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Procedures for the Handling and Processing of Blood Specimens; Approved Guideline—Third Edition

This document includes criteria for preparing an optimal serum or plasma sample and for the devices used to process blood specimens.

A guideline for global application developed through the NCCLS consensus process.

NCCLS...

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Abstract

NCCLS document H18-A3—*Procedures for the Handling and Processing of Blood Specimens; Approved Guideline—Third Edition* considers multiple variables that are involved in handling and processing blood specimens. Its application should enable the user to recognize and control accuracy and precision factors that occur between the time of blood collection and the time of test performance.

Criteria for optimal serum, plasma, or whole blood samples are established, as well as criteria for the performance of *in vitro* devices used to process blood specimens. Implementation of recommended procedures should assist laboratories in the pursuit of excellent performance, with useful, accurate patient test results as the ultimate goal.

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Contents

Foreword

This guideline specifies criteria that should assist the laboratory and other healthcare providers in recognizing and reducing or eliminating preanalytic error resulting from improper handling of blood specimens. The document is the result of considerable discussion and comment; appropriate references are indicated.

Recognition of the need for procedures that address the different areas of specimen collection is evidenced by the following NCCLS documents:

- H1—*Tubes and Additives for Venous Blood Specimen Collection*;
- H3—*Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture*;
- H4—*Procedures and Devices for the Collection of Diagnostic Capillary Blood Specimens*;
- H11—*Procedures for the Collection of Arterial Blood Specimens*;
- H18—*Procedures for the Handling and Processing of Blood Specimens*; and
- H21—*Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays*.

In this document, certain abbreviations have been used: AST (aspartate aminotransferase, SGOT); ALT (alanine aminotransferase, SGPT); LD (lactic dehydrogenase, LDH); CK (creatine kinase, CPK); APTT (activated partial thromboplastin time); T3 (tri-iodothyronine); T4 (thyroxine); and PCV (packed cell volume).

This document replaces the second-edition approved guideline, H18-A2, which was published in 1999. A number of changes have been made in this edition; chief among them is the expansion of the recentrifugation section to include gel and non-gel tubes (see Section 6.2.3). The recommendation that gel tubes should not be used for the collection of ionized calcium specimens has been deleted from Section 7.5 for consistency with NCCLS document C31—*Ionized Calcium Determinations: Precollection Variables, Specimen Choice, Collection, and Handling.*

Key Words

Chilled specimens; criteria for specimen rejection; handling and processing specimens; precentrifugation, centrifugation, and postcentrifugation phases; serum or plasma contact with clot or cells; serum/plasma separator devices; tube closure

Procedures for the Handling and Processing of Blood Specimens; Approved Guideline—Third Edition

1 Scope

This guideline addresses handling and processing of blood specimens for analytical determinations using serum, plasma, or whole blood in the clinical laboratory. The variables associated with precentrifugation, centrifugation, and postcentrifugation phases of specimen handling and processing are emphasized. Where applicable, the recommendations should be considered by the following laboratory areas: chemistry, coagulation, hematology, immunology, ligand assay, serology, toxicology/therapeutic drug monitoring, virology, blood bank, and molecular or DNA analysis.

2 Introduction

This guideline addresses the multiple factors associated with handling and processing blood specimens. These factors can introduce test result inaccuracy or systematic bias after the specimen has been collected but before the test is performed. Performance criteria for *in vitro* diagnostic blood collection devices used to separate serum or plasma from cellular components are also addressed.

Several issues in the handling and processing of blood specimens are documented in the scientific literature.[1-12] Specific concerns relate to prolonged contact of serum or plasma with cells or with tube stoppers; hemolysis; analyte concentration changes due to evaporation; incorrect storage temperature; the use of anticoagulants and serum/plasma separator devices; incorrect transport; and turnaround time (TAT) for patient results. Recognition and control of these variables should reduce error and contribute to the medical usefulness of patient test results.

3 Standard Precautions

Because it is often impossible to know what might be infectious, all patient and laboratory specimens are treated as infectious and handled according to "standard precautions." Standard precautions are guidelines that combine the major features of "universal precautions and body substance isolation" practices. Standard precautions cover the transmission of all infectious agents and thus are more comprehensive than universal precautions which are intended to apply only to transmission of blood-borne pathogens. Standard and universal precaution guidelines are available from the U.S. Centers for Disease Control and Prevention (*Guideline for Isolation Precautions in Hospitals.* Infection Control and Hospital Epidemiology. CDC. 1996;17(1):53-80 and *MMWR* 1988;37:377-388). For specific precautions for preventing the laboratory transmission of all infectious agents from laboratory instruments and materials and for recommendations for the management of exposure to all infectious disease, refer to the most current edition of NCCLS document M29—*Protection of Laboratory Workers from Occupationally Acquired Infections*.

4 Definitions

Accuracy (of measurement) – Closeness of the agreement between the result of a measurement and a true value of the measurand (VIM93).[13]

Analyte – Component represented in the name of a measurable quantity (ISO 17511)[14]; **NOTES:** a) In the type of quantity "mass of protein in 24-hour urine," "protein" is the analyte. In "amount of substance of glucose in plasma," "glucose" is the analyte. In both cases, the long phrase represents the **Measurand**

(ISO 17511)[14]; b) In the type of quantity "catalytic concentration of lactate dehydrogenase isoenzyme 1 in plasma," "lactate dehydrogenase isoenzyme 1" is the analyte (ISO 18153).[15]

Centrifugation phase – The time period when the specimen is inside the centrifuge.

Error (of measurement)//Measurement error – The result of a measurement minus a true value (or accepted reference value) of the measurand (VIM93)[13]; **NOTES:** a) Since a true value cannot be determined, in practice a conventional true value is used (VIM93); b) When it is necessary to distinguish "error" from "relative error," the former is sometimes called **absolute error of measurement**. This should not be confused with **absolute value of error**, which is the modulus of the error (VIM93); c) Formerly, the term "total error" was often used in NCCLS documents.

Postcentrifugation phase – The time period after the centrifuging of the specimen and before removal of an aliquot of serum or plasma for testing.

Precentrifugation phase – Time period after specimen collection and before specimen centrifugation.

Sample – One or more parts taken from a system, and intended to provide information on the system, often to serve as a basis for decision on the system or its production.

Secondary tube – A tube used to contain the resultant plasma/serum yielded by the centrifugation of a primary additive/serum tube containing the patient specimen.

Separated serum/plasma – Serum or plasma that has been completely separated from any contact with cells or a clot; **NOTES:** a) The serum or plasma has either been removed, by pipette, from the cells or contact has been interrupted by a chemical/physical barrier through the use of a serum/plasma separator device (see Section 7); b) The separated serum/plasma should be visually free of erythrocytes; however, 0.1% to 1% intact cells do not contribute to a hemolysis effect.[7]

Specimen (patient) – The discrete portion of a body fluid or tissue taken for examination, study, or analysis of one or more quantities or characteristics to determine the character of the whole.

5 Description of the Product Class

This guideline provides recommended criteria for correct handling of whole blood specimens and serum or plasma samples. Specifications for the optimal performance of *in vitro* diagnostic devices used in the processing of blood specimens are also provided. Devices in this class include integrated blood collection serum or plasma separator devices; devices inserted into the collected blood specimen before initiating centrifugation; and devices used to harvest the serum or plasma after centrifugation.

6 Whole Blood Processed to a Serum or Plasma Sample

Recommendation: Serum or plasma should be physically separated from contact with cells as soon as possible, unless conclusive evidence indicates that longer contact times do not contribute to result error. **A maximum limit of TWO HOURS from the time of collection is recommended.**

NOTE: A contact time of less than two hours is recommended for potassium,[16] ACTH, cortisol,[8] catecholamines,[17] lactic acid,[18] and homocysteine.[19]

For some specimens, temperature affects stability.[4,20,21] However, many analytes have not been studied.

The following studies support a **two-hour** precentrifugation time limit as recommended in this document:

The Laessig, et al study[5] determined that the 17 analytes below were unaffected by a precentrifugation serum-cells contact time as long as 48 hours (room temperature):

- albumin;
- alkaline phosphatase;
- ALT;
- bilirubin;
- calcium;
- cholesterol;
- CK;
- creatinine;
- magnesium;
- phosphorus;
- sodium;
- total protein;
- triglycerides;
- T3;
- T4;
- urea nitrogen; and
- uric acid.

However, altered results were clinically significant for the following:

By **two hours:**

- glucose (decreased);
- potassium (increased); and
- LD (increased).

By **eight hours:**

- iron (increased).

AST showed a slight increase, and chloride a slight decrease, with time. Whereas this study did not indicate any change for magnesium, other investigators report a magnesium increase by four hours at 4 ^{o}C.[22]

In the Chu and MacLeod study of 26 analytes,[23] the effects of a 72-hour contact time at room temperature were determined. Many of the aforementioned 48-hour stable analytes retained stability for an additional 24 hours. In addition, amylase, bicarbonate, cortisol, and gamma-GT were also stable. Analytes adversely affected by 72-hour contact include: glucose (decreased); potassium (increased); phosphorus (increased); creatinine (increased); folate (decreased); and vitamin B12 (increased). Minimally affected analytes include: LD (increased); chloride (decreased); calcium (decreased); ferritin (increased); and sodium (increased). A 72-hour study by Ruby, et al[24] confirmed ALT stability at 4 ^{o}C and 22 ^{o}C.

In addition to the analytes in the previous references, a multianalyte study by Rehak and Chiang[4] determined stabilities of lipase, TBG, and TSH in unseparated serum for 24 hours at seven different temperatures (3 ^{o}C, 10 ^{o}C, 15 ^{o}C, 22 ^{o}C, 25 ^{o}C, 30 ^{o}C, and 38 ^{o}C). Generally, 22 to 25 ^{o}C is considered room temperature, and at 22 ^{o}C, changes were observed for glucose (decreased); potassium (increased); phosphorus (increased); ALT (increased); AST (increased); and creatinine (increased). The higher the

temperature, the more accentuated the alteration. Another time-temperature study (at 0 °C, 23 °C, and 30 °C) by Ono, et al[20] substantiates the instability of many analytes at temperatures above room temperature (30 °C): glucose (decreased by four hours); phosphorus (increased by six hours); ALT and AST (increased by eight hours); and potassium (increased by 24 hours). As expected,[25,26] potassium was significantly increased at temperatures below 15 °C.

In a few studies of selected analytes, ionized calcium in unseparated serum was stable for only two hours at 25 °C, but stable for up to 96 hours at 4 °C.[27,28] Ionized magnesium was stable at room temperature for over six hours and five days at 4 °C.[29] With serum in contact with cells, tricyclic antidepressant drugs are reported to be stable for six days at 4 °C.[30]

In a more recent study, Zhang and fellow investigators evaluated the stabilities of 63 analytes, serum in contact with cells for 24 hours. They determined that serum should be separated from the clot within three hours for glucose, potassium, and phosphorus, and within six hours for albumin, bicarbonate, chloride, C-peptide, HDL-cholesterol, iron, LDL-cholesterol, and total protein. All other analytes in their study were stable for 24 hours without serum separated from the clot. As the authors point out, new laboratory paradigms require greater knowledge of analyte stability. There are increased transportation/time issues from specimen collection site to testing laboratory. It is important to decrease false rejections of acceptable specimens.[31]

For recommended timing on coagulation testing, please refer to the most current edition of NCCLS document H21—*Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays.*

6.1 Precentrifugation Phase

6.1.1 Recommendations

Regardless of the device used for specimen collection, all tubes containing additives except sodium citrate should be gently inverted at least five to ten times to mix the contents, unless otherwise specified by the manufacturer. Tubes containing sodium citrate should be inverted three to four times to mix the contents. (Please refer to the most current edition of NCCLS document H21—*Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays* for more detailed information regarding the precentrifugation phase for coagulation testing.)

6.1.1.1 Serum

Specimens should be clotted before centrifugation. Rimming the tube with a wooden applicator stick to release the clot is not recommended (see Section 6.1.4.1).

Spontaneous and complete clotting normally occurs within 30 to 60 minutes at room temperature (22 to 25 °C).[32] The time to clot will be prolonged if the patient is on anticoagulant therapy. Chilling the specimen (2 to 8 °C) delays clotting. If the time allowed for the specimen to clot is inadequate, latent fibrin formation can cause a problem for many instrument systems leading to erroneous results.

To accelerate clotting, a collection device can be used that contains an activator/accelerator (e.g., snake venom/thrombin, approximately two to five minutes; thrombin, approximately five minutes; and glass or silica particles, 15 to 30 minutes).[6,33-36]

6.1.1.2 Plasma

A collection device containing an anticoagulant is used when plasma is required. Anticoagulated specimens can be centrifuged immediately after collection.

6.1.1.3 Chilled Specimens (2 to 8 °C)

Chilling a specimen inhibits the metabolism of blood cells and stabilizes certain thermolabile constituents. Whole blood specimens are not to be chilled unless there are documented recommendations for so doing. Chilling whole blood beyond two hours is contraindicated for a specimen intended for potassium, because cold inhibits glycolysis, which provides energy for pumping potassium into the cell.[20,25] Without this energy, potassium will leak from the cells, falsely elevating the results. Specimens collected for electrolytes must not be stored at 2 to 8 °C before centrifugation and testing.

To chill a specimen, place it immediately in either crushed ice or a mixture of ice and water. Good contact between the cooling medium and the specimen is essential. Large cubes of ice instead of water are not acceptable because of inadequate contact between the coolant and the specimen. The coolant must cover the specimen level in the device.

The following are examples of tests that require a chilled specimen: catecholamines, ammonia, lactic acid, pyruvate, gastrin, and parathyroid hormone (PTH).[37] Please refer to the most current edition of NCCLS document H11—*Procedures for the Collection of Arterial Blood Specimens* for recommendations regarding specimen collection and handling for pH/blood gas analyses.

6.1.1.4 Metabolic Inhibitors and Preservatives

Collection devices containing an additive (e.g., fluorides) can prevent concentration changes within the specimen over extended periods of time. The antiglycolytic agent, sodium fluoride, is used to stabilize glucose in the presence of blood cells for up to 24 hours at 22 to 25 °C or 48 hours at 2 to 8 °C.[37]

Great care must be taken with newborn and pediatric specimens collected for a glucose determination. It is difficult to inhibit the glycolytic metabolism of these blood cells.[38] This effect is compounded by a high hematocrit or leukocytosis.[39,40] Microcollection devices containing a suitable antiglycolytic agent may be used for pediatric blood glucose collection.

6.1.2 Transportation

For more information, please refer to the most current government regulations and NCCLS document M29—*Protection of Laboratory Workers from Occupationally Acquired Infections.*

6.1.2.1 Blood Specimens Collected On-Site

6.1.2.1.1 Time

Specimens must be transported in the appropriate biohazard bags or containers to the laboratory in as short a time as possible. Unless chilling of the samples is required, all samples should be transported at room temperature. Prompt removal of specimens from the collection area is especially important if the area temperature is above 22 °C, which may cause some analytes to deteriorate.

6.1.2.1.2 Tube Orientation

Where possible, tubes of blood should be kept in a vertical, closure-up position. This positioning promotes complete clot formation and reduces agitation of the tube contents, which in turn reduces the potential for hemolysis.

In the past, tube closures in contact with blood have been a source of contamination in toxicology/therapeutic drug monitoring testing.[41-44] The contaminant, tris-butoxyethyl phosphate (TBEP), interferes in TDM assays by displacing basic drugs from their protein binding sites, resulting in a

redistribution of drug into the erythrocytes with a decreased amount of drug left in the serum or plasma for assay. TBEP has been eliminated by tube manufacturers and is no longer a problem.

Special tubes are now available for trace element studies (e.g., zinc, copper, and selenium). They have minimal closure/tube contamination.[45-47] These tubes are recommended.

6.1.2.1.3 Specimen Agitation and Hemolysis

Gentle handling of collected specimens helps to minimize erythrocyte damage. Hemolyzed specimens may cause chemical interferences and interference with some optical instrumentation. Young, et al[48] have listed the chemical constituents whose concentration in serum and plasma may be affected by hemolysis. It has been reported that plasma containing 20 mg/dL (200 mg/L; 0.012 mmol/L) of hemoglobin is faintly pink, and plasma containing 100 mg/dL (1 g/L; 0.06 mmol/L) of hemoglobin is red.[49] Other studies, however, have reported that as much as 190 mg/dL (1.9 g/L; 0.12 mmol/L) of hemoglobin may not always be visually evident.[50] Elevated bilirubin in the plasma may mask hemoglobin, and a hemoglobin concentration of 200 mg/dL (2 g/L; 0.124 mmol/L) may not be detected by the unaided eye if the plasma contains 20 mg/dL (200 mg/L; 342 μmol/L) of bilirubin;[51] detection of discoloration also depends on the diameter of the tube being viewed.

Tests[7] **seriously** affected (all increased) include:

- LD;
- AST;
- potassium; and
- plasma hemoglobin.

Tests **noticeably** affected include:

- iron (increased);
- ALT (increased); and
- T4 (decreased).

Tests **slightly** affected (all increased) include:

- phosphorus;
- total protein;
- albumin;
- magnesium;
- calcium; and
- acid phosphatase.

Technological advances have made possible the assay of several analytes in whole blood (sodium, potassium, chloride, glucose, BUN, creatinine, ionized calcium, etc.). If hemolysis is present, it is masked when the sample is whole blood, and it may be responsible for an erroneous result. It is recommended that laboratories using whole blood instruments check for hemolysis when results are beyond specific designated concentrations (e.g., potassium >5.5 mmol/L) to determine if the result is erroneous because of the hemolysis. The specimen, or an aliquot of the specimen, should be centrifuged and the plasma visually inspected.

6.1.2.1.4 Exposure to Light

It is important to avoid exposing blood specimens for photosensitive analytes to artificial light or sunlight (ultraviolet) for any length of time. For bilirubin, this is critically important when an icteric newborn is being monitored for the possibility of an exchange transfusion.[52] Further examples include vitamins A and B6, beta-carotene, and porphyrins. These specimens should be protected with an aluminum foil wrap, an amber specimen container, or the equivalent.

6.1.2.2 Off-Site

6.1.2.2.1 Remote Collection Sites

The stability of the specimen dictates conditions for transport from remote collection sites (e.g., physicians' offices, satellite draw stations) to the testing location. If an uncentrifuged whole blood specimen is to be sent to the laboratory for testing, it must reach the laboratory in time to be processed with serum/plasma separation occurring in a time limit to protect the stability of the analytes. If this requirement cannot be met, the specimen must be centrifuged at the collection site, with the serum or plasma separated from the cells and held under appropriate conditions (see Section 6.3) until it can be delivered to the laboratory. Secondary tubes must be leak-proof. Specific handling and processing requirements published by the testing laboratory should also be consulted.

6.1.2.2.2 Courier Services

A courier service used to transport specimens or samples from a physician's office, remote blood-drawing station, or another laboratory, should pay particular attention to adequate packaging and handling to ensure constituent stability for the tests requested. (See the most current government regulations and NCCLS document M29—*Protection of Laboratory Workers from Occupationally Acquired Infections* for specific details.) Of critical importance is attention to transport conditions that are either too hot (summer transportation) or too cold (winter transportation).[8]

6.1.2.3 Automated Delivery Systems

Pneumatic tubes are the predominant automated transport system for specimen delivery to the laboratory. The effects of these systems on the validity of laboratory results have been evaluated.[53-60] The results vary according to the particular tube system, but in general, the tests affected are those influenced by the disruption of red cell membrane integrity. The tests most often cited are LD, potassium, plasma hemoglobin, and acid phosphatase.

Other testing areas that are not appropriate for pneumatic tube delivery systems are samples that must be maintained at body temperature, including cryoglobulins and cold agglutinins.

Reports indicate that transport does not affect the following tests in any of the tube delivery systems:

- albumin;
- alkaline phosphatase;
- AST;
- chloride;
- creatinine;
- glucose;
- sodium;
- total bilirubin;
- total protein;

- urea nitrogen;
- uric acid;
- leukocyte count; and
- thrombin time.

Unless specific documentation already exists, automated transport systems, pneumatic or otherwise, should be evaluated for any effects on laboratory results.

6.1.3 Receipt of Unprocessed Specimens by the Laboratory

6.1.3.1 Time

Upon receipt, specimens should be sorted and prepared for centrifugation. Allow sufficient time for clotting to occur. Blood collected using a tube containing a clotting activator (e.g., thrombin, silica, or glass particles) can be processed as early as 5 to 30 minutes after the blood is drawn. Anticoagulated specimens can be centrifuged immediately (see Sections 6.1.1.1 for serum and 6.1.1.2 for plasma). Also refer to manufacturers' specific recommendations.

Some tests require an unseparated, anticoagulated whole blood specimen (e.g., blood lead, cyclosporin,[61] and glycohemoglobin/A_1C). These specimens are not to be centrifuged. If accidentally centrifuged, do not discard the specimen; send the centrifuged tube to the testing location.

6.1.3.2 Temperature

Chilled specimens (2 to 8 ^{o}C) are to be kept at this temperature until they are ready to be centrifuged. Temperature-controlled centrifuges are recommended (see Section 6.2.2).

6.1.3.3 Tube Orientation

It is recommended that tubes of blood be placed in a vertical, closure-up position upon delivery to the laboratory.

6.1.3.4 Tube Closure

Tubes of blood are to be kept closed at all times. Certain test results can be inaccurate when the tube closure is removed because of an increase in specimen pH resulting from the loss of carbon dioxide (e.g., pH [increased], ionized calcium [decreased], and acid phosphatase [decreased]). Keeping the tube in a closed position eliminates possible exogenous contamination of the specimen and prevents evaporation and the possibility of spills and aerosols.

6.1.3.5 Criteria for Specimen Rejection

Under the following conditions, blood specimens may not be acceptable for testing purposes. Professional judgment at the laboratory director/supervisor level must be exercised in applying these criteria:

- Inadequate Specimen Identification

Example: A tube of blood is not labeled or it is inadequately labeled.

The most current edition of NCCLS document H3—*Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture* should be consulted for recommended labeling requirements. In addition, consult facility policy.

- Inappropriate Volume of Blood

The amount of additive placed into a tube is intended for a certain volume of blood. If less blood than required is drawn, the excess amount of additive has the potential to adversely affect the accuracy of test results.[57,62-64] If more blood is drawn than is required, the amount of additive may be insufficient for its intended purpose and may similarly adversely affect the accuracy of test results.[65] Specific references should be consulted to help avoid short-draw or over-draw additive problems.

Example 1: Sodium citrate: prothrombin time.[66]

Example 2: EDTA: PCV, cell count, cell morphology, lipids.[67-70]

Example 3: Heparin: CK, aminoglycosides (gentamicin, tobramycin).[71,72]

- Using the Wrong Collection Tube

Method-specific specimen requirements must be considered. In particular, tubes with additives are not to be used indiscriminately. An additive can interfere with the analyte to be determined.[48]

Example 1: Sodium fluoride interferes in the urease urea nitrogen method.[73]

Example 2: The wrong order of draw during multiple blood specimen collection can invalidate results because of contamination by the additive.[9]

- Hemolysis

Hemolysis can occur *in vivo* or *ex vivo*. *In vivo* hemolysis may also be the result of a disease process causing intravascular erythrocyte destruction. Repeated blood collection and continued receipt of hemolyzed specimens often indicates intravascular hemolysis and the physician should be notified. Hemolysis can result from a difficult venipuncture or from improper handling of the collected specimen. Hemolysis can occur when blood is drawn from a catheter with connectors having very small or large internal diameters. The changes in the internal diameters may cause turbulence and result in cell disruption with hemolysis. Certain tests will be inaccurate when the specimen is hemolyzed *in vitro*[7] (see Section 6.1.2.1.3).

- Improper Storage/Transportation

Example 1: A specimen that should have been chilled (2 to 8 °C) is received by the laboratory unchilled.

Example 2: A serum or plasma sample that should have been frozen is received thawed by a reference laboratory.

6.1.4 Preparing Specimens for Centrifugation

6.1.4.1 Clot Release (Rimming the Tube)

The use of a wooden applicator stick or similar device for the release of a clot attached to the tube closure or the sides of the tube is not recommended. Rimming the tube is a potential source for laboratory-induced hemolysis.[74] Clot/cell hang-up has been virtually eliminated by technical improvements in tube/closure design and manufacture. If rimming should be necessary, great care should be exercised in removing the closure and in reclosing the tube to prevent aerosol formation. Many laboratories remove tube closures behind a plastic shield or within a biological safety cabinet. It is important to reclose the tube or use a suitable closure and make sure the closure stays in place.

6.1.4.2 Tube Closure

Tubes of blood are to be kept closed at all times. If a separator device must be inserted into the tube before centrifugation (see Section 7.1.3), and the device does not cover the tube opening, a closure must be used.

6.2 Centrifugation Phase

Blood specimens for serum samples should be adequately clotted before centrifugation. Tubes should be centrifuged with their closures in place and with a centrifuge that has an adequate closure.

Tubes of blood intended for whole blood analysis are **not** to be centrifuged and separated (see Section 6.1.3.1).

6.2.1 Centrifuge Time and Relative Centrifugal Force (rcf)

6.2.1.1 Recommendations

The manufacturer's literature, which makes recommendations for specific blood collection devices, should be consulted. Advancements in technology may provide for adequate specimen preparation at different speeds and times of centrifugation.

6.2.1.2 Relative Centrifugal Force (rcf)

Relative centrifugal force (g-force) is a more meaningful term than revolutions per minute (rpm). This document recommends that laboratories describe centrifuge requirements in terms of rcf. The rpm is of limited use to the reader without an indication of the centrifuge model and its specific rotor and head, and the effective radius. The effective radius is the distance measured from the rotor axis to the bottom of the fluid inside the tube at the greatest horizontal distance from the rotor axis.

(a) Calculating rcf

$$rcf = 1.118 \times 10^{-5} \times r \times n^2$$

where:

r = rotating radius (cm); and
n = speed of rotation (rpm).

(b) Relative Centrifugal Force Nomograph (see Figure 1)

6.2.2 Temperature-Controlled Centrifuges

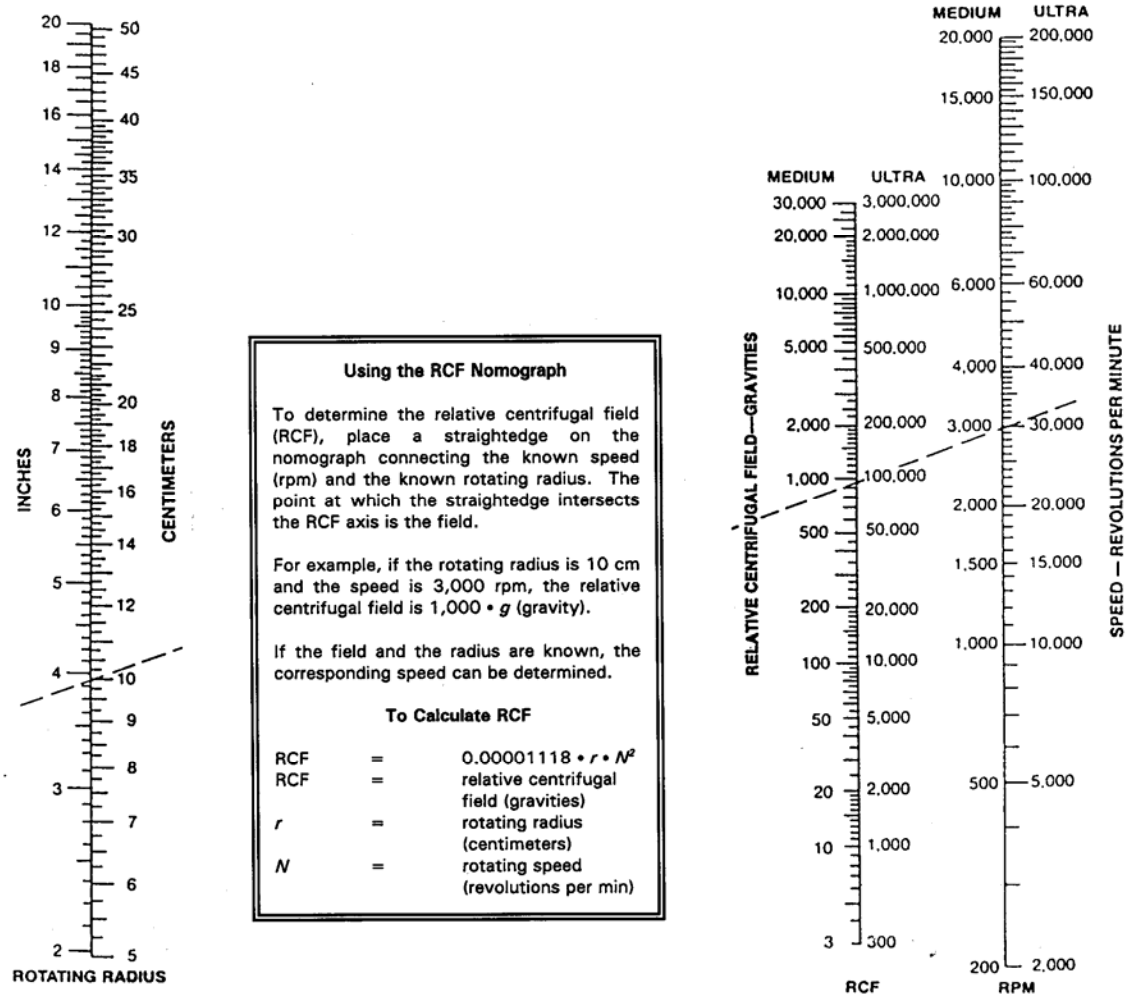
6.2.2.1 Recommendations

Laboratories should have temperature-controlled centrifuges for temperature-sensitive analytes. Centrifuges can generate internal heat that may be inappropriate for analyte stability (e.g., coagulation studies). Specific thermolabile analytes should be separated at 4 °C (e.g., ACTH, cyclic-AMP). Check to make sure the centrifuge is at its intended setting. Unless documentation supports a specific temperature for a specific analyte, a centrifuge setting of 20 to 22 °C is recommended.[2]

6.2.2.2 Chilled Specimens

Specimens that are transported to the laboratory chilled should be centrifuged under temperature-controlled conditions. For a specimen requiring chilled centrifugation, and potassium is one of the requested tests, prompt removal of the specimen from the centrifuge is required (see Section 6.1.1.3).

Temperatures lower than 15 °C can falsely elevate potassium results after two hours.[4,25]



ROTATING TIP RADIUS

The distance measured from the rotor axis to the tip of the liquid inside the tubes at the greatest horizontal distance from the rotor axis is the rotating tip radius. The radius is listed for your convenience in the speed and force tables.

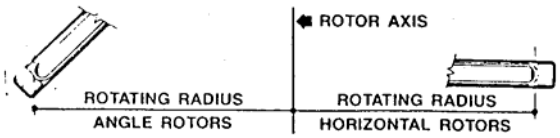


Figure 1. Relative Centrifugal Force Nomograph. Because models and sizes of centrifuges vary considerably, the use of gravity (g) forces instead of revolutions per minute (rpm) is suggested. A nomograph for calculating centrifugal speed is provided. The rotating radius of the centrifuge head is the basis for the calculation and it must be carefully determined. Information on this procedure is also provided. Reprinted with permission from Thermo Electron Corporation, Milford, MA 01757, U.S.A. Tel: (866) 9THERMO; Fax: (508) 634-2199; Email: info.sampleprep@thermo.com.

6.2.3 Recentrifugation

Specimens for potassium measurement should not be centrifuged more than once. Results will be falsely increased.[75-78]

NOTE: A literature search was performed for the years 1966 to 2004. Four references specifically indicate that specimens for potassium should not be recentrifuged.[75-78] No other analytes were cited. However, the area committee cautions that the potential for inaccuracy for other analytes is possible. Laboratories may wish to conduct studies when there are unexplained results possibly attributable to recentrifugation, for example, when the time between centrifugation and recentrifugation has been lengthy, as can occur between offsite blood collection locations and the testing laboratory.

Harvesting of additional serum/plasma AFTER serum/plasma has been removed from non-gel or gel tubes SHOULD NOT be attempted. When serum/plasma has been removed and harvesting of additional serum/plasma is attempted, the volume ratio of plasma to erythrocytes has been altered. Analytes from cellular leakage/exchange, accentuated by clot retraction, will then be centrifuged into the serum/plasma used for testing. This can cause erroneous results.

6.3 Postcentrifugation Phase

6.3.1 Recommendations

6.3.1.1 Serum/Plasma Contact with Cells/Clot

It is recommended that serum or plasma be physically separated from contact with cells as soon as possible with a **maximum time limit of two hours from the time of collection**, unless conclusive evidence indicates that longer contact times do not contribute to error of the results. Shorter contact times may be necessary (see Section 6).

6.3.1.2 Storage

6.3.1.2.1 Separated Serum or Plasma

The growing number of laboratory tests mandates that specific references be consulted to determine exact handling and storage conditions necessary to ensure the stability of specific analytes. It is the responsibility of the individual laboratory to use all available references and/or their own studies to determine specific stability criteria for their laboratory.

In 1965, Winsten[3] recommended that serum and plasma be refrigerated if testing was not complete within five hours after collection. There are now several studies indicating that many analytes are stable at room temperature for 24 to 72 hours, when tubes are stoppered and **serum is in contact** with cells.[4,5,20,23,24] It seems apparent that an analyte that is stable at room temperature in **unseparated** serum/plasma should also be stable at the same temperature for the same length of time in separated serum/plasma. There are also several stability studies of separated serum. At 2 to 8 °C and room temperature, significant stability of separated serum has been observed for 2 to 14 days.[79-85] However, room temperature (20 to 25 °C) in the laboratory is a critical stability parameter with decreased stability observed at temperatures above 22 °C for some analytes.[4] Based on the studies cited, this guideline makes the following 'general' recommendations, recognizing that there can be several exceptions:

- Separated serum/plasma should remain at room temperature for no longer than eight hours. If assays will not be completed within eight hours, serum/plasma should be refrigerated (2 to 8 °C).

- If assays are not completed within 48 hours, or the separated serum/plasma is to be stored beyond 48 hours, serum/plasma should be frozen at or below -20 °C.

CAUTION: Serum/plasma samples are not to be repeatedly frozen and thawed, since this can cause analyte deterioration. They are to be thawed only once. **Frost-free freezers are not suitable for**

storage, as freeze/thaw cycles allow the temperature of the sample to increase and then drop, allowing the sample to refreeze.

- Documented references for analytes that do not fall under these generalized rules are to be followed closely (e.g., insulin, gastrin,[79] ionized calcium,[27,28] catecholamines,[15] and ionized magnesium).[29]

- If these recommendations conflict with instances in which a serum/plasma separator device is used, the directions of the manufacturer are to be followed.

6.3.1.2.2 Serum/Plasma Separator Devices

The manufacturer's directions are to be consulted when a serum or plasma separating device is being used, either as an integral part of the blood collection tube (gel tubes) or inserted into the tube before or after centrifugation. These devices are described in Section 7.

After centrifugation, serum or plasma can be left in contact with the gel barrier for a limited time. Consult the manufacturer's recommendations for specific limitations. Specific references for the device in use are to be consulted. For non-gel devices, the time the serum/plasma can be left in contact with the device is variable. Consult the manufacturer's recommendations for specific limitations.

6.3.1.2.3 Preservatives

Antiglycolytic agents (e.g., fluorides) may stabilize plasma glucose for 24 hours at 25 °C and for up to 48 hours at 2 to 8 °C when plasma is in contact with cells.[37] Inadequate inhibition occurs when erythrocyte, platelet, or leukocyte counts are abnormally high. Newborn glycolysis is difficult to inhibit. The plasma from these specimens should be separated as soon as possible after collection (see Section 6.1.1.4).

6.3.1.3 Tube Closure

Serum, plasma, and whole blood specimens are kept covered (air tight) at all times to prevent possible exogenous contamination, evaporation, concentration changes, or possible spillage and aerosols.

7 Serum and Plasma Separator Devices

The description and functional properties of various devices used to separate serum/plasma from whole blood are covered in this section. Regardless of the type of device, it is important to read the manufacturer's instructions and follow their recommendations carefully. If necessary, request additional performance documentation. Several scientific publications can also be reviewed.[86-99]

Basically, serum/plasma separator devices are divided into two major categories: those that function during centrifugation and those that are used after centrifugation.

7.1 Devices Used During Centrifugation

7.1.1 Integrated Gel Tube Systems

7.1.1.1 Descriptions

- Serum

Integrated gel tube systems incorporate a relatively inert gel material into the blood collection tube. These

gels have a controlled viscosity and a specific gravity intermediate to serum and clot. In addition, a clotting activator (e.g., glass or silica particles) is contained within the tube. The tube walls and tube closure are treated to eliminate cell or clot hang-up at the top of the tube. Integrated tube systems do not require removal of the closure until an aliquot of serum is removed for testing or storage. These systems are available in a variety of tube sizes.

- Plasma

Plasma gel tubes are identical to serum gel tubes, except that they contain an anticoagulant (e.g., heparin, citrate, EDTA) in place of the clotting activator.

7.1.1.2 Function

- Serum

When blood enters the tube, the clot activator begins to activate clotting. Complete and rapid clotting is enhanced by five to ten gentle inversions of the collected blood. During centrifugation, the gel material forms an impermeable barrier between the serum and the clot. The specific rcf and time requirement depend on the device used. Consult the manufacturer's instructions. Barrier formation is more predictable with swing-bucket centrifuges.

When using a fixed-angle centrifuge, and if serum is to be stored on the gel, visual inspection of the tube is necessary to check for completeness of the barrier. Barrier formation is more predictable with swing-bucket centrifuges. However, the gel should be checked for barrier integrity.

- Plasma

The anticoagulant inhibits clotting. Complete anticoagulation is enhanced by five to ten gentle inversions of the collected blood. Separator function is identical to serum gel tubes.

7.1.1.3 Storage

In general, serum can be stored on the gel for up to 48 hours at 4 °C.[82,83] There have been studies for plasma storage on the gel.[6,94] Performance data should be requested from the manufacturer for the device of interest. If storage beyond this time is necessary, refer to the manufacturer's recommendations for specific limitations.

If serum/plasma is to be stored, the gel should be visually inspected for barrier integrity.

7.1.2 Integrated Gel Microcollection Tubes

Serum/plasma microcollection tubes incorporate an inert gel material and are made of plastic. Rapid clotting is retarded by the nonwettable plastic surfaces. Microcollection gel tubes for plasma contain either the anticoagulant lithium heparin or EDTA. Serum/plasma can be separated within 60 to 90 seconds using device-specific rcfs of 2000 to 15 000 x g. Consult the manufacturer's instructions. These devices have been reviewed.[6] (Also see the most current edition of NCCLS document H4—*Procedures and Devices for the Collection of Diagnostic Capillary Blood Specimens*.)

7.1.3 Devices Inserted Into the Collected Blood

7.1.3.1 Gel Devices

7.1.3.1.1 Description

A separator device can incorporate an inert gel material in a small container that is placed into the evacuated blood collection tube after removal of the tube closure. The gel has a controlled viscosity and a specific gravity intermediate to serum and clot. These devices can also be used to separate plasma from cells when the collected blood is anticoagulated. These devices can be used with a variety of tube sizes.

7.1.3.1.2 Function

During centrifugation, the gel material is released from the container into the blood. As centrifugation continues, a chemical/physical barrier is formed between the serum and the clot. Centrifugation should occur at a speed and time recommended by the device manufacturer to ensure release of the gel into the blood to form a barrier.

Serum can be stored on the gel for up to 48 hours. If serum is to be stored, the gel should be visually inspected for barrier integrity and a suitable closure should be placed on the tube after the device has been removed from the tube.

7.1.3.2 Non-Gel Devices

7.1.3.2.1 Description

A variety of non-gel devices have been available over the years. These devices are incorporated into the tube by the manufacturer, or are placed in the tube after specimen collection, and function during centrifugation. The device is inserted gently to avoid hemolysis of the erythrocytes. A suitable tube closure is placed on the tube and kept in place during the centrifugation and postcentrifugation phases until an aliquot of the specimen is removed for storage or testing.

Non-gel devices have been made from a variety of inert materials. They are manufactured to have a specific gravity intermediate to serum and clot. They can also be used to separate plasma from cells when the collected blood is anticoagulated.

Depending on the specific product, these devices can be used with a variety of collection tube sizes.

7.1.3.2.2 Function

During centrifugation, the device moves through the blood to an interface between the serum/plasma and the clot/cells. An rcf of 1000 to 1100 x g is used for ten minutes unless the manufacturer or a scientific reference indicates otherwise. An individualized study may be necessary to determine if the rcf requirement for a specific device influences the accuracy of test results.

These devices are referred to as “barriers” between serum/plasma and clot/cells.[91] The word “barrier” suggests an interruption of contact between the serum/plasma and the clot/cells. If serum/plasma is to be stored on these barriers, the impermeability of the device to the leakage of analytes (e.g., potassium) through the barrier to the serum/plasma should be documented by appropriate studies. In the absence of valid documentation, serum or plasma is removed from contact with the device.

7.2 Devices Used After Centrifugation

7.2.1 Description

Plunger-type filters are devices used to separate serum/plasma from the clot/cells after the blood tube has been centrifuged.

The individual device consists of a plastic tube with a filtering unit on the end. The design and construction of the filtering unit should prevent serum/plasma backflow through the filter and prevent particulate matter (fibrin) from passing into the filtered serum/plasma. Filters that do not prevent serum/plasma backflow are not recommended.

Plunger-type filtering devices are available for a wide variety of blood collection tube sizes.

7.2.2 Function

After centrifugation, the plunger-type filter is inserted into the collection tube and gently pushed down through the serum/plasma to a position above the interface of the serum/plasma and the clot/cells.

It is necessary to keep the filtering unit from touching the clot/cells, because contact is a potential source of cell hemolysis. A gap is created between the device and the tube contents by slightly withdrawing the device from the tube. The air gap is needed to prevent analyte leakage into the filtered serum/plasma at the clot/cell surface. If the air gap cannot be created because the design of the device does not prevent serum/plasma backflow, the separated serum/plasma is removed from the device. For plunger-type devices that can be used for serum/plasma storage, appropriate documentation is used to validate analyte stability and the absence of interference when the serum/plasma is left in contact with the specific device.

7.3 Tube Closure

For any separating device that requires the removal of the tube closure for either insertion of the device before centrifugation or for removal of separated serum or plasma, extreme caution must be exercised to prevent liquid or aerosol contamination of the work area.

The closure is reseated, or a suitable closure is used both before and after centrifuging the specimen.

7.4 Device Shelf-Life

For all serum/plasma separator devices, adhere to the manufacturer's stated storage conditions and expiration dates to ensure proper performance of the device.

7.5 Interferences

Gel device-associated errors have been reviewed.[6]

- LD: Although statistically significant, differences seen for LD (increased) using gel devices are considered clinically insignificant.

- Gel devices should not be used to collect blood for progesterone, tricyclic antidepressant drugs, or direct antiglobulin testing. Gel devices should not be used to collect blood for direct antiglobulin tests, as the gel can cause red cells to stick together simulating agglutination.[100]

- Therapeutic drugs: The laboratory must exercise caution when using gel devices to collect blood for drug classes such as antiepileptic, cardiac, aminoglycoside, etc. Studies have been contradictory and device-specific.

Regardless of device type, gel or non-gel, the scientific literature and the manufacturer's documentation should be consulted before selecting a device to use.

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NCCLS consensus procedures include an appeals process that is described in detail in Section 8 of the Administrative Procedures. For further information, contact the Executive Offices or visit our website at www.nccls.org.

Summary of Comments and Area Committee Responses

H18-A2: *Procedures for the Handling and Processing of Blood Specimens; Approved Guideline—Second Edition*

General

1. Recommendations on the accuracy of hematology results on EDTA tubes spun in error, remixed, and run would be helpful.

- **Documented studies were not found in the literature. Resuspension of spun EDTA tubes could occur if: 1) plasma has not been removed from the tube for other testing; and 2) a smear must be made to determine if any platelet clumping or clot formation or mechanical damage to blood cells has occurred. If platelet clumps or clotting are present, or there is microscopic evidence of cellular damage, the specimen is unacceptable.**

Section 6, Whole Blood Processed to a Serum or Plasma Sample

2. A chart would be helpful in the temperature descriptions.

- **The area committee believes that the current presentation of material on temperature and stability is appropriate due to the wide range of information provided. The user of the guideline may want to extract the information appropriate for the user's laboratory and prepare a chart.**

3. A contact time of less than two hours is recommended for potassium, ACTH, cortisol, catecholamines, and lactic acid. What about glucose?

- **References indicate that a decrease in glucose occurs by two to three hours after collection. This section does indicate that altered (decreased) results were clinically significant for glucose at two hours after collection.**

Section 6.1.2.1.4, Exposure to Light

4. Suggest mentioning the importance of protecting specimens drawn from patients with liver disease as well. Perhaps it should be stressed that blood drawn from any patient for liver function tests should be protected from light during transportation and processing.

- **The area committee believes that the subject of light exposure has been appropriately addressed.**

Section 6.2.2, Temperature-Controlled Centrifuges

5. The use of a temperature-controlled centrifuge is recommended in this section; however, no recommendations are provided if a temperature-controlled centrifuge is not available.

- **The area committee recommends that laboratories routinely receiving chilled specimens purchase a temperature-controlled centrifuge. If chilled specimens are infrequent and a temperature-controlled centrifuge is not available, then handle these specimens as expeditiously as possible. For example, select a centrifuge that has not been operating recently and therefore is at room temperature. Centrifuge for the minimum time recommended. Immediately remove the specimen when the centrifuge comes to a stop. Chill the spun specimen until testing can be completed.**

Section 7.1.1.1, Descriptions

6. This paragraph (serum) seems to be based on the assumption that all gel separator serum tubes contain a clot activator. In fact, glass serum tubes without a clot activator are still widely used.

- **This paragraph for serum refers only to integrated gel tube systems that always contain a clot activator. Tubes without gels are not included in Section 7.1.1.1.**

Section 7.1.1.2, Function

7. This paragraph seems to be based on the assumption that all gel separator serum tubes contain a clot activator. In fact, glass serum tubes without a clot activator are still widely used.

- **See response to Comment 6.**

Section 7.1.3.1, Gel Devices

8. The rcf we normally use is 3500g for ten minutes. Do we need to spin at that speed since there is a minimum of 500g for tubes with gel? Does it matter?

- **The area committee believes the reviewer means 3500 r.p.m. for ten minutes. Regardless, the area committee recommends following the manufacturer's directions for the device in question.**

Section 7.1.3.2.1, Description

9. Given the risk involved in manually inserting such devices, and the prevalence of much safer serum separator options, consider revising to discourage using devices that require the user to open tubes rather than to recognize them as a valid alternative. Even though Section 7.3 urges caution when using such devices, discouraging the use of such devices outright seems more appropriate.

- **Integrated collection devices are certainly safer to use; however, if a laboratory chooses to use nonintegrated devices, particular attention to safety issues must be exercised.**

Section 7.5, Interferences

10. This section states, "…gel devices should not be used to collect blood for ionized calcium or progesterone." Does this include tubes that have an integrated gel tube system or only for devices (gel or otherwise) inserted into the tube after collection? We collect both of these tests into a gel tube.

- **This section has been revised to remove the recommendation that ionized calcium cannot be collected in gel tubes. Please refer to NCCLS document C31-A2—*Ionized Calcium Determinations: Precollection Variables, Specimen Choice, Collection, and Handling; Approved Guideline—Second Edition*, which states, "The difference between plain and "gel separator" serum tubes for ionized calcium and pH is clinically insignificant if manufacturer's instructions for use of tubes are followed; either may be used for ionized calcium measurements."**

Summary of Delegate Comments and Area Committee Responses

H18-A3: *Procedures for the Handling and Processing of Blood Specimens; Approved Guideline—Third Edition*

General

1. No reference to changes in RBC and platelet indices with time from venous sampling for CBC.

- **Young's Preanalytical text cites studies that show RBCs to be stable for at least one week at room temperature and at refrigerated temperatures and that platelets are stable for at least 24 hours refrigerated (studies on room temperature stability are contradictory). No studies are mentioned of platelet indices (MPV). Further information may be available from the specific instrument manufacturer. Reference: Young D. *Effects of Preanalytical Variables on Clinical Laboratory Tests.* AACC Press. Washington, DC; 1997.**

Section 1, Scope

2. Laboratory specialty areas NOT mentioned are chemistry, blood banking, and newborn screening.

- **The text has been revised as follows:**

  "…the recommendations should be considered by the following laboratory areas: chemistry,…virology, blood bank…"

Section 5, Description of the Product Class

3. Suggest wording change. "This guideline provides recommended criteria for the collection of optimal whole blood specimens and serum/plasma samples. Specifications for optimal performance of *in vitro* diagnostic devices used in the processing of blood specimens is also provided..."

- **The text has been revised as follows:**

  "This guideline provides recommended criteria for correct handling of whole blood specimens and serum or plasma samples. Specifications for the optimal performance of *in vitro* diagnostic devices used in the processing of blood specimens are also provided."

Section 6, Whole Blood Processed to a Serum or Plasma Sample

4. Discussion covers most of chemistry issues but lacks in immunology information specifically in reference to storage temperatures of -20 °C are not addressed which is a recommendation for immunology tests.

- **The many immunology tests that are routinely performed make researching the stability of each antibody and antigen almost impossible and it is beyond the scope of this document to assess the stability of every analyte. Please refer to the manufacturer's product insert for specific storage recommendations. In addition, Section 6.3.1.2.1 states: "If assays are not completed within 48 hours, or the separated serum/plasma is to be stored beyond 48 hours, serum/plasma should be frozen at or below -20 ˚C." Immunology specimens can also be stored at -80 ˚C for long-term storage as needed for multicenter clinical trials.**

5. Should state what is needed up front:

   "Serum or plasma should be physically separated from contact with cells as soon as possible unless conclusive evidence that longer contact times do not contribute to result inaccuracy."

   Delete "for some specimens" and replace with:

   "Note: A contact time of less than…
   For some specimens, temperature affects stability. However, many analytes have not been studied."

   Reference(s) is written but only one is cited.

   "The following reference supports a two-hour precentrifugation time limit as recommended in this document:
   The Laessig, et al study[5] determined that 17 analytes were unaffected by precentrifugation serum-cell contact time as long as 48 hours (room temperature)…"

- **The area committee believes Section 6 is accurate as written. The two-hour recommendation is supported by all of the studies described in Section 6.**

  The text has been revised as follows: "The following studies support a two-hour precentrifugation time limit as recommended in this document."

6. However, altered results were clinically significant for the following.

   "By two hours." What does this mean? Up to and two hours? After two hours? If it is by two hours, shouldn't the time then be shortened to prevent spurious results? If it is 'after,' you may want to clarify:

   After two hours post blood collection:

   - Glucose was decreased
   - Potassium was increased
   - LD was increased

   After eight hours post blood collection:

   - Iron was increased.

   Again if using the word 'others' for reference, then cite more references than one. You may want to change wording to reflect citation:

   "…any change for magnesium, another reported a…"

- **The recommended time limit of two hours is considered appropriate as the studies used to arrive at the two-hour time limit do not indicate if changes were occurring just after collection or just prior to or at two hours. A laboratory may wish to use a time limit of less than two hours.**

  The text has been revised as follows to clarify "others": "AST showed a slight increase, and chloride a slight decrease, with time. Whereas this study did not indicate any change for magnesium, other investigators report a magnesium increase by four hours at 4 ˚C."

7. States LD is increased at two hours and is "clinically significant," yet nine lines down states that LD is "minimally affected."

- **H18 states the findings and conclusions of two different studies, References 5 and 23. Both note an increase for LD. One study concluded the increase was clinically significant at two hours and the other concludes the increase to be minimal at 72 hours. The area committee's position is to be conservative in its recommendations.**

Section 6.1.1.3, Chilled Specimens (2 to 8 ˚C)

8. The examples are incorrect: only ammonia needs to be collected on ice.

- **According to Reference 37, the examples are correct. If recent or future studies indicate that a particular requirement for a specific analyte is no longer necessary, a laboratory should document its procedure in its laboratory.**

Section 6.1.1.4, Metabolic Inhibitors and Preservatives

9. The third sentence of the second paragraph states, "Microcollection devices containing a suitable antiglycolytic agent should be used for pediatric blood glucose collection." I suggest changing to "may be used," since there are no published data to demonstrate that there is a difference if other devices are used.

- **The text has been revised as follows: "Microcollection devices containing a suitable antiglycolytic agent may be used for pediatric blood glucose collection."**

Section 6.1.2, Transportation

10. Lacks information on leak proof/screw caps of the transfer tubes.

- **The text has been revised as follows: "Secondary tubes must be leak-proof" has been added after the third sentence in Section 6.1.2.2.1, Remote Collection Sites.**

Sections 6.1.2.1.2, Tube Orientation, and 6.1.2.2.1, Remote Collection Sites

11. Blood tubes do not need to be vertical. All pneumatic tube systems will have random orientations. We have done extensive checks of pneumatic tubes and found no degradation for nearly all analytes. This requirement will cause unnecessary cost and complexity and is not consistent with Section 6.1.2.3.

- **The text has been revised as follows in Section 6.1.2.1.2: "Where possible, tubes of blood should be kept in a vertical, closure-up position."**

Sections 6.1.2.2.1, Remote Collection Sites, and 6.3.1.1, Serum/Plasma Contact with Cells/Clot

12. A maximum limit of two hours is unrealistic for most hospitals. We use three hours which is met most of the time. There are exceptions which require special handling. There are few analytes that are not stable in whole blood for three hours; see Zhang, et al. Effect of serum-clot contact time on clinical chemistry laboratory results. *Clin Chem*. 1998;44:1325-1333.

- **Two hours (Reference 5) to three hours (Reference 31) must be adhered to for glucose and/or potassium. The different studies described under Section 6 are intended to guide the readers of H18-A3 to make informed decisions for their laboratories. When glucose and/or potassium are ordered along with more stable analytes in the same collection tube, the shorter stability times dictate the timeline. The area committee's position is to be conservative in its recommendations.**

  The text has been revised as follows in Section 6.1.2.2.1: "The stability of the specimen dictates conditions for transport from remote collection sites (e.g., physician's offices, satellite draw stations) to the testing location. If an uncentrifuged whole blood specimen is to be sent to a laboratory for testing, it must reach the laboratory in time to be processed with serum/plasma separation occurring in a time limit to protect the stability of the analytes. If this requirement cannot be met, the specimen must be centrifuged at the collection site with the serum or plasma separated from the cells and held under appropriate conditions (see Section 6.3) until it can be delivered to the laboratory…Specific handling and processing requirements published by the testing laboratory should also be consulted."

Section 6.1.2.3, Automated Delivery Systems

13. Recommend how many specimens and a statistical test to be used for a parallel study to qualify a pneumatic tube system.

- **The area committee recommends that the references cited in H18 for pneumatic tube systems be reviewed for how the authors studied these systems as to the number of test comparisons and the statistical tests employed.**

14. In second paragraph, second sentence: "in under-filled tubes." This is confusing. Tubes should be properly filled, especially coagulation tubes. Is the document referring to partial draw tubes? Either way, it should be rephrased since "under-filled" is unclear and potentially misleading.

- **The document is not referring to partial draw tubes but to improperly filled or "short-draw" tubes that lead to the alteration of the blood to additive ratio. The text has been revised as follows: "This is especially important for heparin management by APTT in tubes that are not optimally filled, since a progressive shortening of the heparin APTT value in part related to platelet-released neutralizing substances has the potential to lead to over-anticoagulated patients."**

Section 6.1.3.1, Time

15. In the second sentence, add five minutes (reflects clotting time with thrombin). Also add, "see manufacturer's recommendations" since the clot time of additives listed vary significantly. Correct (see Section 6.1.1.1) to (see Section 6.1.1.2) for plasma reference.

- **The text has been revised as follows: "Blood collected using a tube containing a clotting activator (e.g., thrombin, silica, or glass particles) can be processed as early as 5 to 30 minutes after the blood is drawn. Anticoagulated specimens can be centrifuged immediately (see Sections 6.1.1.1 for serum and 6.1.1.2 for plasma). Also refer to manufacturers' specific recommendations."**

Section 6.1.3.5, Criteria for Specimen Rejection

16. Add "with facility policy" after "consulted for recommended labeling requirements" in the second paragraph.

- **The text has been revised as follows: "The most current edition of NCCLS document H3—*Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture* should be consulted for recommended labeling requirements. In addition, consult facility policy."**

17. Under the Hemolysis bullet point, only Section 6.1.2.1.3 is specifically written for hemolysis.

- **The text has been revised to refer the reader to Section 6.1.2.1.3.**

Section 6.2.2.1, Recommendations

18. Temperature-controlled centrifuges are not necessary for many analytes. The text should be modified to indicate "for temperature-sensitive analytes."

- **The text has been revised as follows: "Laboratories should have temperature-controlled centrifuges for temperature-sensitive analytes. Centrifuges can generate internal heat that may be inappropriate for analyte stability (e.g., coagulation studies). Specific thermolabile analytes should be separated at 4 ^{o}C (e.g., ACTH, cyclic-AMP)."**

Section 6.2.2.2, Chilled Specimens

19. Potassium should not be measured if the specimen was chilled. Potassium changes in whole blood are dramatic and fast when temperature is changed. The two hour caution in the text is incorrect. See the reference cited above.

- **The area committee believes the recommendation is supported by References 4 and 25.**

Section 6.2.3, Recentrifugation

20. The first sentence of the second paragraph should state the findings of the literature search up front. Also, I believe that use of the word 'other' will further clarify that recentrifugation of 'analytes' does not include potassium. Either use 'first and second centrifugations' or 'centrifugation and recentrifugation.' The last sentence is also too long. Try something like this:

    "The working group performed a literature search for the years 1966 to 2004 and found three sources recommending that potassium should not be recentrifuged. Other analytes were not cited. However, the working group cautions that the potential for inaccuracy of other analytes is possible. Laboratories may wish to conduct studies when there are unexplained results seemingly attributable to recentrifugation. For example, when the time between centrifugation and recentrifugation has been lengthy, as can occur between offsite collection locations and the testing laboratory."

- **Section 6.2.3 has been revised as follows:**

  6.2.3 Recentrifugation

  "Specimens for potassium measurement should not be centrifuged more than once. Results will be falsely increased. [75-78]

  NOTE: A literature search was performed for the years 1966 to 2004. Four references specifically indicate that specimens for potassium should not be recentrifuged.[75-78] **No other analytes were cited. However, the area committee cautions that the potential for inaccuracy for other analytes is possible. Laboratories may wish to conduct studies when there are unexplained results possibly attributable to recentrifugation, for example, when the time between centrifugation and recentrifugation has been lengthy, as can occur between offsite blood collection locations and the testing laboratory.**

  Harvesting of additional serum/plasma AFTER serum/plasma has been removed from non-gel or gel tubes SHOULD NOT be attempted. When serum/plasma has been removed and harvesting of additional serum/plasma is attempted, the volume ratio of plasma to erythrocytes has been altered. Analytes from cellular leakage/exchange, accentuated by clot retraction, will then be centrifuged into the serum/plasma used for testing. This can cause erroneous results."

Section 6.2.3.1, Recentrifuging a Non-Gel Tube with Serum/Plasma in Contact with Cells

21. Unclear. Are you saying that this should not be done? If so, then it should be stated up front. Also, one sentence doesn't work. I suggest revising the section as follows:

    "Harvesting of additional serum/plasma after serum/plasma has been removed from the non-gel tube SHOULD NOT be attempted.
    If serum/plasma has been removed from the tube and harvesting of additional serum/plasma is attempted, the volume ratio of plasma water to erythrocytes will be altered. Analytes from cellular leakage/exchange will then be centrifuged into the serum/plasma used for testing, which may cause erroneous results."

- **Section 6.2.3, Recentrifugation, has been revised (see response to comment 20) and Section 6.2.3.1 has been deleted.**

Section 6.2.3.2, Recentrifuging a Gel Tube

22. Unclear. Sentence is too long. Don't understand what is being said. Do you have a recommendation to prevent this or are you telling the reader to just make sure to watch out for clot retraction?

- **Section 6.2.3, Recentrifugation, has been revised (see response to comment 20) and Section 6.2.3.2 has been deleted.**

Section 6.3, Postcentrifugation Phase

23. Lack of information on effect of lipemic, hemolyzed, turbid and icteric serum, and effect of heat inactivation on tests used for determination of IgG/IgM levels for various infectious diseases. No mention of blood sample collection from a cadaver in the document.

- **Postcentrifugation is defined as the time period after the centrifugation of the specimen and before removal of an aliquot of serum/plasma for testing. Lipemia, hemolysis, icterus, and heat inactivation are testing issues.**

  Blood collection from cadavers is beyond the scope of this guideline.

Section 6.3.1.2.1, Separated Serum or Plasma

24. First paragraph, fifth sentence, restate (i.e., insert) 'separated serum' for clarity.

    "At 2 to 8 ˚C and room temperature significant stability of separated serum has been observed for 2 to 14 days."

    Also, a question on room temperature. A 20-25 ˚C range for room temperature is given, but then there is a statement about instability above 22 ˚C. What does that mean? Is the recommended room temperature then between 20-22 ˚C? If so, take out 20-25 and replace with 20-22 ˚C.

- **The following text has been added after the first sentence: "It is the responsibility of the individual laboratory to use all available references and/or their own studies to determine specific stability criteria for their laboratory." This section has been further revised as follows: Two sentences have been added to a new paragraph that reads: "It seems apparent that an analyte that is stable at room temperature in unseparated serum/plasma should also be stable at the same temperature for the same length of time in separated serum/plasma…Based on the studies cited, this guideline makes the following 'general' recommendations, recognizing that there can be several exceptions." The sixth sentence of this paragraph has been revised to read: "However, room temperature (20 to 25 °C) in the laboratory is a critical stability parameter with decreased stability observed at temperatures above 22 °C for some analytes." The first bullet has been revised to state "room temperature" instead of 22 °C.**

  20 to 25 °C is generally considered to be room temperature. However, Reference 4 indicates there can be analyte instability at greater than 22 °C. The reader should consult all of the different references pertaining to temperature.

25. This section contains numerous generalizations that can be misleading and incorrect. Storage conditions to preserve the integrity of blood and serum specimens vary with the analyte. The statement "when tubes are stoppered and serum is in contact with cells" contradicts earlier recommendations in Section 6 to separate serum or plasma from cells within two (three) hours. The generalizations in the first two bullets on page 13 are not applicable to many situations. I suggest this section be completely rewritten to emphasize the importance of setting storage conditions for each analyte which meets its requirements for stable storage.

- **The area committee recognizes that storage conditions can vary with the analyte. This is the reason for the recommendation that laboratories consult specific references and the manufacturer of tests and collection devices. This guideline cites several references which indicate that there are many analytes that are stable with serum in contact with cells. However, there are also several analytes that must be separated from the cells in order to maintain analyte integrity. We believe it should be the responsibility of the individual laboratory to establish its own specific handling policy for different analyte stabilities based on the available science.**

  Section 6.3.1.2.1 has been revised. See response to comment 24.

Section 7.1.1.2, Function

26. An important technical detail needs to be added. Gel barrier tubes should never be respun because serum or plasma that has been in contact with the cells will be expressed into the serum above the barrier. This can lead to serious interference from potassium, phosphate, AST, LDH, etc., which have leaked from the red cells.

- **This information can be found in Section 6.2.3, Recentrifugation.**

27. Sentences 7 and 8 conflict with sentence 6. I recommend keeping sentences 7 and 8, and deleting sentence 6.

- **The text has been revised as follows: "When using a fixed-angle centrifuge, and if serum is to be stored on the gel, visual inspection of the tube is necessary to check for completeness of the barrier. Barrier formation is more predictable with swing-bucket centrifuges. However, the gel should be checked for barrier integrity."**

Section 7.1.1.3, Storage

28. Serum can be stored on gel barriers for several days at 4 ˚C without affecting most analytes. The text should not be so inflexible in stating only up to 24 hours since there is no literature to support that limitation.

- **The text has been revised as follows: "In general, serum can be stored on the gel for up to 48 hours at 4 ^{o}C." Two references have also been added at the end of this sentence. The sentence recommending that tubes should be kept in a vertical closure-up position has been deleted.**

  Some older references (References 82 and 83) indicate two to five days. The number of methods and analytes studied ranged from few to many. As indicated in the guideline, device manufacturers should be consulted for more current stability information. The individual laboratory may also wish to conduct its own stabilities study.

29. Discrepancy for serum storage. This section states up to 24 hours at 4 ˚C (referenced), while Section 7.1.3.1.2 states that serum may be stored for up to 48 hours (no reference).

- **The text has been revised for consistency as described in the response to comment 28.**

Section 7.1.3.1.2, Function

30. Add a reference for storage at 48 hours. The recommendation conflicts with storage time in Section 7.1.1.3.

- **Section 7.1.3.1.2 refers to a nonintegrated gel device. The original manufacturer's documentation indicated 48 hours. Section 7.1.1.3 has been revised to 48 hours for integrated gel tube systems. See response to comment 28.**

31. Add "prior to centrifugation" after "A suitable tube closure is placed on the tube." Or move the fourth sentence in Section 7.1.3.2.1 to the end of 7.1.3.1.2.

- **The text has been revised as follows: "Serum can be stored on the gel for up to 48 hours. If serum is to be stored, the gel should be visually inspected for barrier integrity and a suitable closure should be placed on the tube after the device has been removed from the tube."**

NOTES

The Quality System Approach

NCCLS subscribes to a quality system approach in the development of standards and guidelines, which facilitates project management; defines a document structure via a template; and provides a process to identify needed documents. The approach is based on the model presented in the most current edition of NCCLS document HS1—*A Quality Management System Model for Health Care.* The quality system approach applies a core set of "quality system essentials" (QSEs), basic to any organization, to all operations in any healthcare service's path of workflow (i.e., operational aspects that define how a particular product or service is provided). The QSEs provide the framework for delivery of any type of product or service, serving as a manager's guide. The quality system essentials (QSEs) are:

Documents & Records	Equipment	Information Management	Process Improvement
Organization	Purchasing & Inventory	Occurrence Management	Service & Satisfaction
Personnel	Process Control	Assessment	Facilities & Safety

H18-A3 addresses the quality system essentials (QSEs) indicated by an "X." For a description of the other NCCLS documents listed in the grid, please refer to the Related NCCLS Publications section on the following page.

Documents & Records	Organization	Personnel	Equipment	Purchasing & Inventory	Process Control	Information Management	Occurrence Management	Assessment	Process Improvement	Service & Satisfaction	Facilities & Safety
				H1 H3	X H3						H3 M29

Adapted from NCCLS document HS1—*A Quality Management System Model for Health Care.*

Path of Workflow

A path of workflow is the description of the necessary steps to deliver the particular product or service that the organization or entity provides. For example, NCCLS document GP26—*Application of a Quality Management System Model for Laboratory Services* defines a clinical laboratory path of workflow which consists of three sequential processes: preanalytic, analytic, and postanalytic. All clinical laboratories follow these processes to deliver the laboratory's services, namely quality laboratory information.

H18-A3 addresses the clinical laboratory path of workflow steps indicated by an "X." For a description of the other NCCLS documents listed in the grid, please refer to the Related NCCLS Publications section on the following page.

Preanalytic					**Analytic**		**Postanalytic**	
Patient Assessment	Test Request	Specimen Collection	Specimen Transport	Specimen Receipt	Testing Review	Laboratory Interpretation	Results Report	Post-test Specimen Management
H3	H3	H3 H4 H11 H21	X H3 H4 H11 H21	X H3				

Adapted from NCCLS document HS1—*A Quality Management System Model for Health Care.*

Related NCCLS Publications*

C31-A2 **Ionized Calcium Determinations: Precollection Variables, Specimen Choice, Collection, and Handling; Approved Guideline—Second Edition (2001).** This document addresses preanalytical considerations—such as patient condition, specimen choice, collection, and handling—that can influence accuracy and clinical utility of ionized calcium measurements.

H1-A5 **Tubes and Additives for Venous Blood Specimen Collection; Approved Standard—Fifth Edition (2003).** This standard contains requirements for blood collection tubes and additives including heparin, EDTA, and sodium citrate.

H3-A5 **Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; Approved Standard—Fifth Edition (2003).** This document provides procedures for the collection of diagnostic specimens by venipuncture, including line draws, blood culture collection, and venipuncture in children.

H4-A5 **Procedures and Devices for the Collection of Diagnostic Capillary Blood Specimens; Approved Standard—Fifth Edition (2004).** This document provides a technique for the collection of diagnostic capillary blood specimens, including recommendations for collection sites and specimen handling and identification. Specifications for disposable devices used to collect, process, and transfer diagnostic capillary blood specimens are also included.

H11-A4 **Procedures for the Collection of Arterial Blood Specimens; Approved Standard—Fourth Edition (2004).** This document provides principles for collecting, handling, and transporting arterial blood specimens to assist with reducing collection hazards and ensuring the integrity of the arterial specimen.

H21-A4 **Collection, Transport, and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays; Approved Guideline—Fourth Edition (2003).** This document provides procedures for collecting, transporting, and storing blood; processing blood specimens; storage of plasma for coagulation testing; and general recommendations for performing the tests.

M29-A2 **Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline—Second Edition (2001).** Based on U.S. regulations, this document provides guidance on the risk of transmission of hepatitis viruses and human immunodeficiency viruses in any laboratory setting; specific precautions for preventing the laboratory transmission of blood-borne infection from laboratory instruments and materials; and recommendations for the management of blood-borne exposure.

* Proposed- and tentative-level documents are being advanced through the NCCLS consensus process; therefore, readers should refer to the most recent editions.

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