Specimen-collection standards complete major revisions

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The Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) has been publishing standards and guidelines for clinical-laboratory procedures since the early 1970s. At least six documents detail proper specimen-collection and -processing procedures; most have been recently revised. The revisions reflect significant changes that all specimen-collection personnel, regardless of their professional discipline, must know in order to perform procedures consistent with the most current research and industry regulations.

Because published standards and guidelines carry significant weight in legal proceedings, managers who base their procedure manuals on the most current CLSI documents not only help assure that their staff’s techniques are up-to-date, but also assure the practice of good risk management.

To revise a document, CLSI assembles a volunteer working group consisting of representatives from government, industry, and the medical professions who arrive at a consensus on each revision based on research and other literature published since the last published edition of the document under review. This article discusses key changes to four major specimen-collection and -processing documents recently revised by CLSI — from which passages are reproduced here with permission — including:


**[Note]:** Copies of the current editions may be obtained from CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898.

### H3-A5: Procedure for the collection of diagnostic blood specimens by venipuncture

The specimen-collection document that has undergone the most sweeping changes is the venipuncture standard (see Table 1). This important standard serves as the basis for the routine venipuncture procedure used by most facilities. Since the document was last revised in 1998, changes in tube technology and revised OSHA (Occupational Safety and Health Administration) regulations, as well as an increased awareness of phlebotomy complications, have led to a new recommended order of draw and more detailed language to prevent phlebotomy-related injuries.

The new document reflects recent changes to the Bloodborne Pathogens Standard, which took effect in 2001. Collectors are now advised to discard the collection device without disassembly, which reflects OSHA’s mandate against removing needles from tube holders. As for handling needles attached to syringes, the revised standard cautions against piercing the stopper of collection tubes with the same needle used to perform the puncture. Instead, as recommended by OSHA, CLSI recommends activating the safety feature of the needle used to access the vein, removing and discarding it, and replacing it with a safety-transfer device to fill the tubes.

Also in keeping with OSHA standards, the document no longer recognizes one-handed needle re-sheathing as an acceptable form of concealment and stresses that sharps containers should be easily accessible and positioned at the point of use.

Two additions concerning phlebotomy chairs appear in the latest revision. One passage recommends that chairs in outpatient drawing areas be designed for the ergonomic comfort of the collector. The second modification suggests outpatient drawing chairs have arms both for support and to prevent falls should the patient pass out. Additional provisions for fainting patients include several passages that instruct phlebotomists to anticipate syncope in all patients and to be prepared to react. Also new in this revision is the working group’s caution against the use of ammonia inhalants on fainting patients in case the patient is asthmatic.

### Table 1. History of H3 (venipuncture) standard.

<table>
<thead>
<tr>
<th>Year</th>
<th>Edition</th>
<th>Standard Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003</td>
<td>Fifth</td>
<td>H3-A5: Procedure for the Collection of Diagnostic Blood Specimens by Venipuncture</td>
</tr>
</tbody>
</table>

**Procedural changes**

Many significant changes to the recommended procedure are reflected in the latest revision of the venipuncture standard, beginning with patient identification. Precautions against relying on identification bracelets that are not attached to the patient are now repeated and more prominently placed throughout the document. The standard now permits gloves to be applied just prior to site preparation instead of prior to surveying for veins, and advises collectors to inquire if the patient has a latex sensitivity.

Passages detailing the procedure have been infused with more detailed and precautionary language on site selection,
vein selection, needle insertion, and needle relocation in order to prevent patient injury. For example, whereas tourniquet use was optional in the previous edition of the standard, it is now required unless it interferes with test results (e.g., lactate). An illustration in the standard showing the location of the nerves and brachial artery reinforces the importance of selecting the basilic vein only when the safer median and cephalic veins cannot be found. Without tourniquet application, such safer alternatives may not be located. According to the standard, “Attempt to locate the median cubital vein on either arm before considering alternative veins. Due to the proximity of the basilic vein to the brachial artery and the median nerve, this vein should only be considered if no other vein is more prominent.”

To further prevent nerve injuries, the document now warns against selecting veins on the underside of the wrist, instructs that the needle be inserted at an angle of 30 degrees or less, and forbids lateral needle relocation in an effort to access the basilic vein. To avoid perforating or lacerating the brachial artery, the standard recommends that the pulse be located prior to any attempt to access the basilic vein. It also prohibits performing an arterial puncture as an alternative to difficult venipunctures.

The specimen-collection document that has undergone the most sweeping changes is the venipuncture standard.

To prevent the effects of hemocoagulation from altering test results, the prior revision recommended releasing the tourniquet immediately upon venous access. In the latest edition, the working group responded to concerns from those in the field who pointed out that releasing the tourniquet as soon as the blood begins to flow may result in a disruption in the flow prior to obtaining an adequate volume. The insertion of the words “if possible” now encourages collectors to release the tourniquet upon venous access when it is perceived that doing so will not interfere with the success of the procedure.

Order of draw
First introduced into the literature in 1977, the order of draw was developed to prevent the effects on test results that additives can exert when inadvertently carried over from one tube to the next by the needle making the transfer. Through the years, however, the order has undergone numerous official and unofficial modifications — including the emergence of a separate order to be employed when tubes were filled by syringe — resulting in widespread confusion (see “NCCLS Simplifies the Order of Draw: A Brief History,” MLO, May 2004, p. 26). In 1998, the standards organization recommended a single order of draw for both tube-holder collection and syringe draws. But since then, the widespread use of safer plastic blood-
collection tubes forced those using them to institute a modified order of draw since plastic serum tubes contain a clot activator. As a result, two distinct orders of draw were necessary: one for glass tubes and one for plastic.

In response to this development, the working group revising the standard, which included representatives from every major tube manufacturer, arrived at a consensus that simplified the order of draw to one that could apply to both glass and plastic tubes (see Table 2).

**Note:** Some facilities may find it necessary to alter this order to reflect internal studies that support a modification. When a unique order of draw is supported by reliable evidence, facility policy should take precedence.

### Table 2. Revised Order of Draw: 2003

| First: Blood culture tube or vials |
| Second: Coagulation tube (e.g., blue closure) |
| Third: Serum tube (with/without clot activator or gel separator) (e.g., red closure) |
| Fourth: Heparin tube (with/without gel separator) (e.g., green closure) |
| Fifth: EDTA (e.g., lavender closure) |
| Sixth: Glycolytic inhibitor (e.g., gray closure) |

**Other revisions**

The prior revision cautioned against drawing blood above an active, but temporarily discontinued, IV due to dilution and the presence of analytes in infusing fluids. The standard, however, references a 2002 article that reported several studies that show accurate results can be obtained for some analytes. Given that serious complications can occur when patients are treated according to results obtained from blood drawn above an active IV, CLSI still cautions against draws proximal to existing IVs, but recommends facilities establish their own policies.

Because of complications from excessive bruising, the working group inserted new language into the latest standard to ensure that hemostasis is complete before patients are bandaged. Specifically, the document calls for the collector to observe for hematoma formation. This requires pressure to the puncture to be released and a visual observation of a duration that ensures the detection of subcutaneous bleeding. Hematoma formation not only leads to unsightly skin discoloration and renders the site unacceptable for future venipunctures, but also can exert pressure on the nerves in the area and lead to a disabling compression nerve injury.

Other revisions include the inclusion of chlorhexidine as a site-prep solution, a passage requiring collectors to awaken sleeping patients before drawing blood, a recommendation for the use of hypoallergenic bandages, and the deletion of a passage that encouraged sharply tapping a site to make veins more pronounced.

### H4-A5: Procedures and devices for the collection of diagnostic capillary blood specimens

This revision of the skin-puncture document replaces the prior edition published in 1999. This standard’s major revision includes a technical and organizational overhaul and new illustrations. Most obvious is a title change that recognizes the difference between skin punctures and skin incisions as two means of obtaining capillary blood. The differentiation continues throughout the document. Many changes instituted in the H3-A5 standard were incorporated where appropriate, such as revised passages on patient identification, safety, and so forth.

New OSHA guidelines requiring the use of retractable devices are reflected throughout the revised document, and a procedure was inserted detailing the use of automated (i.e., retractable) devices. Scalpels and wire lancets were removed from the list of skin-puncture devices. In addition, precautions were inserted to recommend using plastic instead of glass capillary tubes and to implement work practices that prevent exposure. Since OSHA and the Centers for Disease Control and Prevention prohibit the use of glass capillary tubes, references to “scorers” have also been removed.

A revision to the section discussing hemolysis includes “excessive milking” of the puncture site as a cause of red-cell rupture. The new edition now recommends prewarming puncture sites for all tests to be collected, not just capillary blood gases. Changes to site selection include the removal of the great toe as a recommended site and the addition of punctures/incisions on the same side of a mastectomy to the list of sites from which capillary blood should not be obtained.

### H21-A4: Collection, transport, and processing of blood specimens for testing plasma-based coagulation assays

This guideline on the collection, transport, and processing of specimens for coagulation tests was published in 2003, updating the prior edition released in 1998. In the 1998 revision (H21-A3), CLSI removed the recommendation to use a discard tube when drawing PT (prothrombin time) and APTT (activated partial thromboplastin time) tests, since studies showed tissue thromboplastin did not affect results when the sodium citrate tube was the first or only tube drawn using a tube holder. The new guideline, however, stresses that proof of necessity for drawing a discard tube for other coagulation tests is “circumstantial at best,” but data suggesting this practice is unnecessary has not yet been published.

In addition, when a winged blood-collection set is used for a venipuncture procedure, a discard tube must be used to fill the blood-collection tube dead space whenever a coagulation tube is the first or only tube drawn. This procedure will prevent underfilling and ensure that the proper anticoagulant:blood ratio is maintained. The discard tube does not have to be filled completely and should be a nonadditive or a coagulation tube.

**An assessment of the patient’s veins should include consideration for the amount of blood to be drawn, the age of the subject, and the size of his veins.**

Whereas the prior revision (H21-A3) stated platelet-poor plasma with counts less than 10 x 10^9/L is acceptable to use for routine coagulation testing (APTT, PT/INR [international normalized ratio] for nonheparinized patients, and thrombin time), the new guideline states platelet counts greater than 10 x 10^9/L are acceptable for the same tests, provided testing is performed on fresh specimens and the patient is not heparinized. Platelet counts greater than 10 x 10^9/L may not be acceptable for other coagulation tests (i.e., for lupus anticoagulants, other phospholipid antibodies, and heparin-monitoring assays). The guideline also recommends that specimens frozen for subsequent testing be platelet-free.
The revision also states that there are no published articles correlating small-gauge needles with platelet activation or hemolysis. It is recommended, therefore, that phlebotomists select the most appropriate needle for the patient’s available veins to minimize clotting and hemolysis. An assessment of the patient’s veins should include consideration for the amount of blood to be drawn, the age of the subject, and the size of his veins.

Finally, the latest edition stipulates that centrifugation performance should be validated every six months or after modification of the centrifuge to ensure plasma platelet counts are within acceptable limits.

H18-A3: Procedures for the handling and processing of blood specimens

This guideline, updated and published in 2004, examines various blood-collection and -processing variables that alter clinical-laboratory test results, including prolonged serum/cell or plasma/cell contact, improper centrifugation, and storage practices for serum- or plasma-separator devices. A substantial portion of the document recommends performance criteria for serum separators. Implementing the recommendations set forth in this document should help facilities reduce error and improve the accuracy and efficiency of patient test results.

Much of what is new in this document focuses on centrifugation and its effect on potassium results. The working group conducted a literature search for articles published between 1966 and 2004 and returned four references indicating that specimens for potassium should not be re-centrifuged. Reflecting the results of the literature search, the revision now states that specimens for potassium measurement “should not be centrifuged more than once because results will be falsely increased.”

The guideline recommends laboratories investigate questionable results of other analytes that may be attributable to re-centrifugation, especially when the time between centrifugation and re-centrifugation is prolonged, which can occur between remote blood-collection locations and the testing laboratory.

In previous editions of the document, recommendations for refrigeration were made for serum and plasma if testing was not completed within five hours following blood collection.

Earlier editions also noted that a few studies indicated that, when tubes were stoppered and serum was in contact with cells, several analytes were stable at room temperature for 24 to 72 hours. The new guideline states that if an analyte is stable at room temperature and is unseparated, the serum or plasma sample should also be stable at the same temperature for the same length of time in separated serum or plasma.

Unchanged is the guideline’s recommendation that serum or plasma exposed to cells in a blood-collection tube prior to centrifugation should not exceed two hours. The update emphasizes, however, that if conclusive evidence indicates that longer contact times between serum and plasma and cellular constituents do not contribute to test error, then that particular processing time is acceptable to use in the laboratory and should be reflected in the facility’s standard operating procedures.

The guideline also proceeds to make the following “general storage recommendations” for serum/plasma:

1. Separated serum/plasma may be kept at room temperature up to eight hours. For assays not completed within eight hours, use refrigeration (2°C to 8°C).
2. Keep separated serum/plasma frozen at or below -20°C if assays are not completed within 48 hours or storage is needed beyond 48 hours. Serum/plasma samples are to be used from one freeze/thaw cycle.
3. Use and follow documented references for those analytes that do not follow these recommendations.
4. Use the manufacturer’s directions if recommendations conflict with serum/plasma separator device practices.

It is therefore the responsibility of the individual laboratory to use all available references and/or its own studies to determine its own specific stability criteria.