Imagine that some well-meaning researchers are testing for “Factor Z” in the blood of various patients, wondering what would happen if the specimens were heated to a high temperature, say above 102°F. Upon heating the tubes containing the blood of these normal patients and running the tubes through their ACME Z-analyzer, the well-meaning researchers note high levels of Factor Z outside the normal range. If they add “Correction Substance Q” to the tubes first, however, the results are normal. Textbooks and literature say nothing about actual patients with high temperatures having high Factor Z levels; in fact, they document the opposite. But a regulatory agency, extrapolating from this data, concludes the tubes for any samples from actual patients with high temperatures should be treated with Correction Substance Q first. And finally, tube manufacturers do not make a tube containing Correction Substance Q.

If this scenario sounds totally unlikely, then you have not looked into the recommendations about correcting sodium citrate tubes (blue tops) for patients with high hematocrits for the APTT (activated partial thromboplastin time). The recommendations from the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) and the College of American Pathologists (CAP) are based on different sources.

**Background**

Since 1980, CLSI has recommended a correction for citrate blue-top Vacutainer-type tubes for patients with hematocrits greater than 55%. The correction only applies to APTT tests, and only to patients with high (not low) hematocrits. The idea is to adjust the amount of citrate anticoagulant or the amount of blood in the tube so that the plasma:anticoagulant ratio (not the blood:anticoagulant ratio) stays fairly constant. Thus, the theory goes, if a patient has a high hematocrit, he has less plasma; therefore, the standard tube contains too much sodium citrate for the amount of plasma and, thus, either the amount of citrate in the tube should be reduced or more blood should be added to the tube in order for a valid result to be obtained.

The specific recommendation from CLSI is contained in NCCLS document H21-A4: “The final concentration in the blood should be adjusted in patients who have hematocrit values above 0.55 L/L (55%). For hematocrits below 0.20 L/L (20%), there are no current data available to support a recommendation for adjusting the citrate concentration. The chart in the appendix [from a 1982 book by Ingram, Brozovic, and Slater called “Bleeding Disorders: Investigation and Management”] can be used to determine the amounts of anticoagulant and blood for hematocrit values above 0.55 L/L.” CLSI does not cite any specific reference for this statement. Also, since 2002, the CAP hematology checklist, HEM.22830, has contained the question, “Are there documented guidelines for detection and special handling of specimens with elevated hematocrits?”

Over the last 25 years, several articles addressing the question of over- or underfilled blue-top tubes have come to form the basis for the continued emphasis on this practice of correction of tubes for polycythemic patients. All of the articles basically address the same question with similar methodologies. Since the tubes and the tests are designed to have a specific anticoagulant: blood ratio, the original questions make good sense: What happens when a tube is underfilled? How much can a tube be over- or underfilled before results are adversely affected? And, how does this apply to actual patient conditions, if it applies at all?

An often-quoted 1974 article by Koecke, et al., entitled “Preinstruments Variables in Coagulation Testing” recommended that a ratio of one part 3.8% anticoagulant to 19 parts blood be used to correct the “spurious elevation of the PT and PTT so often found in specimens with elevated hematocrits, such as those from polycythemic patients” as opposed to the typical ratio of one part citrate to nine parts blood. There is no documentation in the article for the statement regarding polycythemic patients. Furthermore, methods used in this study involved phlebotomizing patients, separating the red cells from plasma, dialyzing the plasma to remove anticoagulant in the collection bags, washing the red cells, then recombining the red cells and plasma in various concentrations to achieve different simulated hematocrits for testing. The number of possible errors and variables in this procedure is obvious.

In “The Effects of Inaccurate Blood-Sample Volume on Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT)” in 1982, Peterson, et al., attempted to study the same question, again using 3.8% sodium citrate, but possible sources of error are present in the study. Normal bloods were drawn into blue-top tubes, but then aliquots were taken out of these tubes and transferred to other tubes, which contained varying concentrations of sodium citrate, to simulate higher hematocrits. Aside from the potential error of transferring aliquots to separate tubes, this process introduces dead-air space in the tube, which Becton, Dickinson and Company, the maker of Vacutainer brand tubes, concluded could introduce error in APTT results.

Also, Peterson’s results differ from Pai’s later studies (see below). Note that Peterson equates underfilling tubes with polycythemia: “The results of this study demonstrate that underfilling (or extreme polycythemia) may produce badly distorted (prolonged) results.” He did not define “extreme polycythemia” and possibly meant extremely high hematocrits.

In “The Effect of Sample Volume on Coagulation Tests” in 1990, Pai, et al., questioned Koecke’s findings because of “artificially manipulated samples” and because only normal donors were used. Pai’s study included both normal patients and patients on warfarin (PTs) and heparin (APTTs). Actually, Pai has a convincing case for not correcting citrate tubes. In only one category, where tubes were underfilled with 3.0 mL of blood versus the correct 4.5 mL, was there a slight prolongation of results for APTT—a mean of 32.88 versus 27.18. These discrepant results were derived from specimens of normal patients. The specimens taken from patients on heparin, with values ranging from 52 to 87 seconds, produced no discrepant results when underfilled tubes were compared to filled tubes. The underfilled volume of 3 mL of blood represents a hematocrit of about 61%, assuming the patient had a normal “crit” of 42%. Pai also infers the connection to actual patients with high hematocrits: “...this [difference] may be accentuated from patients with a higher hematocrit.” But Pai fairly concludes that “a larger number of samples from various patient populations must be analyzed to confirm these findings.”
Yet another article, “Prolonged Prothrombin Time and Activated Partial Thromboplastin Time Due to Underfilled Specimen Tubes With 109 mmol/L (3.2%) Citrate Anticoagulant”9 by Reneke, et al, in 1997 was the first to deal with 3.2% citrate, which is now the recommended concentration. Reneke abstains from theorizing about actual hematocrits, and deals only with underfilled tubes. Similar results were obtained in this study — as in most other studies (Pai being the exception) — that is, underfilling citrate tubes unacceptably prolongs APTT results. The same questionable variables are present in this study, however, as in the 1982 article by Peterson, et al — aliquots are transferred to separate tubes, which introduces air into the tubes.

Finally, Siegel, et al. in “Effect (or Lack of It) of Severe Anemia on PT and APTT Results”10 in 1998, concluded that when 3.8% citrate is used, correction for anemic patients is not indicated, thus echoing Koepke’s and others’ results with anemic patients.

**Summary**

All of the articles discussed here generally make the case that underfilling citrate tubes runs the risk of falsely prolonging APTT results in patients with normal hematocrits when, to begin with, the results are prolonged. With the exception of Reneke, the researchers also make the leap from simulated conditions to actual patients, although many of the simulated high hematocrits are unlikely, if not impossible — some exceed 68%.3,7

Let us look at this as it stands now:

- Researchers manipulate samples with normal hematocrits and extrapolate test results to actual patients with high hematocrits.
- The concept of correction undergoes decades of “carryover” as authors of books and articles repeat the statement without verification.
- CAP relies on CLSI for its correction policy, which in turn relies on statements and inferences from earlier books and articles, none of which deal with actual polycythemic patients.

Could this be, ironically, what Koepke himself refers to as “folklore” regarding coagulation tests?4

We are left, then, with two major difficulties with the current CLSI and CAP guidelines:

- Textbooks and literature contain no evidence to support the concept that patients with high hematocrits have prolonged APTTs if not “corrected.” Koepke and others assert this, but this author cannot find any clinical evidence for it.
- It is an unproven leap of faith to assume that if artificially manipulated samples have prolonged APTTs, then actual patients will also. This would be a reasonable assumption to make as a starting point for actual research into this issue, but laboratory policy should not be based on assumptions.

The case has been made for maintaining the anticoagulant:blood ratio in citrate tubes. The case has not been made for altering that ratio for patients with high hematocrits.

**Recommendations**

**Laboratories:** The literature suggests that if an APTT result on an underfilled tube is in the normal range, it is correct. Therefore, corrections should only be made if the result from a patient with a high hematocrit is prolonged. This reference is to complying with the existing policy, in spite of the aforementioned problems. In actual practice, a reasonable solution to this problem — and one that involves the least manipulation to the tube — would be to overfill the tube to one preset mark to cover the range of “high hematocrits” — about 45% to 65%.

According to Koepke’s data, “The use of lesser amounts of anticoagulant has not been associated with any apparent disadvantage during an ongoing pilot study of a variety of patients, including patients with polycythemia.” And from Siegel, “…in severely anemic patients, there is neither a benefit to nor a need for adjusting the citrate volume in 3.8% collection tubes.” The simple idea of decreasing the amount of anticoagulant to suit all hematocrits may be worth looking into again.

**CLSI and CAP:** It is incumbent on the standards agencies to revisit this issue, using sound research, with actual patients with high hematocrits. If new data cannot prove that high-hematocrit patients have prolonged APTTs, then the recommendations for corrections should be modified or eliminated. But, the issue needs to be resolved with current data to back it up. The customary methods for correction, involving redrawing the patient and manipulating tube vacuum or volume, may introduce error into a system where no error exists and “may not be the best use of time with increasingly limited resources.”

Laboratory regulations, like statistics and primordial ooze, seem to develop a life of their own, given enough time. That is why it is good to question even the obvious. Perhaps the questions raised here will result in some useful answers.

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**References**


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