the lab.

“Under the CLIA ’88 legislation, the laboratory director has responsibility and liability for all clinical diagnostic

testing in their laboratory," he says. "If I introduce something in my lab that hasn't been adequately clinically validated, I could be at risk. Both for CLIA compliance and CAP inspections, they [labs] need to have on file documents that show whatever product they're using does not increase any artifacts."

While this information can be provided by the medical literature or independent laboratory studies, in Dr. Lawrence's experience, it's often provided, or at least augmented, by the manufacturer.

"Most laboratories are extremely short-staffed and it's not cost-effective for them to do their own extensive studies," he says. "By and large, customers find it extremely helpful for us to provide them with the papers."

In contrast, the No. 2 supplier of coagulation tubes worldwide, Greiner Bio-One, Monroe, NC, has found its customers prefer to do their own studies. "'I have to prove it to myself in my own laboratory,'" is what Doug Harris, vice president of marketing and sales, says he hears repeatedly from customers. "At the same time," he says, "most laboratories also want to examine their ranges to ensure that they are appropriate with the particular coagulation test system employed."

Greiner's Vacuette sandwich coagulation tubes have been on the market since 1996. Like BD's tubes, they have an inner tube made of polypropylene and an outer tube made of polyethylene terephthalate. The outer tube has the standard 13 x 75 mm outer dimensions, and the inner tube has a 9.5-mm diameter.

In the United States, Greiner manufactures three draw volumes: 1.8 mL, 2.7 mL, and 3.15 mL. "Our 2.7-mL tube has less headspace than the original 4.5-mL tube, and the 1.8-mL tube is similar as well," Harris says. "Furthermore, we have not yet shown in studies we've formally conducted that any of our tubes produce changes that could be clinically significant."

Greiner is not convinced that headspace is primarily responsible for the phenomena exhibited with partial-draw tubes. "It was not a consistent finding in every tube," Harris says. "No one could exactly say what was causing the problem because coagulation is a very complex science." Instead, Greiner's preanalytical focus has been on activating platelets by glass and the blood-drawing process. Other factors, he says, are the variability between reagents and instruments.

Greiner has started manufacturing its coagulation tubes with harder stoppers. It made this change to improve the stoppers' response to automated cap-piercing instruments.

Greiner's tubes also have a nominal fill line, which is possible with plastic because, unlike glass, it's a molded, not extruded, product. This reference line indicates that the tube is filled properly and helps to avoid variability in the blood draw volume, which is influenced by the tube's shelf life, patient blood pressure, angle of vein entry, and other factors. "It's important that the tube is always filled correctly so that a 1-to-9 citrate-to-blood ratio is achieved," Harris says.

Though many laboratorians were frustrated when BD pulled its partial-draw tubes off the market two years ago, Dr. Lawrence says, "It was an easy decision for us to make. This new product requires no compromise: It give health care workers the safety of plastic; it gives documented clinical performance; and it draws a lower volume of blood without the partial-draw configuration."

Wengert and Anderson, too, believe BD acted appropriately. "They address problems," says Anderson. "From my experience as a laboratory manager, I respect vendors who address problems rapidly so we can rapidly respond, and I know that costs the vendor money."
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