Blood Testing: Choosing the Right Specimen

By Jeffrey J. Chance, PhD

Blood testing is an essential component in the delivery of health care. Each day, healthcare workers collect millions of blood samples, and laboratories test for a wide range of analytes and biological parameters to aid in the diagnosis and monitoring of disease. Testing is generally performed on one of three different specimens — whole blood, plasma, or serum. There are numerous criteria that determine which specimen is most suitable for a particular test and setting.

Centralized Testing vs. Point-of-Care Testing

Today, commercially manufactured diagnostic tests are available for almost every known analyte and blood component. Certain types of tests require a whole-blood specimen, such as hematology, erythrocyte sedimentation rate, blood gases, tests for red cell constituents or surface antigens, and genetic testing. Some drugs, such as cyclosporine, exhibit variable distribution into erythrocytes in vitro and are also most reliably measured with a whole-blood specimen. Serum is generally the preferred specimen for chemistry testing, while plasma is the traditional specimen for coagulation testing. However, whole blood may also be used for these tests.

Generally speaking, whole blood can be considered a “universal specimen” since it contains every analyte, as well as the various types of blood cells. Moreover, advantages to testing whole blood include no waiting time for clotting and specimen centrifugation, and no additional cost associated with the purchase and operation of centrifuges.

But if whole blood can in theory...
Question: We just found out that BD makes two different citrate tube concentrations a 3.2% and 3.8%. We’ve been using both interchangeably. Can we do this and what are the differences in APTT and PT results?

The assumption is that you have collected a quality sample following proper handling conditions. Sodium citrate concentrations can have significant effects on APTT and PT assay results, especially when results are outside of the normal range and responsive reagents are used. Laboratories should determine their normal range of APTT and PT based on one citrate concentration, and must consistently use this concentration for all patient samples until a new normal range is developed. NCCLS recommends the use of 3.2% citrate concentration.

Potential issues between 3.2% and 3.8% sodium citrate concentrations are as follows:

• When responsive reagents (ex. Dade® Actin® FS, Dade® Innovin®) are used, statistical differences in APTT and PT test results between the two citrate concentrations will occur.
• The PT test is consistently higher when responsive reagents and 3.8% sodium citrate are used.
• Normal ranges for APTT and PT may shift higher when 3.8% citrate is compared to 3.2% citrate with responsive reagents. Less variation in the normal ranges occurs between the citrate concentrations when nonresponsive reagents (ex. Dade® Actin®, Thromboplastin C•Plus) are used.
• When nonresponsive reagents are used, varying the citrate concentration has little clinical significance except with patients receiving IV heparin therapy.

*The reference article used for this bulletin conducted studies on the variability and interchangeability of 3.2% and 3.8% citrate concentrations on five populations: healthy volunteers, hospitalized patients not receiving anticoagulant therapy, patients receiving intravenous (IV) heparin therapy, or receiving both IV heparin and oral anticoagulant therapy, and outpatients receiving oral anticoagulant therapy. Please refer to this paper for information on these study groups.

Reference:
1. Adcock, Dorothy M; Kressin; Marlar, Richard A PhD; Preanalytical Variables in the Routine Coagulation Laboratory. ASCP Teleconference Series Sep. 12, 2000; Program No. 6064
3. Adcock, Dorothy M ; Kressin; David C; Marlar, Richard A PhD; Effect of 3.2% vs 3.8% Sodium Citrate Concentration on Routine Coagulation Testing. Am J Clin Pathol. 1997; 107:105-110

<table>
<thead>
<tr>
<th>Citrate Concentration</th>
<th>Dade® Actin® FS APTT</th>
<th>Dade® Innovin® PT</th>
<th>Dade® Actin® APTT</th>
<th>Thromboplastin C•Plus PT</th>
</tr>
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<tr>
<td>3.2%</td>
<td>22-31</td>
<td>8.6-10.7</td>
<td>23-33</td>
<td>12-14</td>
</tr>
<tr>
<td>3.8%</td>
<td>24-33</td>
<td>9.2-11-4</td>
<td>22-31</td>
<td>11-14</td>
</tr>
<tr>
<td>ρ</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
<td>Non-significant</td>
<td>Non-significant</td>
</tr>
</tbody>
</table>
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• **Innovative tube geometry** that minimizes tube headspace and associated platelet activation to optimize APTT monitoring of unfractionated heparin patients. You always get a full draw with standard outside tube dimensions, for 1.8mL and 2.7mL draw volumes.

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continued from front cover

be used for all types of blood testing, then the question arises, why isn’t it? Certainly, historical limitations and conventions have played a role in the current widespread use of serum and plasma. Assay development has long focused on serum-based methodologies due to the ease of handling, manipulation, and storage associated with the relatively clean matrix of serum. The chemical reactions that take place between the reagent and the target analyte are also easier to develop and optimize in a serum-based format. In recent years, many chemistry tests traditionally performed on serum have also become available for plasma.

While the turnaround time (TAT) is longer for tests that require a clotted and centrifuged specimen, this wait has been tolerable for routine analysis done in central laboratories. Furthermore, improvements in TAT have been realized with sample transport initiatives and the shortened time it takes to perform analysis on modern analyzers. Consequently, serum and plasma have become de facto standards for many tests in the central lab, and the long-standing acceptance of these conventions has given diagnostic manufacturers little incentive to develop whole-blood assays for routine testing.

Although the technology now exists to perform most serum or plasma tests on whole blood, there are barriers other than instrumentation that make it difficult to accept this specimen type for many tests in the central lab. Over time, cell lysis in whole-blood specimens alters certain analyte concentrations such as potassium, lactate dehydrogenase, and phosphate, and cellular metabolism alters analytes like glucose and lactate. Consequently, whole-blood specimens require analysis within a limited time window to ensure accurate results for several common tests. Numerous other analytes exhibit some degree of reduced stability in whole blood, and clot formation and loss of specimen integrity over time severely limit the storage of whole blood for delayed or repeat testing. Taken together, these constraints and conventions indicate that current practices in specimen choice for central lab testing will likely continue in the foreseeable future.

When testing is performed outside the central lab, the criteria used for specimen selection change considerably. In settings such as the patient bedside, critical care and emergency room (ER) satellite labs, operating rooms, and physician office labs (POLs), the results need to be available as quickly as possible in order to make decisions on patient management. As a result, convenience and short TAT are the most important factors for point-of-care testing (POCT). Because whole blood requires no processing and is available for immediate analysis, it is often the specimen of choice in such settings. Consequently, the aforementioned limitations regarding short testing windows and stability over time are less relevant.

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The extent to which decentralized testing using whole blood might continue to expand remains to be seen. While the technology exists to perform most tests on whole-blood samples, the technology and test volumes associated with POCT tend to increase the cost per test—in some cases significantly—compared to centralized testing. In some institutions, a reduction in satellite laboratory personnel requirements and a shift to testing performed by nursing staff could possibly offset the cost burden. However, there may be limits to which nursing can accommodate increased testing duties, and the financing of new POCT supervisory positions may be necessary to ensure that quality assurance procedures are enforced. In institutions where centralized testing is available, it is clear that strong arguments based on medical, financial, and outcome studies will be needed to justify expansion of POCT. Perhaps the biggest hurdle to expansion of decentralized whole-blood testing is the consolidation and automation of core laboratories—powerful trends that tend to pull testing back to serum- or plasma-based high-volume analyzers.

Whole-Blood Tests at the Point of Care

As discussed above, most POCT uses whole blood, while tests conducted in the central laboratory utilize serum or plasma. The differences in sample matrix are not trivial as it turns out, because in some instances the results for certain tests are not equivalent.

One particularly good example is prothrombin time (PT) measurement, which is used to monitor patients on warfarin therapy in order to maintain an optimal therapeutic dose. While whole blood is arguably the more suitable specimen for coagulation assay because it contains plasma and platelets, plasma-based PT measurement remains the standard method. With the development of small whole-blood analyzers as a near-patient alternative to the conventional plasma method, laboratories have encountered the challenge of demonstrating equivalence of international normalized ratio (INR) values between the two methods.

POCT for glucose has also received much attention. The question is not whether whole blood gives better results, but rather if more frequent testing can reduce the incidence of adverse outcomes. Whole blood is regarded as the most convenient specimen for diabetics who need to test themselves daily.

For both of these tests, passing the equivalency hurdle is essential for success. In the hospital setting, strict equivalence with central lab methods—as defined by each institution—may be required to avoid having different reference ranges for the same test, which might confuse medical staff. Given the potential for negative sequelae due to incorrect INR results, self-testing using whole blood PT devices is being examined perhaps even more rigorously than it has been with home glucose testing.

Serum vs. Plasma in the Central Lab

The choice between serum and plasma for centralized chemistry testing is an area of great interest in terms of specimen selection. As noted earlier, serum has been the conventional standard for most chemistry tests, including routine, special chemistry, therapeutic drug monitoring (TDM), and tumor marker assays. One of the reasons for this convention—the historical development and availability of tests for serum only—is to an extent no longer applicable since plasma assays are now available for most common tests.
Today, the choice between serum and plasma is in large part a decision based on the unique requirements and priorities of an individual lab. When the priority is TAT, plasma has a clear advantage since it can be centrifuged immediately upon collection. However, in settings where the mean time between phlebotomy and specimen arrival at the laboratory exceeds recommended serum specimen clot times, or where the laboratory does not otherwise hold specimens, the advantages in TAT with plasma are not realized. Consequently, some institutions may limit plasma chemistry testing to settings where the specimen is immediately available for processing, such as an ER lab.

Labs may also find that serum samples lead to more instrument downtime than plasma samples as a result of fibrin formation. This claim needs to be verified by each laboratory, since specimen quality is highly dependent on adherence to recommended collection and handling conditions for both serum and plasma tubes. Aside from logistic considerations, another rationale for the use of plasma has been that it is free from several coagulation-induced changes in analyte concentrations, and more accurately reflects the composition of circulating blood.

A number of different anticoagulants are available for obtaining plasma samples. For chemistry testing, lithium heparin has been the preferred anticoagulant since both EDTA and

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**NCCLS Order of Draw GUIDELINES**

Under Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture, the current approved NCCLS standard for Multiple Specimen Collection order of draw (7.13.3.2) recommends the following:

The following order-of-draw, which is recommended when drawing several specimens during a single venipuncture, is based on pragmatism. Its purpose is to avoid possible test result error due to cross contamination from tube additives. This procedure should be followed for both evacuated tubes and syringe transfer of blood to multiple tubes.

1. **Blood culture tube**
2. **Plain tube, nonadditive**
   (e.g., red stopper)
3. **Coagulation tube**
   (e.g., blue stopper)
4. **Additive tubes:**
   - Gel separator
   - Heparin
     (e.g., green stopper)
   - EDTA
     (e.g., lavender stopper)
   - Oxalate/fluoride
     (e.g., gray stopper)

**BD Vacutainer™ Order of Draw for Multiple Tube Collections**

**Designed for Your Safety**

<table>
<thead>
<tr>
<th>Closure Color</th>
<th>Collection Tube</th>
<th>Mix by Inverting</th>
</tr>
</thead>
<tbody>
<tr>
<td>BD Vacutainer™ Glass Tubes</td>
<td>BD Vacutainer™ Plus Plastic Tubes</td>
<td>Blood Cultures - SPS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Serum Tube (glass)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Citrate Tube</td>
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<tr>
<td>or</td>
<td>BD SST™ Gel Separator Tube</td>
<td>5 times</td>
</tr>
<tr>
<td></td>
<td>Serum Tube (plastic)</td>
<td>5 times</td>
</tr>
<tr>
<td>or</td>
<td>Heparin Tube</td>
<td>8 to 10 times</td>
</tr>
<tr>
<td>or</td>
<td>BD PST™ Gel Separator Tube With Heparin</td>
<td>8 to 10 times</td>
</tr>
<tr>
<td></td>
<td>EDTA Tube</td>
<td>8 to 10 times</td>
</tr>
<tr>
<td></td>
<td>Fluoride (glucose) Tube</td>
<td>8 to 10 times</td>
</tr>
</tbody>
</table>

**Note: Always follow your facility’s protocol for order of draw**

Handle all biologic samples and blood collection “sharps” (lancets, needles, luer adapters and blood collection sets) according to the policies and procedures of your facility. Obtain appropriate medical attention in the event of any exposure to biologic samples (for example, through a puncture injury) since they may transmit viral hepatitis, HIV (AIDS), or other infectious diseases. Utilize any built-in used needle protector if the blood collection device provides one. BD does not recommend reshielding used needles, but the policies and procedures of your facility may differ and must always be followed. Discard any blood collection “sharps” in biohazard containers approved for their disposal.

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citrate bind calcium and have commonly-measured counterions (potassium, sodium). EDTA and citrate also cannot be used for enzymatic assays that require calcium as a cofactor, such as alkaline phosphatase. For accurate lactate determination, a fluoridated oxalate plasma specimen is often recommended to minimize glycolysis.

However, a number of analyte biases between serum and heparin plasma exist and must be assessed. Due to large positive biases, ammonia is typically not determined using serum. The clotting process also increases concentrations of potassium and inorganic phosphate, as well as activity of lactate dehydrogenase and alanine aminotransferase. However, the biases may be clinically acceptable or may be handled via an adjustment to the reference range. Total protein results are higher in plasma due to the presence of fibrinogen, and heparin itself may interfere with certain assays (e.g. amylase).

Many analyte biases are also highly analyzer-dependant. Published evaluations in the literature are few, and the assessment of equivalence and acceptability tends to be a very individual and variable process. In addition, storage stability of plasma for each analyte must be documented, since acceptable testing windows are smaller for many tests compared with serum. Thus, it is essential that each lab perform an independent evaluation when considering a switch to plasma testing.

It is interesting to note that the use of heparinized plasma is presently only a small fraction of that for serum. The percentage growth in plasma in recent years has, however, been slightly higher than with serum, and certain countries, particularly in Europe, tend to do considerably more plasma testing than others. Making the switch from serum to plasma is certainly feasible, but not without overcoming some hurdles. Given the workload involved in validation, laboratories need to make a very careful assessment of the benefits in order to ensure that any conversion to plasma testing makes sense for a given institution.

Future Trends in Blood-Based Testing

Choosing the right specimen is not merely a clinical decision, but a combination of clinical, financial, technological, and logistical considerations. Where a choice exists, the choice of specimen will be closely aligned with other trends in diagnostic testing. POCT outcome studies, pneumatic tube systems, core laboratory consolidation and automation, and continued biosensor development all have the potential to influence decisions on the choice of specimen. Certainly, it will be important for laboratory operators to monitor these trends.

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SUGGESTED READINGS

- Doumas BT, Hause LL, Simuncak DM, Breitenfeld D. Differences between values for plasma and...
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Working Group on Preanalytical Variables of the German Society for Clinical Chemistry and the German Society for Laboratory Medicine. Serum, plasma or whole blood? Which anticoagulants to use?


• Harr R, Bond L, Trumbull D.


• NCCLS. Document H 1-A4:

• Sainato D. PO CT moves to coagulation.

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• Sainato D. PO CT moves to coagulation.

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