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FORUM

Guidance on the critical shortage of sodium citrate coagulation tubes for hemostasis testing

Abstract

Recent manufacturing problems and increased utilization has created a shortage of 3.2% sodium citrate blood collection tubes used for coagulation testing, causing stakeholders such as hospitals, clinics and laboratories, to find suitable alternatives. Considerations for in-house citrate blood collection tube preparations or purchasing commercial products from unknown manufacturing sources is of particular concern to laboratories that perform coagulation testing. It is well recognized that variability exists between citrate blood collection tube manufacturers, thereby making any transition to new blood collection methods more challenging than simply switching to a new source. This document provides provisional guidance for validating alternative sources of sodium citrate blood collection tubes (commercial or in-house preparations) prior to clinical implementation.

1 | BACKGROUND

In mid-June 2021 the Food and Drug Administration (FDA) and the College of American Pathologists (CAP) notified United States (US) laboratories of pending supply shortages for 3.2% (0.109 M) sodium citrate tubes due to "several tube recalls" in addition to the "unprecedented levels of demand"[1,2] presumably due to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections and testing for assessing coagulopathy associated with severe coronavirus disease 2019 (COVID-19) illness.[3,4] CAP recommended to the three primary stakeholders (ordering providers and nurses, phlebotomy and the clinical laboratory), several strategies including efforts to 1) reduce standing orders for coagulation testing, 2) reduce routine (non-essential) coagulation testing, 3) avoid using citrate tubes as discard tubes, 4) reserve smaller volume tubes for select patient populations, and 5) consider point-of-care testing (POCT) where suitable and available. As a last resort, consider using citrate tubes beyond their expiry date. Later in July 2021, the US Food & Drug Administration (FDA) issued a second statement related to the interruption of supply for sodium citrate tubes.[5] The FDA recommended the same conservation practices recommend by CAP. In addition, the FDA initiated an Emergency Use Authorization (EUA) to Becton Dickinson to allow US sites the ability to acquire and use 3.2% citrate tubes manufactured in the United Kingdom for evaluating "coagulopathy", including those associated with SARS-CoV-2 infections.[6] However, this EUA is vague as allowing usage of these citrate tubes in other clinical scenarios, including bleeding disorder evaluations and drug monitoring which may or may not fall under the "coagulopathy" umbrella.

While the immediate impact appeared to affect mainly the US marketplace, there is a trickling effect now being seen outside the confines of the US. As laboratories worldwide consider alternative options, there is growing concern amongst hemostasis laboratory opinion leaders about these alternative options. Although the vast majority of laboratories are using plastic citrate blood collection tubes, glass collection vials are suitable from the same manufacturer, though biases between glass and plastic are well-known.[7,8] Moreover, the glass citrate tubes supply chain has also been affected.

Another major concern has recently developed, in which 3.2% sodium citrate tubes can be obtained from online retailers. While these citrate tubes appear similar to traditionally used commercial sources using "Carolina blue" or "light blue" caps, and information suggesting they are "CE marked" (indicating conformity with the European Union marketing requirements) and "IVD" (indicating for in-vitro diagnostic use), the labels do not provide the source or manufacturer of these products nor information for interim distributors or storage conditions. This raises concerns about the suitability of these products as the manufacturing characteristics such as the chemical composition, vacuum draw volume consistency, plastic or glass tube composition, tube stopper composition, lot variability, and

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lot stability. It has been previously demonstrated that variability exists between 3.2% sodium citrate manufacturers[9,10] and thus merely replacing an existing product with a newer product that is available may not be as problem-free as one would desire. Laboratories with senior staffing may recollect the old days of preparing on-site sodium citrate tubes, (which some sites in the US are doing again during this current crisis), but those procedures may have been long archived or eliminated from the regulated environment of most clinical laboratories. While sodium citrate may be commercially available, the preparation, storage and validation of these locally prepared collection tubes are currently not adequately described.

It is suggested that this citrate tube shortage may last until the end of 2021 and the supply recovery may not be uniform worldwide. While there may be regulatory requirements for on-site preparation of 3.2% sodium citrate blood specimen collection methods, this provisional guidance provided is based on expert opinion and prior experience ('wisdom of the aged') or local coagulation research study practices. These recommendations are not intended to supersede any local or regional requirements. Note that all preanalytical variables associated with coagulation testing still exist with locally prepared citrate collection tubes, including proper collection, blood collection volume and blood to citrate ratio as well as sample transportation, processing and storage.[11–13]

1.1 | Recommendation #1: each laboratory must evaluate and validate new 3.2% sodium citrate blood collection tubes from commercial sources

The following recommendations are also in keeping with those of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM), which has defined basic requirements, technical aspects (e.g. evaluation of physical integrity and correct functioning of the vacuum, avoidance of safety issues and so forth), along with clinical aspects (precision, accuracy, stability of test results) that shall support laboratory professionals during the organization of blood tenders for blood collection tubes or the process of local validation before replacement of blood collection tubes.[14] Therefore:

- The laboratory, at a minimum, should order an anticipated volume (3 months) of a single lot of commercially available tubes.
- The laboratory should coordinate paired sample testing with their existing lot of 3.2% sodium citrate tubes with new source citrate tubes. At least ideally 40 samples should be tested for prothrombin time/International Normalized Ratio (PT/INR), activated partial thromboplastin time (APTT), fibrinogen (FBG), D-dimer (DD), anti-Xa and if available, antithrombin (AT). This proposal would address the three commonly used instrument test platforms (clot-based, immunoassay, and chromogenic). The evaluation should include:

  a. Samples that cover the measurement range of each test.
  b. Samples that cover anticoagulant monitoring, in particular heparins and oral vitamin K antagonists.

Further detailed indications could also be found in the EFLM document.[14]

NOTE: While commercially available citrate blood collection tubes appear similar in size, the collection volume (amount of tube vacuum to aspirate blood volume) may differ so refer to the fill volume markings as noted on tube labels.

NOTE: 3.8% sodium citrate blood collection tubes are not an acceptable alternative to 3.2% sodium citrate blood collection tubes, as significant differences in clotting time exist between these two citrate concentrations that may require reference interval changes and geometric mean of normal for PT, required for accurate INR reporting.[15]

1.2 | Recommendation #2: laboratories creating local 3.2% sodium citrate blood specimen collection tubes

After preparation of these tubes, they should undergo the same comparison evaluation ('validation') as noted with new source commercial tubes. The quantity of citrate tubes to be made locally should be predicated on the estimated use over a protracted period, based on prepared citrate stability (estimated to be 2 months). The tubes should be stored capped and in a cool dry area at room temperature or refrigerated. Refrigerated tubes should be brought to room temperature before use. Prefilled syringes containing 3.2% citrate may be a suitable alternative to plastic or glass blood collection tubes, but we recommend this blood collection technique only for sites experienced with using this blood collection method. Regardless of which approach is used, specific training and procedures are needed for blood collection to assure proper blood volume to sodium citrate ratios are achieved and to mitigate risks of potential downstream errors on relabeling secondary tubes.

- For plastic tubes or vials, only use polypropylene material.
- For glass tubes or vials, only use siliconized glass material.
- Preparation of 3.2% (0.109 M) buffered Sodium Citrate (pH 7.0) anticoagulant tubes:

  a. In a one (1) liter volumetric flask (or adjust volume and reagents as appropriate), add 31.57 g of trisodium citrate (3.2%) and 11.92 g HEPES to 1 liter flask and q.s. to 1000 ml using deionized water. Mix well (ideally using magnetic stirrer). Adjust pH to 7.0 (with 0.5 M HCL or 0.05 M NaOH as appropriate based on the initial pH).

NOTE: If unbuffered preparation is required, then eliminate the HEPES and pH adjustment steps.

- The proper ratio is 1 part citrate:9 parts whole blood when hematocrit is within 25–55%. Citrate volume adjustment for hematocrits outside the aforementioned range can be adjusted as previously described.[12,13] For preparing local blood collection tubes:

a. 2.0 ml tubes: add 0.2 ml of prepared citrate solution
   - Will then require 1.8 ml of collected whole blood
b. 3.0 ml tubes: add 0.3 ml of prepared citrate solution
   - Will then require 2.7 ml of freshly collected whole blood
c. 5.0 ml tubes: add 0.5 ml of prepared citrate solution
   - Will then require 4.5 ml of freshly collected whole blood

- After citrate is aliquoted into each blood collection tube, they must be capped using appropriate rubber stopper (optimal), polypropylene cap, or suitable alternative. Failure to appropriately seal the collection vial may result in leaks during inversion or erroneous citrate concentration or pH due to evaporation or gas exchange with room air.

a. In-house citrate blood specimen collection tubes should be appropriately labeled indicating citrate concentration, a lot identifier (e.g. preparation date) and expiry date. Tubes should be stored in a cool dry place or refrigerated after capping.
b. A space for labelling with the patient's name and other appropriate identifiers, date and time of blood collection must also be available.
c. All information on the tube label must be in permanent, water-insoluble ink.

- When required for patient testing, the blood must be collected via syringe technique as these collection tubes will contain no vacuum, and extreme caution, using as little pulling pressure as possible using butterfly or straight needles is required to avoid injury or shear in the sample. It is important to remember here that blood collection with syringes shall be tentatively standardized (e.g., trying to always apply the same aspiration pressure, so preventing the risk of various degrees of blood coagulation and platelet activation), with additional standardized blood collection precautions when needles without safety devices are used. Samples collected for platelet function studies may require alternative anticoagulant or collection techniques.[16]

a. The blood must be collected and placed into the locally prepared citrate tube within 60 s of collection.
   - As there is no vacuum in the citrate blood collection tubes prepared locally, the caps must be removed prior to adding blood. Do not use excessive pressure or force to expel blood from the syringe into the citrate tube.
b. After dispensing the blood into the citrate tube to the appropriate fill volume, safely cap the tube and gently invert (upside-down) 4–6 times to allow adequate mixing between blood and anticoagulant mixture, avoiding vigorous shaking that may cause blood cell injury, as well as activation of platelets and blood coagulation.[17] Tubes should be transported at room temperature to the laboratory using method that would avoid potential leakage (e.g. transport rack) should the cap not be adequately applied.

NOTE: It is likely any post-capping mechanism may not be sufficient to allow for transport of in-house prepared citrate blood collection tubes via a pneumatic tube systems (PTS). If PTS is being considered, evaluating this transport mechanism prior to using is strongly recommended (e.g. sending the tube type containing water up the fill line, cap the tube, then send) although this trial assessment may not guarantee future blood transports and we would suggest manual transporting of blood samples when using vacuum-less, locally prepared citrate blood collection tubes.

c. The stability of the blood sample using locally prepared citrate collection tubes should be equivalent to existing recommendations.[18]

1.3 | Recommendation #3

Statistical acceptability for citrate tube verification of performance. There are numerous statistical evaluations to determine equivalence including regression, paired *t*-test, analysis of variance or others. Limits of bias acceptability may be regionally regulated but fall within 5–15% difference between comparator or predicate and new method or assay. In the end, 'clinical significance' is more useful measurement but can be more challenging to define and clinical significance is more likely predicated on local patient population and test utilization and thus requires approval by designated authority in the clinical laboratory. Clinical or statistically significant differences may require reassessment of the reference interval, including the geometric mean of normal used for accurate INR calculation.

1.4 | Recommendation #4

Training of nursing staff and phlebotomists as to the appropriate fill volume for the tube type(s) validated and approved by the system should be performed. The proportion of the tube filling and appropriate fill volume indicator may vary by manufacturer. For lab-created sodium citrate tubes or pre-loaded sodium citrate syringes, a standardized permanent mark indicating the fill volume is recommended. Especially in a system using more than one specimen tube type, education in the form of didactics or visual/job aids should be provided to the individuals drawing the sample and documented in the laboratory system.

1.5 | Recommendation #5

Citrate blood samples may originate from sources outside the laboratory, such as clinics, doctor offices, and satellite phlebotomy stations which may not be readily aware of FDA or CAP notifications of citrate blood collection tube shortages. As such, reference laboratories should notify their clients of likely citrate tube shortages, the impact they may experience as well as precautions when seeking alternative sources from online retailers. Results obtained from samples collected and sent to reference laboratories using alternative, non-regulatory approved 3.2% sodium citrate blood collection tubes should be interpreted with caution and unexpected results be repeated after resolution of tube shortage.

2 | CONCLUSION

The local validation of new blood collection tubes is a necessary part of assuring quality testing in the clinical laboratory. The recent citrate blood collection tube shortage presented significant challenges due to the acute nature of the shortage with the concomitant increased usage during the COVID pandemic. Any change in citrate blood collection tube source, whether it be commercial or local preparations, require due laboratory diligence to assess for equivalence to existing platform prior to the transition of any new blood collection tube platform. This can be particularly challenging for laboratories that receive samples from multiple entities for centralized testing, such as specialty hemostasis and reference laboratories. Notification and edification of primary stakeholders (clinical, phlebotomy and laboratory staff) about blood collection tube changes may be required prior to implementation. We are cognizant the recent shortage of blood collection tubes is fluidic and additional guidance for addressing these emergent situations may continue to evolve.

KEYWORDS

3.2% sodium citrate, coagulation testing, D-dimer, fibrinogen, PT/APTT, validation

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CONFLICT OF INTEREST

Authors state no conflicts of interest.

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