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Pre-analytical sources of error in coagulation testing

Historically, coagulation testing has demonstrated considerably more variability (both within a single laboratory and between different laboratories) compared to other laboratory sections. Early coagulation tests relied on "tilt tube methods". While trained medical technologists achieved remarkable precision within a given laboratory, slight variations in technique could produce significantly different results. (Those of you who remember performing "Lee White clotting times" with glass test tubes and Dixie® cups as medical students can appreciate this. It was common practice to perform common coagulation tests in duplicate to help assure reproducibility.) In recent years, coagulation testing has become more automated, and there have been efforts to improve standardization (e.g. the use of the international normalized ratio (INR) in prothrombin time (PT) testing.) As a result, analytic error in coagulation testing has decreased significantly and pre-analytic error has become the major cause of "laboratory error" (just as it is for most other sections of the laboratory).

While all laboratory tests are dependent upon the quality of the specimen submitted, coagulation testing is exquisitely sensitive in this regard. The very act of collecting the specimen may trigger the complex coagulation cascade that forms the basis for *in vitro* testing. The goal of coagulation testing is to prevent the cascade from occurring until it can be monitored in a laboratory setting. The lability of many coagulation factors, the calcium dependence of the coagulation cascade, and the "highly excitable nature" of blood platelets all present challenges to successfully achieving that goal.[1] An understanding of the sources of pre-analytic error is helpful in evaluating the possibility of spurious abnormal or unexpected coagulation test results. It may eliminate the need for expensive follow-up testing and prevent unnecessary or potentially dangerous therapy.

Patient Factors: Is it the right patient? **All laboratory specimens should be labeled at the bedside or surgical suite and compared to the patient's identification bracelet.** The practice of collecting specimens and returning to a central clerical station to apply labels persists in some nursing units and physician offices. This practice invites clerical error and should be condemned. Is the patient receiving any drugs or therapy that might interfere with coagulation testing (e.g. aspirin, warfarin, or fresh frozen plasma)? If coagulation testing is required to monitor therapy, then the effects of such exogenous elements may be anticipated in reviewing the results. However, if coagulation testing is being performed to evaluate the possibility of a primary coagulation defect – the testing should be deferred until such exogenous factors are no longer capable of interfering with the results. Likewise, the patient's underlying clinical condition may interfere with testing for a primary coagulation defect. Recent thrombotic episodes may transiently decrease coagulation factor levels. On the other hand, many coagulation factors (including fibrinogen, factor VIII and von Willebrand's factor) are "acute phase reactants" and may be increased during acute illness or pregnancy. Mild congenital deficiencies could be missed if testing is carried out during these times.

Site of Collection: Peripheral venipuncture is preferred for obtaining specimens for coagulation testing. While capillary specimens can be used, a great deal of skill is required to obtain a suitable anticoagulated specimen. Indwelling catheters are not optimal for coagulation testing for several reasons: risk of dilution by IV fluid, risk of hemolysis, risk of triggering coagulation cascade, and risk of heparin contamination (if heparin flushes are used to keep the line "open"). Having said that, there are occasions when indwelling catheters represent the only realistic option for specimen collection. The line should be flushed (with 5–10 ml. of saline) followed by withdrawal and discard of 10 ml of blood (or at least 6 dead space volumes). If abnormal results are obtained from a heparin line draw, there are two options. The simplest (and most straightforward) is to obtain a peripheral specimen. If this is not possible, the coagulation laboratory (784-2159) can treat the specimen with a heparinase

enzyme to remove the heparin effect (if so requested by the patient's physician or nurse). (The heparinase will not discriminate between heparin line contamination and systemic heparin, so this procedure should **NOT** be used for patient's receiving systemic heparin anticoagulation.)

Specimen collection tubes: Sodium citrate binds calcium, thereby (theoretically) preventing the coagulation cascade from occurring in the specimen collection tube. Prior to 1998, two concentrations of sodium citrate tubes were commonly used: 3.2% and 3.8%. The higher citrate concentration tubes bind more calcium and result in longer clotting times. The differences are often trivial in normal subjects, but may be clinically significant in patients with clotting factor deficiencies or on anticoagulant therapy. In 1998, the National Committee for Clinical Laboratory Standards (NCCLS) recommended adoption of 3.2% citrate tubes in a move to improve interlaboratory standardization. Rex Laboratory switched to 3.2% citrate tubes in December 2001. Our assays have been standardized based on this concentration. (We occasionally receive (and will assay) physician office specimens collected in 3.8% citrate. For such specimens we add a comment that the reference ranges may not be valid – and we will not report an INR for PTs.) Assays are standardized on a ratio of blood to anticoagulant of 9:1, assuming a normal hematocrit. Patients with either elevated (>55%) or reduced (<20%) hematocrits have less or more plasma volume respectively, and may have abnormal results on this basis. In such cases the amount of citrate should be altered by applying the following formula: [2]

$$X = \frac{100 - Hct}{595 - Hct}$$

where X = volume of 3.2% citrate per unit volume of blood
Hct = hematocrit (in %)

Multiply X by volume of blood to be drawn to determine the volume of 3.2% citrate to place in the tube.
For example: If the hematocrit is 70%

$$X = \frac{100-70}{595-70} = 0.057 \text{ ml citrate per unit volume of blood}$$

For 5 ml tube: 0.057 x 5 = 0.286 ml citrate
For 10 ml tube: 0.057 x 10 = 0.571 ml citrate

For pediatric and newborn infants, the following formula may be substituted:

$$C = (0.00185) \times (V) \times (100 - Hct)$$

where C = volume of 3.2% citrate (ml)
V = volume of whole blood to be collected (ml)
Hct = hematocrit

For example, if the hematocrit is 57% and the draw volume is to be 2 ml,

$$C = (0.00185) \times (2) \times (100 - 57)$$
$$= 0.16 \text{ ml of 3.2\% citrate}$$

Finally, many laboratories (including Rex) have also recently converted from glass collection tubes to plastic (under pressure from OSHA). Prior to making this switch, the plastic tubes Rex uses were tested to assure that the plastic would not produce different effects than the glass in terms of specimen integrity. Different vendor's products may produce different results in this regard.

Method of Collection: Nothing beats a good clean venipuncture. Prolonged or traumatic venipuncture or delay in anticoagulating the entire specimen may result in triggering of the coagulation cascade or hemolysis – either of which will produce invalid results. Initiation of the clotting cascade depletes coagulation factors and may prolong coagulation times. Hemolysis may also trigger the coagulation cascade. Furthermore the hemoglobin pigment may interfere with photo-optical detection systems. The patient should be seated or recumbent. A suitable vein, which will enable free blood flow, is desirable. Prolonged (> 1 min) tourniquet application should be avoided to minimize blood stasis (may increase chances of triggering coagulation cascade). For adults, 22 to 19 gauge needles are recommended. For pediatric patients, 23 to 21 gauge needles are suggested. The specimen should be

collected directly into the Vacutainer tube rather than a syringe (reduces chance of hemolysis or clotting, eliminates variable of operator technique in filling tube(s), and is safer for the phlebotomist). If multiple tubes are to be collected, the citrate (blue top tube) for coagulation studies should be collected 2nd or 3rd, following a non-additive tube (e.g. red top or serum separator tube). Although the risk of anticoagulant carryover from EDTA (purple top) or heparin (green top) tubes is small, specimens for these studies should be collected after the citrate tube has been collected. For single specimen draws, discard tubes are no longer considered necessary prior to collection of coagulation specimens in well-performed venipunctures, using needles. [3] (Nevertheless, NCCLS continues to recommend use of a the discard tube. If a "butterfly"-type winged infusion needle is used, a discard tube must be used to prevent dead space air in the butterfly tubing from partially filling the tube and decreasing the blood:anticoagulant ratio.) The citrate tube should be filled completely (by the vacuum in the tube) and then inverted gently 5-10 times (neither shaken nor stirred). Underfilled tubes reduce the blood:citrate ratio and may produce spuriously increased coagulation times. Because of this, underfilled tubes will be rejected. (We had one instance where an individual collected 2 incompletely filled tubes and combined the contents – resulting in markedly abnormal PT and PTT results. The cause was not determined until the collector (not a Rex employee) was questioned in great detail about obtaining the specimen.)

Specimen processing: Specimens should be delivered to the laboratory promptly. In the past some advocated placing specimens on ice following collection, but this is no longer recommended. Specimens for PT are stable at room temperature for 24 hours. [3] All other types of coagulation tests should either be analyzed or spun down and frozen within 4 hours of specimen collection. If there is a delay in transporting the specimen to the laboratory, degradation of clotting factors may produce erroneous results. Samples frozen at -20ºC are stable for 2 weeks, while those stored at -70ºC are suitable for analysis for up to 6 months. [2]

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References

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3. Brigden ML *et al*. Prothrombin time determination: the lack of need for a discard tube and 24-hour stability. *Am J Clin Pathol* 1997;108:422-6.

Correct site surgery but wrong site labeling: So what's the big deal?

The Joint Commission on Accreditation of Healthcare Organizations generated significant interest on August 28, 1998, when it released "Lessons Learned: Wrong Site Surgery" sentinel event alert. The Commission followed up on December 5, 2001 with a review of wrong site surgery. Rex operating room personnel have devoted many hours and expended a great deal of energy to devise a process to obviate wrong site surgery. However, the process should not stop here and continued effort must be dedicated to finalizing and closing the loop for wrong site surgery by assuring that specimens from the operating rooms are appropriately and correctly labeled. "Right" vs. "left" may not seem like a big deal to busy hospital staff but to the middle aged woman with cancer present in only one of her concurrent bilateral breast biopsies, it is HUGE. Both biopsies were undertaken for mammographic abnormalities due to microcalcifications and not for palpable masses. Needle-localization excisional biopsies from the left and right breasts were approximately the same size and both contained microcalcifications. The first specimen showed extensive comedo-intraductal carcinoma and was labeled "Right Breast Biopsy" and the second specimen showed benign fibrocystic changes and was labeled "Left Breast Biopsy". However, the accompanying requisition designated the first specimen as "Left Breast Biopsy" and the second specimen as "Right Breast Biopsy". Both specimens were submitted in their entirety, so the surgeon could not identify the difference in consistency between the two specimens that he noted to himself during the surgery. Due to the extent of the comedo-intraductal carcinoma and the central location in the breast, the recommendation would include a mastectomy rather than a lumpectomy on the involved side and no further therapy for the opposite breast. Everyone's thought is about the lawsuit that could ensue, but how could we let this patient down this way when she trusted us? Fortunately, there were certain histologic features that would clearly demonstrate

which side the respective biopsies came from. The right breast biopsy contained central duct structures designated lactiferous sinuses along with the comedo-intraductal carcinoma. In this case, the specimen containers were labeled appropriately and the requisition was the incorrectly labeled document.

This case is fictitious, but a very similar situation has occurred here and involved right and left breast biopsies in a middle aged female with bilateral abnormal mammograms. The real case specimens were of different sizes and the surgeon knew which side each specimen came from based on the size difference between the two tissue specimens. In this case, both biopsies were benign.

At this point, the reader's stress level should be significantly reduced and rightfully so, since no harm came to the patient. The histology department processes over 28,000 cases annually from the Main and Same Day Surgery operating rooms, endoscopy suite, emergency department and outpatient setting. The personnel in all involved areas should be congratulated, since our "error" rate in this type of situation is less than 5 cases/year or less than 0.02%. Even in the best circumstances, errors will occur but what have we done to prevent the type of errors described above? Continuing education discussions have been held with operating room and laboratory personnel. We have stressed that "wrong site surgery" does not stop with simply assuring that the correct site has been identified appropriately prior to the actual surgery. The process is only complete when the correct site has been identified, the surgery performed successfully, the tissue specimens labeled correctly and brought to and received in the laboratory by laboratory personnel. In the laboratory, pathologists, histotechnologists, cytotechnologists, pathology aides, and transcriptionists are all responsible for verifying patient and specimen identification and notifying the operating room, endoscopy or physician office staff of any inconsistencies in a timely manner. In addition, our department is responsible for limiting and correcting dictation and/or typographical errors that confuse or mislead those caring for the patient.

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BNP to RCH (Willard, this one's for you)

Effective immediately, all specimens for B-type natriuretic peptide (BNP) will be referred to Raleigh Community Hospital (RCH) for analysis. As many of you know BNP has become a popular test for the evaluation and management of patients with congestive heart failure. We hope to be able to offer either BNP or pro-BNP testing here at Rex at some point in the future. In the interim, we have contracted with RCH to provide testing for Rex patients/clients in order to decrease the current turnaround time from Mayo Medical Laboratories. A regular courier run from Rex to RCH is scheduled for 1300 daily. The expected turnaround time is 1 hour once the specimen reaches RCH. Results will be phoned/faxed to the appropriate nursing station/physician's office. The test requires a 5 ml. EDTA (purple) top tube. If the specimen cannot be forwarded to RCH within 4 hours, a frozen 1 ml aliquot of plasma is necessary.

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For further information, call the Laboratory. Telephone extensions are: Pathologists' Direct Line (3201), Sharon Logue (Lab Director 2400), Robin Ivosic (Microbiology Lab Manager 3053), Elaine Patterson (Core Lab Manager 3054), Jackie Okoth (Core Lab PM Manager 4248), Diane Young (Anatomic Pathology Manager 3888), Nga Moore (Customer Service Manager 3396)